A085 Folliculogenesis, oogenesis and superovulation

Differences in oocyte and cumulus cells gene expression in Nelore heifers with low and high antral follicle count

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Antral Follicle Count (AFC) has been associated with reproductive performance in cattle. In taurine females, higher AFC is positively associated with pregnancy rates, and is linked to greater *in vivo* and *in vitro* embryo production, both in number and efficiency. Contrarily, in indicine females, AFC is negatively correlated with pregnancy rates after IVP-ET or TFAI. How the transcriptional patters in ovarian follicular cells correlate with the AFC remains unknown. This study aimed to investigate the expression of 95 genes in oocytes and cumulus cells in females of low AFC (LAFC) and high AFC (HAFC) using Nelore heifers (*Bos taurus indicus*) as model. Nellore heifers (n=48) had the ovarian follicular wave synchronized. On Day 5, AFC was determined by ultrasound examination. The bottom 10 and top 10 females were assigned to experimental groups LAFC (number of follicles<30, AFC = 24 ± 4.73) and HAFC (number of follicles >60, AFC = 72.3 ± 15.67), respectively. On Day 5 cumulus-oocyte complexes (COCs) were retrieved by ovum pick-up. Cumulus cells and oocytes were separated by pipetting. Twenty samples (pools) of 10 oocytes (one per animal) and 20 samples (pools) of cumulus cells from 10 COCs (one per animal) were used for RNA extraction, cDNA synthesis and gene expression assessment of 94 genes by RT-qPCR. Means of normalized gene expression, using *PPIA* as the reference gene, were compared by Student's t-test and were considered significant when P<0.05. In oocytes, a total of 11 genes were differentially expressed, including eight upregulated in LAFC group (*GAPDH*, *HSF1 BMP15*, *HAS2*, *EGFR, NPR3, H1FOO* and *IGFBP2)* and three downregulated in LAFC (*TFAM*, *XBP1* and *PRDX3*) compared with HAFC. Collectively, these genes are related to COC differentiation, meiotic control, epigenetic modulation, follicular recruitment and cellular responses to stress. In cumulus cells a total of 27 genes were differentially expressed between the groups. Cumulus cells from LAFC animals had higher expression of genes associated with meiotic control (*EGFR*, *RGS2*, *NPR3* and *NPR2*) and epigenetic modulation (*DNMT3A*, *HDAC2* and *PAF1*), as well as genes associated with energetic metabolism, cellular responses to stress and others (*CDK6*, *PA2G4*, *CASP9*, *STAT3*, *XBP1*, *HSP90AA1*, *HSPA5*, *HSPD1*, *SOD1*, *IGF1R*, *GSK3A*, *ATPL5*, *TFAM* and *PFKP)*. Contrarily, *FSHR*, *GAPDH*, *SREBF1*, *NFKB2*, *ARO* and *PTGS2* were downregulated in LAFC compared with HAFC group. These results indicate that distinct cellular compartments within ovarian follicles have dissimilar transcriptional patterns comparing LAFC and HAFC females. Such variations are potentially linked to reproductive performance and are suggestive of differential modulation in terms of cumulus-oocyte complex differentiation, meiotic control and oocyte competence acquisition. Financial support: Sao Paulo Research Foundation (FAPESP; grants 2012/50533-2 and 2018/13155-6) and CAPES - Brazil - Finance Code 001.

A086 Folliculogenesis, oogenesis and superovulation

Amburana cearensis leaf extract attenuates cisplatin-induced ovarian damage in mice

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Cisplatin is one of the most effective chemotherapeutic drugs, but it has a proven ovarian toxicity by increasing oxidative stress (Barberino et al., Biology of Reproduction, 96:1244–55, 2017). Thus, the pretreatment with natural compounds with antioxidant properties may reduce this toxicity. Recently, *Amburana cearensis,* a medicinal plant, has attracted attention because of its antioxidant actions (Gouveia et al., Theriogenology, 86:1275-84, 2016).The aim of this study was to evaluate the effects of pretreatment with the extract from the leaves of *A. cearensis* before cisplatin chemotherapy on ovarian follicle morphology, oxidative stress markers (reactive oxygen species [ROS] and glutathione [GSH] levels) and metabolically active mitochondria in mice. The adult female mice (n=25) were divided into five groups: the first group acting as a control, received orally by gavage (p.o.) saline solution (0.15 M, 0.3 mL/mouse) and after 1 h, intraperitoneal injection (i.p.) of saline solution (0.15 M, 0.15 mL/mouse). The second group received cisplatin (5 mg/kg body weight, i.p.) at 1 h after saline solution treatment (0.15 M, 0.3 mL/mouse; p.o.). The third group was a positive control, which received *N*-acetylcysteine (150 mg/kg body weight, p.o.) 1 h before cisplatin treatment (5 mg/kg body weight, i.p.). For the fourth and fifth groups, mice received leaf extract of *A. cearensis* at 50 or 200 mg/kg body weight (p.o.), respectively, and after 1 h, mice received cisplatin (5 mg/kg body weight, i.p.). All groups were treated once daily for 3 days. The ovaries were harvested from the mice 24 h after the last pharmacological administration and destined to histological (follicular morphology) and fluorescence (ROS, GSH, and active mitochondria levels) analyses. The percentages of normal follicles (total or in the different developmental stages) were submitted to the Chi-square test. Data from ROS, GSH, and mitochondrial activity were compared by Kruskal-Wallis nonparametric and Student Newmans Keuls tests ($P<0.05$). The results showed that cisplatin treatment decreased ($P<0.05$) the percentage of normal follicles (30%) in comparison to the control (72.12%) and *N*-acetylcysteine (67.33%) groups. Nevertheless, *A. cearensis* extract pretreatment (50 mg/kg: 58.1%; 200 mg/kg: 54.82%) prevented ($P<0.05$) the reduction in the percentage of normal follicles induced by cisplatin, maintaining it similar to *N*-acetylcysteine group. Cisplatin treatment increased ($P<0.05$) ROS levels and decreased ($P<0.05$) both levels of GSH and active mitochondria in comparison to the control and *N*-acetylcysteine groups. Pretreatment with *A. cearensis* (50 or 200 mg/kg) prevented ($P<0.05$) the oxidative stress induced by cisplatin. In addition, *A. cearensis* extract (50 or 200 mg/kg) increased ($P>0.05$) the mitochondrial activity compared to control and *N*-acetylcysteine groups. In conclusion, the leaf extract of *A. cearensis* may attenuate the ovarian damage caused by the cisplatin treatment.

A087 Folliculogenesis, oogenesis and superovulation

Autophagy is a pro-survival adaptive response to heat shock in bovine oocytes

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Autophagy is a physiological mechanism that can be activated under stress conditions to recycle protein aggregates and damaged organelles for cellular metabolism. The cascade of events triggered during fertilization activates the autophagic pathway essential for early preimplantation development. However, the role of autophagy during oocyte maturation has been poorly investigated. Therefore, the objective of this study was to characterize the impact of autophagy inhibition on developmental competence and gene expression of bovine oocytes exposed to heat shock (HS). COCs were matured at Control (38.5ºC for 22 h) and Heat Shock (41ºC for 16 h followed by 38.5ºC for 6 h) temperatures in the presence of 0 and 10 mM 3-methyladenine (3MA; autophagy inhibitor). This model was used for all experiments described below. Parametric data were analyzed by ANOVA procedure of SAS and non-parametric data were analyzed by the Kruskal-Wallis test. The first study determined whether HS induces autophagy on bovine COCs. After 22 h IVM, a total of 180 oocytes and cumulus cells of 150-210 COCs were processed for microtubule-associated protein 1A/1B-light chain 3 (LC3: autophagy marker) protein abundance by 3 replicates of western blotting. High LC3-II/LC3-I ratio is indicative of autophagy induction. Heat shock increased ($P<0.05$) LC3-II/LC3-I ratio in bovine oocytes as compared to control. However, for oocytes matured with 3MA, there was no difference in LC3-II/LC3-I ratio between control and HS. On the other hand, there was no effect of temperature on LC3-II/LC3-I ratio in cumulus cells. Although, the addition of 3MA reduced ($P<0.05$) LC3-II/LC3-I ratio under control and HS. The second study determined the role of autophagy on developmental competence of heat-shocked oocytes. After IVM, a total of 150 COCs were subjected to 5 replicates of IVF and IVC. Inhibition of autophagy during IVM reduced ($P<0.01$) cleavage rate of heat-shocked oocytes as compared to all the other groups. Exposure to HS reduced ($P<0.05$) the proportion of oocytes that reached the blastocyst stage. However, this deleterious effect of HS was increased ($P<0.01$) for oocytes matured with 3MA. The third study determined the effect of autophagy inhibition on mRNA expression in heat-shocked oocytes. Gene expression analysis of 150 bovine oocytes total was performed 5 times using Applied Biosystems™ TaqMan® Assays. The qPCR was performed in the Biomark HD System. Inhibition of autophagy in heat-shocked oocytes reduced ($P<0.06$) mRNA abundance for genes related to different biological processes such as oocyte maturation (*BMP15*, *HAS2*, and *GREM1*), lipid and energy metabolism (*SREBF2* and *MTIF3*), cellular growth (*IGF2*), and heat shock response (*HSF1* and *HSPA1A*). In conclusion, autophagy is a stress response induced on oocytes exposed to HS during IVM and its inhibition modulated important functional processes in the bovine oocyte rendering the oocyte more susceptible to the deleterious effects of HS.

A088 Folliculogenesis, oogenesis and superovulation

The effect of a non-steroidal anti-inflammatory drug on PGF and PGFM concentrations in bovine follicular fluid after GnRH treatment

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Although it is known that prostaglandins are essential for ovulation, some studies suggest that PGF could also be used as an ovulation inducer in timed artificial insemination protocols. The present study tested whether an i.m. injection of PGF reverts the effect of a non-steroidal anti-inflammatory drug (NSAID) administered 17h after GnRH-induced ovulation in cattle. The procedures were approved by UFPel and UFSM Animal Ethics Committee. Nineteen non-lactating cyclic Jersey cows had their follicular growth synchronized by a hormonal protocol consisting of insertion of an intravaginal device (IVD) containing progesterone (P4; 1g) on D0 along with i.m. injections of estradiol benzoate (2mg) on D0 and of cloprostenol (150µg) on both D0 and D8. The IVDs were removed on D9 and GnRH injections (gonadorelin acetate; 100µg i.m.) were performed in all the cows 20h after IVD withdrawal (hour 0). Then, 17h after GnRH, cows were allocated to three treatments: control (saline i.m.; n=6); NSAID: 2.2mg/kg flunixin meglumine (FM) i.m. (n=6); and NSAID+PGF: 2.2mg/kg FM (n=6) and 25mg dinoprost tromethamine (i.m.), 6h later. The groups were normalized according to follicular diameter and follicular aspirations were performed 24h after hour 0. The concentrations of PGF and of PGF metabolite (PGFM) in the follicular fluid (FF) were evaluated through ELISA Prostaglandin F2α and 13,14-dihydro-15-keto Prostaglandin F2α kits (Cayman Chemical, Ann Arbor, USA), respectively. Follicular vascular flow was evaluated at 0 and 24h after GnRH administration using Color Doppler Ultrasonography (Mindray M5; 6,5 MHz) and subjectively evaluated by three trained technicians using a 4-point score. The effects of treatments on PGF and PGFM concentrations were analyzed by ANOVA, with comparisons of means using the Tukey test. The median score of follicular vascular flow was compared among treatments by the Kruskal-Wallis ANOVA for non-parametric data. The concentration of PGF in the FF was greater (P=0.0032) in the control (49563.2±7727.7 pg/ml) than in NSAID (5664.3±3473.1 pg/ml) and NSAID+PGF (3198.4±770.5 pg/ml). The PGFM concentrations in the FF were greater (P<0.0001) in NSAID+PGF (3450.4±269.6 pg/ml) than in the control and NSAID groups (1228.2±45.7 and 204.1±568.5 pg/ml, respectively). Decreased vascular flow was observed in NSAID+PGF group (P<0.05). In conclusion, systemic NSAID treatment inhibits GnRH/LH-induced PGF secretion in preovulatory follicles which, in the conditions of the present study, was not reverted by systemic PGF administration. The use of NSAID after GnRH treatment provides an adequate model to investigate the effect of PGF on ovulation in cattle. The authors are thankful to FAPERGS and CNPq (Edital PRONEX-16/2551-0000494-3) and CAPES for financial support (financial code 001).

A089 Folliculogenesis, oogenesis and superovulation

Effects of in vitro prematuration of bovine COCs on the levels of maturation-related transcripts and meiosis resumption

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During growth, oocytes synthesize large quantities of mRNA that accumulates to form the stock of maternal mRNA, which will have a crucial role during oocyte maturation and early embryo development. However, it is still not known if in vitro prematuration of oocytes increase the levels of these maternal mRNAs and have a positive impact on oocyte maturation. The present study evaluated the effects of prematuration and maturation of COCs on the levels of mRNA for growth and differentiation factor 9 (*GDF9)*, cyclin B1, oocyte-specific linker histone *(H1FOO*), quinase *cMOS*, poly(A) ribonuclease *(PARN)* and eukaryotic initiation factor 4E (*eIF4E)*. Cow ovaries (n= 40) were obtained from a local abattoir and the cumulus-oocyte complexes (COCs) of medium antral follicles (3.0 to 6.0 mm in diameter) were aspirated and classified according to the morphology of oocyte and cumulus cells. Fresh COCs were collected and used as uncultured control. For prematuration and maturation protocols, grade 1 and 2 COCs with a visible compact and intact cumulus cells and a dark cytoplasm were selected. The prematuration medium (pre-IVM medium) consisted of TCM-199* supplemented with 10 µM cilostamide (Sigma, St. Louis, MO, USA) and follicular hemi-sections (n=8). The COCs were pre-matured individually for 20 h. The *in vitro* maturation medium (IVM) was designated as TCM-199* supplemented with L-glutamine (Sigma) 0.2 mM pyruvic acid (Sigma), 5.0 mg/mL LH (Bioniche, Belleville, ON, Canada), 0.5 mg/mL FSH (Bioniche), 0.4% BSA (Sigma), 10 µM of cilostamide (Sigma) and 100 IU/mL penicillin and 50 µg/mL streptomycin sulfate (Sigma). The COCs were cultured individually for 22 h at 39°C in a saturated humidity atmosphere containing 5% CO2 and 95% air. To evaluate the levels of mRNAs, oocytes from uncultured COCs (group 1), as well as from prematured COCs (group 2) or both prematured and matured COCs (group 3) were collected and stored at -80 °C, until RNA extraction. Quantification of mRNA for *GDF9*, *cyclin B1*, *H1FOO*, *cMOS*, *PARN* and *eIF4E* was performed by real-time PCR. The results were analyzed by the Kruskal-Wallis test, followed of the post hoc nonparametric Dunn's Multiple Comparison Test. Differences were considered significant when $P<0.05$. After prematuration of COCs, only 35.0% of the oocytes had meiosis resumption, whereas after IVM a meiosis resumption rate of 90% was observed. Prematuration and IVM of COCs increased the levels of mRNA for *cMOS* and *H1FOO* in oocytes when compared to oocytes before the IVM period. The levels of mRNA for *GDF9*, *PARN* and *eIF4E* did not show significant differences, but the levels of *cyclin B1* presented a significant increase after prematuration period, when compared to oocytes before prematuration. In conclusion, prematuration and maturation of oocytes from medium antral follicles increase expression of *eIF4E*, *PARN*, *H1FOO*, *cMOS*, *GDF9* and *cyclin B1*.

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Effects of in vitro culture of secondary follicles on the levels of mRNAs for *GDF9*, *cyclin B1*, *H1FOO*, *cMOS*, *PARN* and *eIF4E* in bovine oocytes

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During oocyte development a prolonged phase of intensive RNA synthesis is required for the production of transcripts essential for oocyte growth and pre-implantation embryo development. In this way, the investigation of genes that are involved with the translation process, as well as transcripts that are stored in the oocytes are quite important for understand oocyte development *in vitro*. Thus, the aim of the this study was to evaluate the effect of *in vitro* culture of secondary follicles from cattle on the levels of mRNAs expression of *GDF9*, cyclin B1, *H1FOO* (oocyte-specific linker histone), quinase *cMOS*, *PARN* (poly(A) ribonuclease) and *eIF4E (*cap binding protein) in oocytes. Thus, secondary follicles (~ 0.2 mm) were isolated from the ovarian cortex and part of these follicles were ruptured and oocytes were collected and stored at -80 ºC (control group). The remaining follicles were individually cultured at 38.5°C, 5% CO2, for 18 days only in TCM-199 supplemented with 10 µg/mL insulin, 5.5µg/mL transferrin and 5 ng/mL selenium (ITS), 3.0 mg/mL bovine serum albumin (BSA), 2mM glutamine, 2 mM hypoxanthine, 50 µg/mL of ascorbic acid and 100 ng/mL FSH. After 18 days of culture, as in the control group the follicles were ruptured and the oocytes were collected and stored at -80 °C, until RNA extraction. In both groups, the levels of mRNAs were quantified by the RT-qPCR technique. The primers used specifically amplified the mRNAs for *GDF9*, cyclin B1, H1FOO, *cMOS*, *PARN* and *eIF4E* while glyceraldehyde3-phosphatedehydrogenase (*GAPDH*) was used as an endogenous control for normalization of mRNAs expression. The delta-delta-CT method was used to demonstrate the relative expression of the mRNAs studied. The data of expression for the genes evaluated in oocytes from uncultured and cultured secondary follicles were analyzed by the Kruskal-Wallis test, followed by the post hoc non-parametric Dunn's Multiple Comparison Test. Differences were considered significant when $P<0.05$. The results showed that in vitro culture of bovine secondary follicles significantly increased their diameter (232.29 ± 16.36µm) when compared with those uncultured follicles (183.19 ± 15.44). In vitro grown follicles had an increase in the levels of mRNA for *GDF9*, *cMOS*, *PARN*, *eIF4E*, *cyclin B1* and *H1FOO* in their oocytes when compared to those before culture. In conclusion, the *in vitro* culture of bovine secondary follicles for 18 days can increase the expression of mRNA for *GDF9*, *cyclin B1*, *H1FOO*, *cMOS*, *PARN* and *eIF4E* in their oocytes.

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The effects of culture media and FSH on PI3K-Akt pathway during bovine IVM

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Oocyte quality is a key factor determining reproductive success. Culture conditions during *in vitro* oocyte maturation may influence the oocyte's developmental potential. Some signaling pathways important for oocyte competence as Phosphatidylinositol-3-kinase/Protein kinase B (PI3K-Akt) can be altered by supplements routinely used in *in vitro* culture medium as IGF-1, EGF and FSH. Our hypothesis is that FSH supplementation and different maturation media during IVM modifies the expression of PI3K-Akt-related genes in bovine cumulus cells. In order to test this hypothesis, we performed IVM with two different maturation media supplemented with high (0.01 UI/mL; HIGH group) or low (0.000875 UI/mL; LOW group) human recombinant FSH (hrFSH) concentrations. High or low hrFSH concentrations were tested in maturation medium 1 (MM1 – TCM199 supplemented with pyruvate, gentamicin, LH and bovine fetal serum) or maturation medium 2 (MM2 – TCM 199 supplemented with pyruvate, gentamicin, BSA, AREG, IGF-1, progesterone and estradiol). Cumulus cells from immature COCs, 12 h of IVM and cumulus cells from matured COCs (at metaphase II stage) 24 h of IVM were used to evaluate gene expression of PI3K-Akt pathway related genes. Pools (n=5) of cumulus cells from 10 or 20 COCs per group were submitted to total RNA extraction using TRizol, followed by DNase treatment and cDNA synthesis using High Capacity cDNA Reverse Transcription Kit. The relative gene expression of *PI3K, AKT1, AKT3, PTEN, FOXO3, BAX, BCL2, BRCA, CDK6, EIF4B, EIF4E* and *MAPK* were determined using three genes (*PPIA, RPL15* and *YWHAZ)* as reference. Expression levels were calculated using the $2^{-\Delta Ct}$ method and data were tested by ANOVA in a 2x2x2 factorial design (time x hrFHS x MM) with additional control group (immature). Means were compared by Tukey's test. A level of 5% significance was used. After 24 h of IVM maturation rates were similar between groups. High hrFSH at 12h of maturation decreased *PI3K* and *CDK6* gene expression and increased *MAPK1* relative expression and at 24h high hrFSH treatment reduced *PTEN* and increased *BCL2* and *CDK6* gene expression, independent of maturation medium. Regarding the *PI3K* gene, lower expression was observed in MM1 group at 12h and 24h of IVM. Additionally, when COCs were maturated with MM1 the cumulus cells expression of *FOXO3*, *BRCA*, *CDK6* were higher and the expression of *PTEN* lower at 12h. No differences were observed between MM1 and MM2 after 24h of IVM. In summary, the hrFSH possibly stimulate the PI3K-Akt pathway in both maturation media studied and the maturation medium (MM1) has an inhibitory effect on PI3K-Akt based on *PTEN* and *PI3K* gene relative expression. These results demonstrated the influence of culture conditions on cumulus cells gene expression at 12 and 24h and the possibility to modulate important pathways involved in oocyte competence acquisition during IVM. Financial support: FAPESP grants 2014/22887-0, 2018/01431-9, 2018/13155-6.

A092 Folliculogenesis, oogenesis and superovulation

Repeatability of the antral follicle count and its relationship with productive and reproductive parameters in prepubertal heifers with early induction of puberty

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This study evaluated the repeatability of antral follicle count (AFC) and its relationship with productive and reproductive parameters in prepubertal heifers submitted to early puberty induction. Nelore heifers (G-N=15, *Bos indicus*) and Nelore x Angus heifers (G-NA=15, *Bos indicus-taurus*) with 12 to 14 months old and without corpus luteum (CL) were kept under the same management conditions and nutrition for this study. The AFC, body weight, body condition score (BCS), ovary and dominant follicle diameter were determined in each animal on Days -15, 0, 12 and 21. The puberty induction was performed on Day0, by the insertion of a progesterone device (previously used 3 times, CIDR®, Zoetis, Madison, USA) which remained until the 12th. On the removal of the device 1mg of estradiol benzoate was administered IM (Gonadiol®, Zoetis, Madison, USA) and an adhesive was fixed in the sacrococcygeal region (Estrotect™ Breeding Indicator, Spring Valley, USA) for estrus intensity classification (0= absent, 1=low and 2=high intensity). At Day21, the presence and diameter of the CL were registered. In all evaluations, a 7.5 MHz ultrasound with a rectal linear transducer was used. Data were analyzed by the generalized linear model (GLM) followed by the Tukey test. The Fisher exact test was used to evaluate ovulation rate and the repeatability was calculated as δ2 animal / δ2 animal + δ2 error (P<0.05). Regardless of hormonal induction, the AFC was highly repeatable during the study in G-N (r=0.79) and G-NA (r=0.90). The AFC average throughout the study was higher (P<0.05) in G-N than G-NA (24.2±8.5 vs. 17.7±9.0 follicles). BCS was similar (P>0.1) between G-N (2.6±0.2) and G-NA (2.7±0.3) on D-15, but on Days 0, 12 and 21 the G-NA showed higher BCS (2.6±0.2 vs. 3.0±0.2, 2.6±0.2 vs. 3.1±0.1 and 2.6±0.2 vs. 3.2±0.2, P<0.001). A variation of BCS throughout the study occurred in G-NA (P<0.0001), but not in G-N (P>0.1). The weight average gain was higher (P=0.014) in G-NA (0.69±0.33 kg/day) than in G-N (0.40±0.29 kg/day). The G-NA exhibited higher (P<0.01) weight than G-N in the initial (Day-15 - G-NA: 238.4±28.9 vs. G-N: 220.0±25.6 kg) and final period (Day21 - G-NA: 262.9±27.2 vs. G-N: 233.9±30.8 kg). The dominant follicle diameter was similar (P>0.1) between G-N and G-NA in Days -15, 0 and 21, but in Day12 G-NA had a larger diameter (9.3±1.5 vs. 11.6±2.7 mm, P=0.007). The estrus intensity (1.5±0.5 vs. 1.7±0.5), ovulation rate [41.67% (5/12) vs. 50.00% (7/14)] and CL diameter (11.2±2.4 vs. 11.2±5.5 mm) were similar (P>0.1) between G-N and G-NA, respectively. Early puberty induction had a low response and was similar between Nelore and crossbred Nelore x Angus heifers. The AFC was higher in Nelore heifers, although in both breeds AFC exhibited highly repeatability throughout puberty induction. Crossbred heifers had higher BCS, average weight gain and diameter of the dominant follicle.

A093 Folliculogenesis, oogenesis and superovulation

Follicular development of fetal bovine ovarian cortex fragments submitted to xenotransplantation

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Xenotransplantation of ovarian tissue in immunosuppressed animals may be an option to promote follicular development and maturation, allowing the preservation of preantral follicles. The conservation of bovine fetal ovarian tissue contributes to the maintenance of evolutionary intention, even if this fetus is not compatible with life. Conservation of genetic material of fetuses, by xenotransplantation and their subsequent *in vitro* maturation, decreases the generation interval, increasing genetic gain. Ovarian tissue from bovine fetuses is a good candidate for preservation of preantral follicles because it has suffered less environmental interference, besides having a greater reserve of primordial (quiescent) follicles, which is more tolerant to cryopreservation, due to its small size, relatively unspecific morphology, and the low metabolism. The aim was evaluate the follicular development and numbers of blood vessels in fetal bovine ovarian tissue fragments submitted to xenotransplantation for 7 or 14 days (X7 and X14, respectively), and *in vitro* culture for 1 or 7 days (C1 and C7, respectively), compared to freshly analyzed fragments (FC). Ten pairs of fetal bovine ovaries collected in local slaughterhouse, fragmented (3 x 3 x 1 mm) and fresh xenotransplanted, were used in 20 female mice of the Balb C line, so that each ovarian pair provided 5 fragments for X7 and 5 fragments for X14, therefore each mice received 5 fragments. *In vitro* culture and the histological analysis were performed in the Animal Reproduction Laboratory of the Federal University of Uberlândia. The number of blood vessels per section was analyzed and the follicles were classified in normal or degenerate and primordial, transitional, primary, secondary or antral follicles, according to morphological characteristics. The data was compiled and analyzed with Sigma Plot software. Follicular viability was higher in the FC (88.2 ± 3.2), with similar results to C1 and C7. X14 presented better follicular viability (67.6 ± 4.3) than X7 (62.2 ± 4.0), with similar response to the culture groups ($p<0.05$), and there was a positive correlation between the number of blood vessels and the follicular viability ($r = 0.23$, $p<0.1$). FC presented the highest number of vessels (16.7 ± 3.1) in comparison with the other groups, which presented similar results. The follicular activation in X7 and X14 presented lower results (37.0 ± 3.7 and 34.4 ± 5.8, respectively) than FC (55.5 ± 4.9), being that X7 presented a similar result to C1 ($P <0.05$). These findings suggest that xenotransplanted fetal bovine ovarian tissue fragments for 14 days had satisfactory follicular viability and preservation of the follicular reserve (CEUA/UFU/Protocol 006/17). Financial support: FAPEMIG and CNPq.

A094 Folliculogenesis, oogenesis and superovulation

Effect of adding ascorbic acid in cultured ovarian preantral follicles for twelve days in cattle

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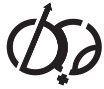
This study aimed to evaluate the effect of antioxidant ascorbic acid addition to the media for *in vitro* culture of preantral follicles of *Bos taurus indicus* females. Five ovaries of five cyclic adult Nelore females were collected at a local slaughterhouse, with a body score ranging from 3 to 3.5 (range 0 to 5). Ovaries were washed in 70% ethanol, and ovarian cortex was divided into five fragments about 3x3x1mm. One fragment per animal was immediately fixed in Bouin (non-cultured control, D0). The others fragments (n=4) were individually cultured in 24-well culture dishes containing 1ml of minimum essential medium (MEM, Gibco BRL, Rockville, MD, USA; osmolarity 300mOsm/l, pH 7.2) supplemented (MEM+) with ITS (6.25mg/ml insulin, 6.25mg/ml transferrin, and 6.25ng/ml selenium; (Sigma, St. Louis, MO, USA)), 0.23mM pyruvate (Sigma, St. Louis, MO, USA), 2mM glutamine (Gibco BRL, Rockville, MD, USA), 2mM hypoxanthine (Sigma, St. Louis, MO, USA), 1.25mg/ml bovine serum albumin (BSA Gibco BRL, Rockville, MD, USA), 20IU/ml penicillin (Sigma, St. Louis, MO, USA) and 200mg/ml streptomycin (Gibco BRL, Rockville, MD, USA). The culture was tested only with MEM+ (cultured control), as well as different concentrations (50, 100 and 200ng/mL) of ascorbic acid (Sigma, St. Louis, MO, USA). Ovarian fragments were cultured in the media for twelve days. The culture media were replaced by fresh aliquots every two days. To the analysis of the integrity and degree of development of the follicles, the classical histology with Periodic Acid-Schiff (PAS) and Hematoxylin staining was used. The classification of follicles was based on the evaluation of the morphological integrity (normal or degenerated) and the development stage (primordial, primary and secondary). Data were submitted to ANOVA tests ($p \leq 0.05$). We evaluated 750 preantral follicles (normal or degenerated), of which 142 were primordial follicles and 608 developing follicles. After 12 days of culture, fragment treated with 200ng/ml of ascorbic acid had 55.33% (83/150) of intact follicles, did not differ from D0 ($p=0.94$; 64% (96/150)). Fragments treated with 50ng/ml, 100ng/ml of ascorbic acid and MEM+ had bottom number of intact follicles (23.33% (35/150), 30.66% (46/150) and 24% (36/150), respectively) compared to D0 and 200ng/ml ($p < 0.05$). The concentration of 200ng/mL ascorbic acid resulted in a higher percentage of developing follicles (52%, 78/150) when compared to D0, MEM+ and 50ng/mL ($p<0,05$), which had 17.33% (26/150), 18% (27/150) and 21.33% (32/150) of developing follicles, respectively. On the other hand, the concentration of 100ng/mL of ascorbic acid did not change the number of developing follicles (30.66%, 46/150) compared to all other treatments. Thus, MEM+ supplemented with 200ng/mL of ascorbic acid for twelve days of culture, was capable of preserving morphological integrity and promote the development of bovine preantral follicles.



A095 Folliculogenesis, oogenesis and superovulation

Effects of epidermal growth factor (EGF) and progesterone (P4) on the levels of maturation-related transcripts in oocytes of bovine secondary follicles cultured in vitro

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The objective of this study was to investigate the effects of EGF and P4 on growth and on the levels of mRNA of of *GDF9*, cyclin B1, *H1FOO* (oocyte-specific linker histone), quinase *cMOS*, *PARN* (poly(A) ribonuclease) and *eIF4E (*cap binding protein) in oocytes from cultured bovine secondary follicles. Therefore, secondary follicles (~ 0.2 mm) were isolated from the ovarian cortex and individually cultured at 38.5°C, 5%CO2, for 18 days in TCM-199 alone (cultured control) or supplemented with 10 ng/ml progesterone (P4), 10ng/ml EGF or both EGF and progesterone (10ng/ml each). Follicular diameters were evaluated and the levels of mRNA were quantified at the end of 18 days of cultures by real-time PCR technique. The primers used specifically amplified the RNAs for *cMOS*, *Cyclin B1*, *H1Foo*, *eIF4*, *GDF-9* and *PARN*. The housekeeping gene *β-tubulin* was used as an endogenous control. The delta-delta-CT method was used to demonstrate the relative expression of the mRNAs studied. Follicular diameter and the levels mRNAs evaluated in oocytes from secondary follicles were compared by the Kruskal-Wallis test, followed by the Dunn's test. The differences were considered significant when P <0.05. The results showed that there was a progressive increase in follicular diameter in all treatments, but only the follicles cultured in EGF-supplemented medium showed significantly larger diameters than those grown in the control at the end of culture period. The presence of EGF in the culture medium also promoted a significant increase in mRNA levels of *cMOS*, *eIF4*, *GDF-9* and *PARN* compared to follicles cultured with both the EGF and P4 (P <0.05). On the other hand, oocytes from follicles cultured in the presence of progesterone showed higher mRNA levels for mRNAs of *cyclin B1* and *GDF-9* when compared to the control medium (P <0.05). Levels *of eIF4* mRNA were also higher in the oocytes from follicles cultured in the presence of both EGF and P4 (P<0.05). Regarding mRNA *H1Foo* expression, oocytes from follicles cultured with P4 or both EGF and P4 had higher levels of mRNA of *H1Foo* than those cultured in medium containing only EGF (P <0.05). According to this results, it can be concluded that the presence of EGF in the culture medium promotes follicle growth and increase the levels of mRNA for *cMOS*, *eIF4*, *GDF-9* and *PARN*, while progesterone increase the mRNA expression of *cyclin B1* and *GDF-9* in oocytes from cultured bovine secondary follicles.

A096 Folliculogenesis, oogenesis and superovulation

Aspects of superovulatory response and embryo production in holstein heifers

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The objective of this study was to correlate characteristics of the superovulatory response (SOV) of Holstein heifers with total and viable embryo production submitted to Multiple Ovulation and Embryo Transfer (MOET). Data of SOV and embryo production from 309 donors, from the same farm, over a period of 12 months were used. All donors were superovulated in a same SOV protocol with 180 mg of Folltropin™ (Vetoquinol-Brazil), in decreasing doses. Donors were flushed by the same veterinarian. The number of ovulations (CLs) was determined by ultrasonography (Mindray M5™-China) immediately before flushing. Flushing was performed in all females with two or more ovulations (281 donors - 90.9%). In these animals the mean CLs were 9.6±5.6. A total of 6.7±3.7 embryos and 4.3±3.3 viable embryos per donor were obtained. The rate of embryonic recovery, defined by total recovered embryos divided by total CLs was 62.7%. The percentage of viable embryos in relation to the total was 70.4%. The donors were allocated into 4 groups according to SOV response. G1: 2 to 5 CL (N = 50-17.9%); G2: 6 to 10 CL (N = 97-34.6%); G3: 11 to 15 CL (N = 75-26.8%) and G4: up to 16 CL (N = 56-20.0%). Embryo production, embryo viability and recovery rate in donors of the different groups were analysed by anova and compared using Tukey's test at 5% probability. The number of total embryos was 2.5 ± 1.4^{d}; 5.8 ± 2.0^{c}; 8.3 ± 3.2^{b} and $9.7+4.1^{a}$ ($P<0.05$) and viable embryos was 1.8 ± 1.5^{c}; 4.5 ± 2.2^{b}; 5.7±3.2 and 6.2±4.2 ($P<0.05$) for groups 1, 2, 3 and 4, respectively. The mean recovery rate was 64.9 ± 33.4^{ab}; 73.6 ± 23.7^{a}; 64.5 ± 23.9^{ab} and 53.9 ± 23.3^{b} ($P<0.05$) and the embryo viability 61.1±41.4; 71.9±27.6; 65.3±28.7 and 60.1±28.9 ($P>0.05$) for groups 1, 2, 3 and 4 respectively. The production of embryos is directly related to SOV. The number of viable embryos per donor was positively related to the SOV until the SOV response of 15 CLs per animal. From this, no relation was found. The recovery rate of donors with SOV above 16 CLs (G4) is lower than those with SOV between 6 to 10 CLs (G2). It is concluded that donors that present very large responses to SOV, above 16 CLs, are less efficient in relation to the process, and it may be necessary to reduce the superovulatory stimulus for these animals in a next MOET procedure.
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Varying COC density and culture medium volume during IVM: effects on meiosis progression

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In vitro maturation (IVM) of cumulus-oocyte complexes (COC) is a crucial and limiting step for successful *in vitro* production of bovine embryos. Oocyte developmental competence is linked to the ability to achieve full cumulus expansion and to complete meiosis. Depending on COC yield following "ovum pick up" (OPU) for a given donor, COC density in the IVM medium may vary largely. However, it is still not clear if individually cultured COC would have their developmental capacity hampered, nor how varying COC density and culture media volume impact on IVM. We hypothesized that individual COC culture and low COC density would reduce the percentage of MII oocytes at the end of IVM. In four replicates, COCs aspirated from slaughterhouse ovaries were grouped to provide different COC density and total medium volume as follows: 1 COC in 20µl (G1; 1:20); 1 COC in 400µL (G2; 1:400), 5 COCs in 400µL (G3; 1:80), 10 COCs in 400µL (G4; 1:40) or 20 COCs in 400µL (G5; 1:20). IVM medium was TCM199 with Earle's salts, bovine serum albumin, amikacin, pyruvate, recombinant human follicle stimulant hormone, amphiregulin, insulin-like growth factor 1, estradiol and progesterone. After 24h of IVM, we assessed cumulus cell expansion and oocyte meiotic status with Hoechst 33342 staining. All groups were similar for cumulus cell expansion. Rates of MII were higher ($P<0.05$) in G3 (65.40%), G4 (61.53%) and G5 (59.69%) compared to G1 (26.34%) and G2 (36.04%). Therefore, individual COC culture provided lower MII rates, suggesting that the interaction between COCs via secreted factors benefit competence to resume meiosis during IVM. Supported by FAPESP 2016/21671-9.