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Differentially expressed miRNAs in follicular cells and cell-secreted vesicles into the bovine ovarian follicular environment

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Keywords: extracellular vesicles, follicular cells, microRNAs.

During folliculogenesis the crosstalk between oocytes and follicular cells is crucial to induce cell proliferation and differentiation, for the proper oocyte development and for the acquisition of competence. Extracellular vesicles (EVs) such as exosomes and microvesicles were identified within the follicular fluid and suggested as mediators of intercellular communication. The EVs can transfer bioactive molecules such as lipids, proteins, mRNA and miRNAs. The objective of this study was to analyze differential expression of miRNAs between granulosa cells (GCs) and cumulus-oocyte-complex (COCs) as well as between its respective cell-secreted vesicles. GCs and COCs were collected from 3-6 mm follicles and cultured with medium DMEM supplemented with bovine seric albumine, ITS liquid media supplement (100x), fungizone, peniciline, MEM non-essential amino acids solution (100x) and androstenedione. After 48h the culture media of GCs and COCs was supplemented with FSH (1ng/mL). This treatment aims to mimic the existing FSH stimulation during the phase of follicular growth. Cells and culture medium were collected 48 h after the FSH treatment and stored at -80˚C. The culture medium EVs were isolated by ultracentrifugation and total RNA isolation were performed using Trizol® reagent (Invitrogen). Reverse transcription was carried out using the miScript PCR System (Qiagen). Real-time PCR analysis with custom plates of 351 bovine miRNAs was performed, in triplicate, in cells (GCs and COCS) and in its respective EVs (GC-EVs and COC-EVs). Expression levels were calculated using the 2-ΔCt method and data were tested by ANOVA and compared by Tukey's test at 5%. Bioinformatics analysis of miRNAs present in follicular cells and respective EVs isolated from culture media identified putative regulated pathways in GC and COCs and both EVs. Initially, we identified sixteen miRNAs present only in COCs and eight only in granulosa cells. GCs and COCs had in common 302 miRNAs whose ten were increased ($P < 0.05$) in COCs: let7a-3p, miR-128, miR-15b, miR-196b, miR-342, miR-411a, miR-497, miR-502b, miR-542-5p, miR-592; and one was increased ($P < 0.05$) in GCs: miR-454. Functional enrichment indicated regulation of pathways as oocyte meiosis by miRNAs increased in COCs, and signaling pathways such as mTOR, ErbB, TGF-beta by the miRNA increased in GCs. Thirteen miRNAs were identified only in GC-EVs and 30 in COC-EVs. Twenty-four miRNAs were common between GC-EVs and GC-EVs, two of which (miR-127 and miR-433) were higher ($P < 0.05$) in GC-EV, regulating oocyte maturation mediated by progesterone, adherens junctions and cell cycle; and two (miR-631 and miR-323) were higher ($P < 0.05$) in COC-EVs, regulating pathways such as gap junctions and ErbB signaling. The results suggest that miRNAs present in the follicular environment are part of a complex communication network and cellular interactions that regulate and modulate cellular pathways associated with oocyte and follicular development.

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Effect of white tea intake (Camellia sinensis (L.) kuntze) on the gene expression of VEGF in the corpus luteum of superovulated rats

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Keywords: vascular endothelial growth factor, corpus luteum, RT-PCR.

Tea is an extremely popular drink, being the second most commonly consumed in the world. People ingest teas on average two to three times a day, the majority being derived from the Camellia sinensis plant. The beneficial health effects of the consumption of tea from this plant are well known, such as the prevention of cancer, cardiovascular disease and osteoporosis. Despite this, little is known about the action of white tea on reproduction. It is important to evaluate the possible consequences of consumption of catechin main in the luteal development. The epigallocatechin gallate (EGCG) present in the tea, influences the expression of VEGF gene in tumors and is an important angiogenic factor in the reproductive organs. This study aimed to verify the effects of prolonged intake of white tea on the relative abundance of VEGF mRNA in superovulated rats. For this purpose, the rats were divided into two groups, control group (n = 30), which received water, and white tea intake group (n = 30). The ovaries of 10 animals in each group at the end of each month (three months) were dissected and the corpora lutea(CLs) stored in Trizol at -80°C. The CLs were subjected to RNA extraction protocol for Trizol (Invitrogen) and the relative abundance of VEGF mRNA was evaluated by real-time PCR as the endogenous controls: beta-actin, GAPDH , HPRT1 and RPS18. The data were evaluated for the assumption of normality (Shapiro-Wilk) and the statistical comparisons were performed using the unpaired t test between groups and at at different collection moments ($P < 0.05$). The relative abundance of VEGF mRNA was changed by white tea consumption in all analyzed periods: 30 days (CT= 0.99 ± 0.31 , CB= 2.29 ± 0.35) ; 60 days (CT=0.83 ± 0.32 , CB= 1.74 ± 0.73) ; 90 days (CT= 1.02 ± 0.26 , CB= 2.24 ± 0.91), as in the total period of 3 months: CT= 0.95 ± 0.30 and CB= 2.13 ± 0.75. We conclude that prolonged intake of white tea alters VEGF expression in the corpus luteum of Wistar rats.

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MiRNA profile of exosomes isolated from bovine ovarian follicles: association with oocyte competence

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Keywords: miRNAs, exosomes, bovine.

Folliculogenesis is the result of cell proliferation and differentiation of follicular cells (granulosa and cumulus). Oocyte maturation is the result of RNA accumulation within the oocyte and epigenetic nuclear reprograming. Intercellular communication is crucial to complete these two processes. Extracellular vesicles such as exosomes mediate part of this cellular crosstalk within the ovarian follicular fluid. Exosomes are small cell secreted vesicles (30-150nm) that contain protein, mRNAs and miRNAs. MiRNAs are small non-coding RNA molecules between 18-16 nucleotides that are involved in blocking target mRNA translation. Our hypothesis is that exosomes from bovine follicular fluid present different miRNA molecules associated with oocyte competence. In order to test this hypothesis bovine ovarian follicles, between 3-6 mm were individually isolated. Follicle contents were separated under a stereomicroscope to allow the collection of FF and the cumulus-oocyte-complex (COC), each complex was cultivated individually. Follicular fluid was submitted to differential centrifugation for removal of cellular components and debris before freezing at -80C. COCs were in vitro matured for 18h and then evaluated for the presence of the 1st polar body (1stPB). Denuded oocytes were activated parthenogenetically 26h post-maturation to generate parthenogenetic embryos. Follicular fluid was pooled according to oocyte competence, where the non-competent were the non-matures (Nmat) by the absence of the 1stPB and the competent were the ones that reached the blastocyst (Blast) stage. Based on the competence groups we isolated EVs from pools of 10 follicular fluids (Exo-Blast and Exo-Nmat) by ultracentrifugation at 100.000 xg. Total RNA was extracted from the pools of EVs and analyzed by Real-Time PCR utilizing an Array of miRNAs customized for bovine. MiRNA profile according to oocyte competence identified 185 miRNAs unique for Exo-Blast and 107 miRNAs unique for Exo-Nmat. Bioinformatic analysis of the 13 highly detected miRNAs predicted their involvement regulating signaling pathways such as ECM-receptor interaction, PI3K-AKT signaling pathway and Focal Adhesion. Bioinformatic analysis of miRNAs unique to Exo-Blast determined their involvement regulating pathways such as RNA Transport, Cell Cycle and Oocyte Maturation Mediated by Progesterone. Bioinformatic analysis of miRNAs unique to Exo-Nmat determined their involvement regulating pathways such as RNA Transport, Endometrium Cancer and mTOR signaling pathways. Thus, our results demonstrated that exosomes carry different miRNA molecules involved in regulation of different cellular pathways. Additionally, the miRNAs content of exosomes, according to oocyte competence, suggests their role modulating folliculogenesis and oocyte maturation processes. Further experiments are necessary to explore the different mechanisms of action mediated by exosomal contents during follicle growth and oocyte maturation.

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Effect of high and low antral follicle count on the growth rate and follicular diameter from Nelore (*Bos indicus*) cows subjected to synchronization of ovulation

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Keywords: antral follicle, follicular diameter, *Bos indicus*.

The aim of this study was to evaluate the influence of high or low antral follicular count (AFC) on the growth rate and follicular diameter in Bos indicus cows subjected to timed artificial insemination (TAI). Multiparous and suckling (n = 250; 40 to 60 days postpartum) Nelore cows were evaluated using an intravaginal microconvex array (Aquila PRO, Pie medical, Maastricht, The Netherland) to determine (AFC, follicles ≥ 3 mm) seven days before the start of the study (D-7). After assessment of the AFC, 43 cows of high count (G-high; ≥ 40 follicles) and 32 cows of low count (G-low; AFC ≤ 10 follicles) and BCS 2.8 ± 0.2 (1 to 5 scale) were for monitoring of ovarian follicular dynamics. Randomly during the estrous cycle (D0), cows received a synchronization of ovulation protocol using an ear implant of norgestomet (3 mg, Crestar®, MSD Animal Health, São Paulo, Brazil) and were intramuscular (i.m.) injected with estradiol benzoate (2 mg, Gonadiol®, MSD Animal Health, São Paulo, Brazil). At ear implant removal (D8), they were injected with D-cloprostenol (0.150 mg, Prolise®, Tecnopec, São Paulo, Brazil), equine chorionic gonadotropin (300 IU, Novormon®, Syntex SA, Buenos Aires, Argentina) and estradiol cypionate (1 mg, ECP®, Zoetis, São Paulo, Brazil), i.m. The TAI was performed 48 h after ear implant removal. Ultrasound exams were performed at D-7, D4 and D18 to determine AFC, at D0 and daily (D4 to D10) to follicular diameter control, and every 12 h (D10 to D12) to ovulation control. In each scan the follicles ≥ 3 mm were identified, measured and recorded in individual registers for subsequent evaluation and calculation of follicular growth rate. Data were analyzed using ANOVA followed by Tukey test ($P < 0.10$) and the results are presented as means ± standard deviation. The mean AFC observed in D-7, D4 and D18 were 48.9 ± 5.7, 49.5 ± 5.7 and 49.6 ± 6.8 follicles to G-high and 12.5 ± 4.1, 12.4 ± 4.3 and 12.9 ± 3.8 follicles G-low, respectively ($P = 0.01$). The follicular growth rates remained similar between the G-low and G-high at D4 to D8 (0.40 ± 0.22 vs. 0.32 ± 0.21 cm/day; $P = 0.30$), D8 to D9 (0.11 ± 0.08 vs. 0.12 ± 0.08 cm/day; $P = 0.73$) and D8 to D10 (0.11 ± 0.06 vs. 0.14 ± 0.06 cm/day; $P = 0.16$), as well as there was no difference in ovulation time (70.2 ± 6 vs. 69.8 ± 7 h, $P = 0.85$). However, the dominant follicles showed greater diameter in G-low than G-high at D4 (0.73 ± 0.22 vs. 0.62 ± 0.14 cm, $P = 0.06$), at D8 (1.12 ± 0.18 vs. 0.94 ± 0.18 cm, $P = 0.01$), at D9 (1.23 ± 0.17 vs. 1.06 ± 0.16 cm, $P = 0.01$), and D10 (1.34 ± 0.13 vs. 1.22 ± 0.18 cm, $P = 0.01$), being that, the estimated ovulatory follicle diameter was greater in the G-low (1.44 ± 0.15 cm) than in the G-high (1.34 ± 0.2 cm, $P = 0.08$). In this study AFC had no effect on the follicular growth rate. However, cows with low count had larger dominant follicles and estimated ovulatory follicle diameter, suggesting that the AFC may have some effect on the conception rate of Nelore cows submitted to TAI.

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Effect of IGF-I in *in vitro* maturation of bovine oocytes subjected to heat shock

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Keywords: mitochondrial activity, reactive oxygen species, heat shock

The heat stress leads to an increased in reactive oxygen species (ROS) production in the cell. The IGF-I showed antioxidant activity, reducing the damaging effect of hydrogen peroxide in developing mouse embryos (Kurzawa et al., Zygote, 2004; 12:231-240). Thus, the objective of this study was to evaluate the effect of different concentrations of IGF-I added to the in vitro maturation (IVM) medium on mitochondrial activity and reactive oxygen species (ROS) production of oocytes subjected to heat shock. Immature oocytes aspirated from ovaries obtained from a slaughterhouse were selected and randomly allocated in a factorial experiment design 3 x 2. Were tested three concentrations of IGF-I (0, 25 and 100 ng/mL - Sigma-Aldrich, São Paulo, BR) added to the medium and two IVM incubation conditions (conventional: 24 hours at 38.5ºC and 5% CO2; or heat shock: 12 hours at 41ºC followed by 12 hours at 38.5ºC and 5% CO2). The IVM was performed in Nunc plate containing 400 µL of TCM-199 (Invitrogen, São Paulo, BR) supplemented with 20 µg/mL of FSH (Pluset®, Hertape Calier, Juatuba, BR) and 10% of estrus cow serum. After the MIV period, the oocytes were denuded in a solution of 0.1% hyaluronidase in PBS (Sigma, BR) by vortexing for 5 minutes and washed twice in PBS containing 0.1% PVP. For mitochondrial activity analysis the oocytes were incubated in PBS supplemented with PVA (0.1%) and MitoTracker Red CMX-Ros (50 nM) (Invitrogen, São Paulo, BR) for 30 minutes at 38.5 ºC. Additionally, the oocytes were washed in PBS-PVA and incubated in drops of PBS containing 10 µM of diacetate 2',7'-dichlorofluorescein (Sigma-Aldrich, São Paulo, BR) for 15 minutes at 38ºC. Oocytes were washed three times in PBS-PVA droplets and mounted on slides to assay fluorescent emission of the mitochondrial activity (n = 351) and ROS (n = 331). The intensity emitted was quantified using the software Image J. Three replicates were performed. Data were analyzed by Proc Mixed of SAS software (version 9.3, SAS Inst., Inc., USA) and oocyte was considered as a random effect. Were considered the effects of IGF-I concentration, incubation condition, replicate and interaction between IGF-I and incubation condition. Values are shown as the mean ± s.e.m. There was no interaction ($P > 0.05$) between IGF-I concentration and incubation condition on mitochondrial activity and ROS production. The mitochondrial activity was increased ($P < 0.01$) by IGF-I (15015 ± 757 a; 21448 ± 994 b and 21425 ± 1042 b arbitrary units (AU) with 0, 25 and 100 ng/mL IGF-I, respectively) and was reduced ($P < 0.01$) by heat shock (conventional: 21274 ± 899 vs heat shock: 17387 ± 652 AU). ROS production was increased ($P < 0.05$) by IGF-I (9170 ± 457 a; 14869 ± 727 b; and 15205 ± 723 b AU with 0, 25 and 100 ng/mL IGF-I, respectively) and heat shock ($P < 0,05$) (conventional: 11590 ± 509 vs heat shock: 15025 ± 622 AU). The reduction of ROS concentration was expected once the IGF-I can enhance the activity of glutathione peroxidase (Higashi et al., 2013, Biochim. Biophys. Acta., 2013; 1832:391-399). However, IGF increased production of ROS and reduced the prejudicial effect of heat shock by the increasing of oocyte mitochondrial activity, but its thermoprotective effect was not related to antioxidant activity.

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Restricted intake and lipid inclusion in Santa Inês ewe lambs diet: age, weight and progesterone concentration at first ovulation

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Keywords: energy, lipid supplementation, ether extract.

The age at first ovulation is influenced by several factors, and nutrition has an essential role on it. Lipids provide essential fatty acids that are positively associated to reproductive aspects. The aims of this study were to evaluate the effects of lipid inclusion and restricted intake on age and weight at the first ovulation and the serum progesterone (P4) concentration at the sixth day after first ovulation. Thirty-five Santa Inês ewe lambs with initial body weight (BW) of 21.5 ± 0.3 kg and age of 91.6 ± 1.4 days were used. Animals were blocked according to initial BW and distributed to one of four treatments: animals receiving diet without lipid inclusion (3.5% ether extract – EE), ad libitum intake (WLI-ALI, control group, n = 9); diet without lipid inclusion and restricted intake (WLI-RI, n = 9); lipid inclusion diet, represented by toasted and broken soybean (9.8% EE), ad libitum intake (LIP-ALI, n = 8); and lipid inclusion diet and restricted intake (LIP-RI, n = 9). Ewe lambs subjected to restricted intake had 84% of the diet offered to those in ad libitum treatments. The individual daily dry matter intake (DMI) and EE intake (EEI) were measured and the animals were weekly weighted until 35 kg BW. For P4 analysis, blood samples were collected once a week after the animals reached 25 kg BW and twice a week after 30 kg until 35 kg BW. Ovulation was estimated to occur on the sixth day before P4 ≥1 ng/mL. DMI, EEI, average daily weight gain until first ovulation (DWG), weight and age at first ovulation, serum P4 concentration and days in experiment until first ovulation were analyzed by GLM PROC of the SAS software (version 9.3). Ovulation occurred in 60% (21/35) of the ewe lambs (5; 5; 6; 5 from WLI-ALI; WLI-RI; LIP-ALI; and LIP-RI, respectively). DMI of ewe lambs from WLI-ALI group was greater ($P < 0.01$) than the other groups and the intake of ewe lambs from LIP-RI group was lower ($P < 0.01$) than those from WLI-RI and LIP-ALI (802.9 ± 12.9, 678.0 ± 15.8, 726.5 ± 22.9, 661.3 ± 21.2 g/day) groups. EEI differed among groups ($P < 0.01$), and the highest was observed in LIP-ALI and the lowest in WLI-RI. Weight (30.0 ± 0.8; 29.3 ± 1.3; 30.5 ± 1.3; 28.5 ± 1.6 g/day) and age at first ovulation (153.2 ± 5.9; 170.0 ± 9.5; 165.2 ± 10.5; 170.6 ± 15.5 days), DWG (132.2 ± 21.1; 94.4 ± 6.4; 128.2 ± 12.6; 93.4 ± 12.3 g/day), serum P4 (3.1 ± 0.6; 2.0 ± 0.6; 2.1 ± 0.5; 2.7 ± 0.6 ng/mL) and days in experiment until first ovulation (60.6 ± 5.8; 81.0 ± 8.9; 62.8 ± 9.8; 69.8 ± 14.6 days) did not differ ($P > 0.05$) among WLI-ALI, WLI-RI, LIP-ALI, and LIP-RI treatments, respectively. The first ovulation in Santa Inês ewe lambs occurs at 30 kg BW. The restricted intake imposed in this study did not delay the age at fist ovulation. The greater lipid intake did not favor reproductive parameters. Serum P4 did not increase with the soybean inclusion in the diet.

Acknowledgments: CNPq and FAPEMIG.

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Restricted intake and lipid inclusion: effects on ovarian follicular development in Santa Inês ewe lambs

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Keywords: puberty, ovarian activity, lipids.

The reproductive efficiency is directly related to the nutritional management. The diet lipid inclusion, especially with enhanced sources of omega 6 and omega 3 fatty acids, is positively associated to the ovarian follicular development of ruminants. The objective of this study was to evaluate the effects of toasted soybean grain addition on restricted or ad libitum feeding on ovarian follicular development of Santa Inês ewe lambs. Thirty-five ewe lambs with initial body weight 21.5 ± 0.27 kg and age of 91.6 ± 1.36 were blocked according to initial body weight and allocated to the following treatments: ad libitum intake of a diet without lipid inclusion (ALI-WLI, 3.5% Ether Extract – EE, n = 9); restrict intake of the WLI diet (RI-WLI, n = 9); ad libitum intake of a lipid inclusion diet represented by toasted and cracked soybean grain (ALI-LID, 9.8% EE, n = 8); and restrict intake of the LID diet (RI-LID, n = 9). Diets that were offered to the animals from RI-WLI and RI-LID was limited to 80% of the intake of the animals from the respective ad libitum treatments. The ovarian activity was evaluated in all animals by ultrasonographic exams (ALOKA, SSD 500, 7.5 MHz), on alternate days during 10 days at the ages of three, four and six months, while a single evaluation was performed at the ages of five and seven months. Follicles with diameter ≥3 mm were measured in real time. The diameter of the follicles was calculated as the average of the two greatest antral cavity diameters. The diameter of the largest follicle was analyzed throughout time using MIXED (SAS®).The number of class ≥3-5 mm and >5 mm follicles was also analyzed throughout time by general linear mixed models, considering Poisson distribution and log function, using GLIMIX (SAS®) procedure. The diameter of the largest follicle, the number of ≥3-5 mm and >5mm follicles did not differ among treatments ($P > 0.05$). The number of ≥3-5 mm follicles increased with age, reached its maximum of 2.49 follicles at the age of 168 days and then decreased ($\hat{y}= 0.00027x2+0.09148x-6.83727$, $P < 0.05$). The number of follicles >5 mm presented marked increase after 150 days ($\hat{y}=-0.00037x2+0.1639x-18.0185$, $P < 0.05$). The follicle diameter increased linearly according to the age of the animals ($\hat{y}=15.68177+0.1599x$, $P > 0.05$). Probably with the increased LH pulse frequency approaching puberty provided the follicular development, which grew in size until reaching class >5 mm follicles. In conclusion, the diameter of the largest follicle and the number of >5 mm follicles were not influenced by the restricted intake diet nor the lipid inclusion; however, they increased in a direct relationship with the age of the animals.

Acknowledgments: CNPq and FAPEMIG.

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Effect of the FSH dose in superovulatory protocols on ovulatory follicle dynamics in ewes

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Keywords: FSH, superovulation, ovine.

The objective of this study, was to evaluate the exogenous FSH dose effect on gonadotrophic treatment over ewes ovulatory follicle dynamics. Twenty four Santa Inês ewes were submitted to estrus synchronization with intravaginal progesterone device (CIDR®, Pfizer, Hamilton, New Zealand) inserted on Day 0 and remaining until the Day 8. On Day 0 and Day 8 were intramuscular (IM) administered 0.125 mg of PGF2α synthetic analogue (Sincrocio®, Ouro Fino, Cravinhos, Brazil). The gonadotrophic treatment started 48 hours before the progesterone removal (Day 6) when females were randomly allocated into three experimental groups according to the total dose of exogenous porcine FSH (Folltropin-V®, Bioniche, Belleville, Canada): G200 (n = 8) - 200 mg; G133 (n = 8) - 133 mg, and G100 (n = 8) - 100 mg. Total doses were administered in eight IM injections with 12 hours intervals (20, 20, 15, 15, 10, 10, 5 and 5% of a total amount). On Day 6, the females received 300 IU of eCG IM (Novormon®, Shering-Plough S. A., Syntex S.A., Buenos Aires, Argentina). B-mode ultrasonography was performed to assess the follicular growth and ovulation moment of the ovulatory wave. The statistical analysis was performed with software R® (R Foundation for Statistical Computing, Vienna, Austria) and results were compared using the Kruskal Wallis test and Dunns posttest ($P < 0.05$) (mean values ± standard deviation). Rates were compared by Chi-square test. The day of emergence (6.17 ± 0.92), maximum diameter (mm; 5.96 ± 0.86), day of maximum diameter (9.38 ± 0.58), duration of growth (h; 78.76 ± 18.38), rate growth (mm/day;1.06 ± 0.30) and ovulatory diameter (mm; 5.76 ± 0.78) showed no statistical difference between the experimental groups ($P > 0.05$); ovulatory moment occurred later in animals from G200 (h; $P = 0.0037$; 45.36 ± 11.46) compared with G100 subjects (40.39 ± 12.19) and G133 (39.61 ± 11.11); preovulatory follicle size reduction and preovulatory follicle size reduction rate were greater (mm; mm/day; $P = 0.0027$ e 0.0024, respectively) in the G200 ewes (0.26 ± 0.38; 0.52 ± 0.77, respectively) compared with the G100 (0.13 ± 0.30; 0.25 ± 0.59, respectively), however were similar on G133 (0.23 ± 0.39; 0.46 ± 0.83, respectively). It was concluded that different exogenous FSH doses (100, 133 and 200 mg) does not interfere in the ovulatory follicle dynamics in superovulatory protocols, exception for ovulatory moment, preovulatory follicle size reduction and preovulatory follicle size reduction rate.

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Parameters blood and viability oocyte Girolando's breed supplemented with linseed (*Linum usitatissimum L.)* - preliminary results

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Keywords: cryopreservation, embryos , poli unsaturated fat acids.

The aim of this study was to evaluate the effect of supplementation with linseed on plasma concentrations of glucose, albumin and cholesterol, as well as the quality of oocytes obtained by ovum pick-up (OPU). Therefore, a total of 12 cows Girolando's breed, were randomly divided into 2 groups: control group (CTRL, n = 6) and linseed group (LINH, n = 6), differing only by the supply of 0,800kg/animal/day of linseed in the diet of LINH group. The supplementation period was 126 days, because in cattle the development of preantral follicles to the pre-ovulatory follicles takes three months (Webb, R. et al., Journal of Animal Science v. 82, p.63- 74, 2004). Thus, all oocytes were completely under the effect of feeding. Seven OPUs sessions were performed with 20 days intervals between each session. Blood samples were collected from the females at each of the OPU sessions, centrifuged and the metabolites were quantified in serum by automated colorimetric enzymatic method by spectrophotometry. Only oocytes classified as grade I and II were considered viable. The analyzes of blood metabolites and viability rates were performed using SAS PROC GLIMMIX the version 9.3. There was no effect ($P > 0.05$) linseed supplementation on plasma concentrations of glucose, albumin, cholesterol and oocyte viability rate (60.4% vs 64.4% control group and linseed, respectively). So as the time of supplementation did not interfere in any of the variables evaluated. Therefore, the supplementation with 0.800 kg of linseed in the diet of Girolando cows was not sufficient to alter the serum concentrations of glucose, albumin and cholesterol as well as the quality of the oocytes during the supplementation period, possibly due to the high initial body score condition of the animals.

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Epigallocatechin-3-gallate maintains follicular survival similar to fresh control after *in vitro* culture of ovine ovarian tissue

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Keywords: ovary, antioxidant, folliculogenesis

Epigallocatechin-3-gallate (EGCG) is natural polyphenol with antioxidant potential (JIANG et al., International Journal of Clinical and Experimental Medicine, 9, 2.479-2.485, 2016). Studies showed that this substance decreases oxidative stress (ÓRTIZ-LOPEZ et al., Neuroscience, 322, 208-220, 2016) and has lower toxicity (FIORINI et al., Liver Transplantation, 11, 298-308, 2005) in different types of cell. However, it is not known whether EGCG could influence on the in vitro culture of ovine ovarian follicles. The aim of this study was to verify the effect of epigallocatechin-3-gallate on the morphology and activation of ovine preantral follicles cultured in ovarian tissue. Ovine ovaries (n = 10) were fragmented (approximately 3x3x1 mm), being one fragment fixed for histology (fresh control) and the remaining fragments were individually cultured in 1 mL of medium for 7 days at 39º C with 5% CO2. The basic culture medium (control medium: α-MEM$^+$) consisted of α-Minimum Essential Medium (α-MEM) supplemented with 10 ng/mL insulin, 5.5 µg/mL transferrin, 5.0 ng/mL selenium, 2 mM glutamine, 2 mM hypoxanthine, 1.25 mg/mL de BSA, 100 µg/mL penicillin and 100 µg/mL streptomycin. To evaluate the effects of EGCG, ovarian fragments were cultured in α-MEM$^+$ alone or added by different concentrations of EGCG (0.01, 0.1, 1, 10 or 100 µg/mL – diluted in ultrapure water). After culture, the fragments were destined to histological analysis and classified as morphologically normal when the follicles did not show cytoplasmic shrinkage or nuclear pyknosis or organized granulosa cells and/or theca cells; atretic follicles showed one of those parameters. Follicular activation (transition from primordial to developing follicles) was also evaluated. Chemicals used in the culture and histology were purchased from Sigma Chemical Co. (St. Louis, USA) and Dinâmica (São Paulo, Brazil), respectively. The percentages of morphologically normal, primordial and growing follicles were submitted to ANOVA and Tukey test ($P < 0.05$). The results showed that after in vitro culture, only treatment containing 1 µg/mL EGCG maintained the percentage of morphologically normal follicles similar ($P > 0.05$) to that observed in fresh control. However, 100 µg/mL EGCG reduced ($P < 0.05$) the percentage of morphologically normal follicles when compared to α-MEM$^+$ and other EGCG concentrations. In all treatments (α-MEM$^+$ and different concentrations of EGCG), it was observed a reduction ($P < 0.05$) in the percentage of primordial follicles concomitant with an increase ($P < 0.05$) in the percentage of developing follicles, compared to fresh control. In conclusion, 1 µg/mL EGCG maintain follicular survival similar to fresh control. In addition, all EGCG concentrations, except 100 µg/mL, showed follicular survival similar to α-MEM$^+$ after in vitro culture of ovine preantral follicles.

A152 Folliculogenesis, Oogenesis and Superovulation

Response prepubertal Nelore heifers to two ovulation induction treatments - preliminary data

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Keywords: corpus luteum, preovulatory diameter, zebu cattle.

Puberty is the beginning of the productive and reproductive process of a bovine female, because their anticipation can affect the productivity of the herd as a whole. Many hormones can be used for the induction of puberty in heifers, among which can be cited progesterone, estradiol, progesterone and estradiol association, or also GnRH and its analogues, but each one with its different efficiencies (EMERICK, L.L. Brazilian Journal of Animal Reproduction, v. 33, p.11-19, 2009.). The aim of this study was evaluate the ovarian response to two ovulation induction treatments in Nelore heifers (Bos indicus). Fifty-seven prepubertal heifers (absence of corpora lutea in two ultrassonographic evaluations with seven day interval between them) with average weight of 294.63 ± 26.41 kg and age 17.47 ± 0.81 months were divided into two treatment groups: one group performing the ovulation induction with intravaginal device progesterone and ovulation inducer (GP4+GnRH) and another group with only ovulation inducer (GGnRH). In GP4+GnRH (n = 29) intravaginal progesterone device (one gram/device) was used (third use) (Primer®, Tecnopec, São Paulo, Brazil), which was kept inside the animal for ten days. Two days after the implant had been withdrawn, an ovulation inducer was applied - 0.02 mg of buserelin acetate (GnRH) (Sincroforte®, Ouro Fino, Sao Paulo, Brazil) by intramuscular injection. In GGnRH (n = 28) the ovulation induction was performed using only the ovulation inducer, this being applied at the same concentration, administration methods and days as the previous group. Ninety-six hours after GnRH administration the Doppler ultrasonography monitoring was performed to determine the ovulation, formation and function of the corpus luteum (CL). Data were subjected to analysis of variance (ANOVA) and the means were compared by the Tukey test, with 5% significance level. The follicular diameter on the application of GnRH in the group GP4+GGnRH was 12.21 ± 2.27 mm, and GGnRH of 10.12 ± 1.16 mm. In all animals that ovulated had the formation of CL, but there was no difference in the ovulation and formation of CL between the groups GP4+GnRH (89.66%) and GGnRH (89.29%), P = 0,08. In this study the both ovulation induction treatments was efficient, since the follicular diameter, at the time of GnRH application, was between 10 and 12 mm, values are greater than ovulatory capacity of Bos indicus (BARUSELLI, P.S. Brazilian Journal of Animal Reproduction, v. 31, p. 205-211, 2007).

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Regional distribution of preantral follicles in equine ovaries

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Keywords: ovarian follicles, follicular location, mare.

The objective of this study was to define the distribution of the equine preantral follicles in the ovarian tissue. Ovaries (n = 7) of mares from slaughterhouse were dissected and sectioned in the saggital plane (greater curvature), resulting in two hemiovaries. In the experiment 1 (n = 3), each hemiovary was cut vertically into six fragments, totaling 12 fragments for each entire ovary, measuring about 1,5x0,5x0,5cm. These fragments are defined in three regions that include the extension of the ovary, named as inner region of parenchyma (C1), the middle region (between the inner region and the outer, C2) and the third section, the outer region (C3). In the experiment 2 (n = 4), each hemiovary was sectioned into four symmetrical fragments, totaling eight fragments for each entire ovary, in which they were located in distant or near the ovulatory fossa (NOF and FOF). The fragments related to lower ovarian curvature were defined as close to ovulation fossa (NOF1+NOF2) and fragments of larger ovarian curvature were defined as distant from ovulatory fossa (FOF3+FOF4). The preantral follicles were classified as intact or degenerated according oocyte morphology and granulosa cells. Statistical analysis for comparison of data was performed using Fisher's exact test, with 5% level of significance using the statistical software Minitab 16.1.1. A total of 1130 follicles were found in the first experiment, being 1054 (93.3%) primordial follicles and 76 (6.7%) developing follicles. In the experiment 2, 938 follicles were found, being 894 (95.30%) primordial follicles and 44 (4.7%) developing follicles. There were differences ($P < 0.05$) between the proportions of pre-antral follicles in the inner (C1 41.6%, 470/1130), middle (C2 32.4%; 366/1130) and external (C3 26%; 294/1130) regions of the equine ovary. These three regions differed among each other, although C1 region had the highest percentage of pre-antral follicles in relation to others ($P < 0.05$). The C2 and C3 regions showed a higher percentage of intact primordial follicles and developing ones than the C1 region. The inner region revealed an increased ($P < 0.05$) amount of degenerated follicles compared to the others. The regions close to ovulation fossa (NOF1+NOF2 58.7%; 551/938) had higher follicular concentration in relation to the region far from ovulatory fossa (FOF3 + FOF4 41.3; 387/938; $P < 0.05$). The FOF region showed a lower proportion of degenerated follicles (primordial and developing) than the NOF region, demonstrating a greater presence of intact ovarian follicles ($P < 0.05$). We conclude that the distribution of preantral follicles in the equine ovary occurred heterogeneously, with larger amounts of preantral follicles in the inner area and in regions near of ovulatory fossa. This study suggests new concepts about the location of equine ovarian follicles, such as the small delimitation between ovarian layers and the follicles presence in the external layer.

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Effect of prostaglandin administration at the moment of TAI in buffalo heifers submitted to the synchronization of ovulation during the non-breeding season (preliminary results)

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Keywords: heifers, synchronization, ovulation.

It was evaluated the effect of prostaglandin administration ($PGF_{2\alpha}$) in buffalo heifers submitted to the synchronization of ovulation and timed artificial insemination (TAI) during the non-breeding season (spring to summer; 24º 26' 15" South and 47º 48' 45" West). The hypothesis was that $PGF_{2\alpha}$ administration at the moment of TAI increases the synchronization of ovulation, as well as ovulation rate. Prostaglandins are important local mediators to the oviduct functions and are involved in ovulation and fertilization processes (Małysz-Cymborska et al., Reproduction in Domestic Animals, 48:1034-1042, 2013). At random stage of the estrous cycle (D0), 35 buffalo heifers received an intravaginal progesterone device (P4; Sincrogest®, Ourofino Agronegócio, Brazil) and 2mg im of estradiol benzoate (EB, Benzoato HC®, Hertape Calier Saúde Animal S.A., Brazil). In D9, the animals received 0.53mg im of $PGF_{2\alpha}$ (sodium cloprostenol, Sincrocio®, Ourofino Agronegócio, Brazil) and 400IU im of eCG (Folligon®, MSD Saúde Animal, Brazil), followed by P4 removal. After 24h (D10), the heifer's ovulation was induced by the application of 1mg im of EB (Benzoato HC®) and 32h later, all heifers were subjected to TAI (D11). The animals were submitted to ultrasonographic examinations (Mindray DP2200Vet, China) carried out in D0 to check ovarian activity, in D9 and D10 to measure the follicular diameter (Ø) and from D11 to D14 (12/12h for 60h) to check the moment of ovulation and the ovulatory follicle Ø (OF). The heifers which present follicles < 9mm in D10 (n = 10) or ovulated before the TAI (n = 3) were removed from the experiment. The remaining females were divided according to age, weight, body condition score, ovarian activity and the largest follicle Ø verified in D10 into two groups: Control (n = 10) and $PGF_{2\alpha}$ (n = 12). The heifers of the $PGF_{2\alpha}$ group received 0.53mg im of $PGF_{2\alpha}$ (Sincrocio®, Ourofino Agronegócio, Brazil) at the moment of TAI. In D19 and D41, the animals were submitted to ultrasonographic examinations (Mindray DP2200Vet) for the measurement of CL Ø and to access the pregnancy rate, respectively. The statistical analysis was performed by GLIMMIX of the SAS®. There was no difference between the experimental groups (Control vs. $PGF_{2\alpha}$) for the analyzed variables: OF Ø (13.3 ± 0.4 vs.13.0 ± 0.5 mm; P = 0.82); moment of ovulation (68.7 ± 1.8 vs. 66.0 ± 0.0 h, P = 0.13); ovulation rate [90.0% (9/10) vs. 100.0% (12/12); P = 0.98]; CL Ø (17.9 ± 0.7 vs. 17.9 ± 0.7 mm; P = 0.45) and pregnancy rate [40.0% (4/10) vs. 75.0% (9/12); P = 0.12]. However, in evaluating the variances homogeneity, greater synchronization of ovulation was found in $PGF_{2\alpha}$ group than in Control (P < 0.00001). It was concluded that the $PGF_{2\alpha}$ administration at the moment of TAI increases the synchronization of ovulation, however, does not provide higher ovulation rate in buffalo heifers submitted to the synchronization of ovulation and TAI during the non-breeding season.

A155 Folliculogenesis, Oogenesis and Superovulation

The effect of lipopolysaccharides on the expression of genes involved in steroidogenesis and inflammatory response in bovine granulosa cells

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Keywords: estradiol, ovarian activity, immune response.

The lipopolysaccharides (LPS) induce inflammatory response and have been detected in follicular fluid (FF), uterine fluid and plasma of cows with metritis and with infectious mastitis. LPS acts in the hypothalamus or pituitary gland, inhibiting the release of gonadotropins. However, LPS can have also a direct effect in the ovary, especially in the theca and granulosa cells, affecting the steroidogenesis. The objective of this study was to evaluate the effect of a LPS challenge on the expression of genes involved in steroidogenesis and activation of the inflammatory response in granulosa cells of the dominant follicle in cows. For this, 20 Jersey cows were randomly assigned to 2 groups: control group (n = 10) and LPS group (n = 10). The follicular wave in both groups was synchronized using an intravaginal progesterone releasing device (CIDR; Eazi-Breed CIDR®, Zoetis Animal Health, NJ, USA) and 2 mg of estradiol benzoate (Hertape Calier, MG, Brazil) on day 0. Twelve hours before CIDR removal (day 8), cows received an IM injection of PGF2α (25 mg of dinoprost tromethamine, 5 ml of Lutalyse, Zoetis Animal Health). Two hours after the CIDR removal a single dose of intramuscular LPS (2.5 ug / kg BW, Sigma-Aldrich® Inc., MO, USA) was administered in the cows of LPS group and a placebo IV solution (2 mL NaCl 0, 9%) in the control group. Six hours after LPS challenge, the dominant follicle of each animal was identified by ultrasound, and aspirated. The granulosa cells were recovered by FF centrifugation. Rectal temperature was measured five hours after the LPS challenge. Total RNA from granulosa cells was extracted using Trizol® and mRNA expression of target genes was carried out using qRT-PCR (Applied Biosystems, Foster City, CA, USA) using the H2α gene as endogenous control. For statistical analysis the Mann-Whitney test was used (GraphPad Software Inc., La Jolla, CA, USA). The cows receiving LPS had a systemic inflammatory response, supported by higher rectal temperature (40.4 ± 0.1°C) compared to the control group (38.8 ± 0.1°C) ($P < 0.05$). Furthermore, there was a 91% reduction in the expression of *TLR4* ($P = 0.002$) and 89% reduction in the expression of TNF ($P = 0.001$) in cows challenged with LPS, both genes are important regulators of the immune response. Moreover, mRNA expression of *STAR*, an important regulator of steroidogenesis, was decreased 93% ($P = 0.01$) in cows receiving LPS. No difference was observed in the expression of the genes *NF-kB*, *CYP19A1*, *P450C17*, *LHCGR*, *CYP11A1* and *HSD3B1* between groups ($P > 0.05$). Intrafollicular estradiol concentration was not different between groups ($P > 0.05$). In conclusion, LPS induced an inflammatory response in the granulosa cells of dominant follicle, mediated by the *TLR4*, but through mechanisms that should be further investigated. Moreover, LPS can affect the production of steroid hormones in the long term, since it drastically reduced *STAR* expression.

A156 Folliculogenesis, Oogenesis and Superovulation

Profile of lipid metabolism-related genes in granulosa cells from Nelore cows submitted to ovarian superstimulation

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Keywords: superovulation, granulosa cells, lipid metabolism.

Superovulation with exogenous gonadotropins affects oocyte and embryo quality as well granulosa cells differentiation. Lipids play a significant role in energy storage, cell structure, modification of physical properties and function of biological membranes; they also have potent effects on cell-cell interactions, cell proliferation and intra and inter-cellular transport. Furthermore, superstimulated Nelore (Bos taurus indicus) cows had differences on the profile and abundance of phospholipids (PF) in follicular fluid. Thus, to gain insight into the effects of superstimulation with FSH (P-36 protocol) or FSH combined with eCG (P-36/eCG protocol) was quantify the expression of lipid metabolism-related genes (ACSL1, ACSL3, ACSL6, SCD, ELOVL5, ELOVL6, FASN, FADS2, SREBP1, CPT1B) in granulosa cells from cows submitted or not to superstimulatory protocols, Nelore cows were treated with two superstimulatory protocols: P-36 protocol (n = 14) or P-36/eCG protocol (n = 16; replacement of the FSH by eCG administration on the last day of treatment). Non-superstimulated cows (n = 14) were only submitted to estrous synchronization without ovarian superstimulation. Nelore cows were slaughtered 12 hours after the progesterone device was removed. The follicular fluid was aspirated, granulosa cells removed by centrifugation at 1.200g. The mRNA abundance was measured by real time RT-PCR using bovine-specific primers. The data were normalized by the expression of endogenous gene, peptidyl isopropyl isomerase (PPIA). Effects of the superstimulatory treatments were tested by ANOVA and means were compared with Tukey-test. Differences were considered to be significant at $P \leq 0.05$. Ovarian superstimulation did not affect the expression lipid metabolism-related genes in bovine granulosa cells. Only CPT1B mRNA abundance was affected by ovarian superstimulation showing lower levels in granulosa cells from cows submitted to P-36/eCG (2.25 ± 0.47) treatments compared to the non-superstimulated cows (6.48 ± 1.51). In conclusion, although previous data showed that abundance of some PL in bovine follicular fluid were affected by ovarian superstimulation, only the expression of CPT1B in granulosa cells seems to be affected by ovarian superstimulation in Nelore cows submitted to P36/eCG protocol.

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A157 Folliculogenesis, Oogenesis and Superovulation

Effects of somatotropin injection on gene expression of follicular cells from the dominant follicle in cows

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Keywords: dominant follicle, gene expression, somatotropin.

The aim of this study was to determine the effect of somatotropin injection on gene expression of follicular cells from the dominant follicle of dairy cows. Twenty Jersey cows managed under the same conditions and nutritional regimen (pasture-based system) were used. Cows were randomly assigned to two treatments: somatotropin; (ST; n = 10), that received one dose of somatotropin (500 mg, subcutaneo, Lactotropin, Elanco, SP, Brazil) at -60 days relative to start the protocol of synchronization (day 0) and Control; (CN; n = 10) that did not receive somatotropin application. On day 0, cows received one injection of GnRH (100 mg, intramuscular, of gonadorelin hydrochloride; 2 mL of Factrel, Zoetis Animal Health, NJ, USA) and received a controlled internal drug-release insert (CIDR; Eazi-Breed CIDR, Zoetis Animal Health). On day 8, 12h before CIDR was removed, cows received one injection of PGF2a (25 mg, intramuscular of dinoprost tromethamine; 5 mL of Lutalyse, Zoetis Animal Health) and the dominant follicle from each cow was aspirated 12h after CIDR removal. To determine the number of follicles and identify the dominant follicle, follicular development was monitored via ultrasound at days -60, -53, -46, -14, -7, 0, 8 and at the moment of aspiration. The follicular fluid from the largest follicle for each cow was aspirated and follicular cells were retrieved immediately by centrifugation, frozen in liquid nitrogen and stored at −80°C until RNA extraction. Total RNA was extracted and gene expression of LHCGR, STAR, HSD3B, P450scc, CYP19A1, IGFr and PAPPA was measured by real-time PCR. T-tests were performed using GraphPad Prism 5 to compare means between groups. Average follicular cells STAR mRNA expression was higher in ST treated cows (2.88 ± 0.85 fold higher) than in CN cows ($P = 0.02$). However, ST cows had a lower ($P < 0.05$) mRNA expression of PAPPA, P450scc and CYP19A1 (0.19 ± 0.09, 0.39 ± 0.17 and 0.24 ± 0.10 fold, respectively) than CN cows. Expression of HSD3B, IGFr and LHCGR mRNA was not different between treatments ($P > 0.05$). In conclusion, injection of somatotropin in dairy cows has long term effects on follicle development and was able to increase STAR mRNA expression of and decrease PAPPA, P450scc and CYP19A1 on follicular cells of the dominant follicle 60 days after injection, potentially increasing the chance of ovulation in this class of follicles.

A158 Folliculogenesis, Oogenesis and Superovulation

Follicular population on the estimation of the superovulatory response in Santa Ines sheep

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Keywords: FSH, ewe, Santa Ines.

The aim was to verify the correlation between follicular population count, superovulatory response and the recovery of viable structures in the in vivo production of sheep embryos. Twenty-five nulliparous Santa Inês ewes (11.9 ± 1.1 months old, body score of 2.8 ± 0.3) were superovulated using the Day 0 protocol concept. For previous wave synchonization, intravaginal progestagen sponges (Progespon®, Zoetis, Campinas-SP, Brazil) were kept for 6 days and on Day 5, 300 IU eCG(Novormon®, Schering Plough, São Paulo, Brazil) and 0.24mg cloprostenol (Estron®, Tecnopec, São Paulo, Brazil) were given. Thirty-six hours after sponge removal, 25 µg licerelin was administered.The superovulation started 80 hours after sponge removal by the use of 200 mg of FSH/ per ewe (Folltropin-V ®, Bioniche Animal Health, Ontario, Canada) in six declining doses, every 12 hours (50/50, 30/30, 20/20 mg). At the first FSH dose, a new sponge (Progespon®, Zoetis, Campinas-SP, Brazil) was inserted and removed at the time of the fifth dose. At the last FSH dose, 0.24 mg of cloprostenol (Estron®, Agener Union, São Paulo, Brazil) and, 24 hours later, 25 µg of lecirelin (Gestran Plus®, Tecnopec, São Paulo-SP, Brazil) were administered. Ewes were mated every twelve hours from the last FSH dose to the end of estrus. An ultrasound equipment (Sonoscape S6®, SonoScape, Shenzhen, China) coupled to a 7,5 MHz linear transducer, by transrectal via was used for the quantification of the follicular population (PF) in two moments: at the beginning of the estrus synchronization (PFESTRUSBASE) and at the time of the first FSH dose (PFFSH-1). Embryo collections were carried out by surgical method, six to seven days after mating, and the viable structures (VS) quantified. The number of CLs (NCLs) was determined by laparoscopy previously to the embryo recovery. The PFESTRUSBASE and PFFSH-1 variables were compared with the NCLs and EV through the Pearson's correlation coefficient and Simple Linear Regression Analysis. For all tests, $P < 0.05$ was considered as statistically significant. An average of 7.5 ± 4.8 CLs and 4.0 ± 3.5 viable structures were obtained per donor. Significant correlations, medians and low, were found between: PFESTRUSBASE and NCLs ($r = 0.45$; $r2 = 0.17$; $P < 0.05$); PFFSH-1 and NCLs ($r = 0.41$; $r2 = 0.13$; $P < 0.05$); PFESTRUSBASE and VS ($r = 0.55$; $r2 = 0.27$; $P < 0.05$) and PFFSH-1 and VS ($r = 0.41$; $r2 = 0.13$; $P < 0.05$). In conclusion, there is a median correlation between follicular population observed by ultrasonography and viable recovered structures after superovulation protocol. Therefore, this tool is not indicated as a screening tool, alone, in the selection of Santa Inês sheep embryo donors.

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A159 Folliculogenesis, Oogenesis and Superovulation

Exosomes of follicular fluid from superstimulated Nelore cows modulate gene expression in bovine cumulus cells and blastocysts

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Keywords: ovarian superstimulation, extracellular vesicles, in vitro maturation.

Superstimulation can affect cellular and molecular events in ovarian follicular cells, *cumulus*-oocyte complexes (COCs), oviduct and embryo, as well as cell-to-cell communication through secretion and uptake of exosomes (Exo). The aim of this study was to investigate the effects of the extracellular vesicles (specifically, Exo), recovered from follicular fluid (FF), on the oocyte meiosis progression and apoptosis; and also on gene expression of COCs and blastocysts. Exo were recovered by ultracentrifugation and filtration of the FF from Nelore cows submitted to ovarian superstimulation with FSH (Exo/FSH); with FSH combined to eCG (Exo/eCG) or from non-superstimulated cows (Exo/NS). The presence of Exo was confirmed by Transmission Electron Microscopy. COCs from slaughterhouse were *in vitro* matured (IVM) for 22-24h with TCM199 medium enriched with 10% Exo from each group (Exo/FSH, Exo/eCG or Exo/NS) or without Exo (Control). IVM-oocytes were assessed for meiosis progression (Hoescht-33342 staining) and apoptosis (TUNEL assay). Pools of 20 oocytes were separated from their *cumulus* cells and each cell type was submitted to RT-qPCR quantification of *GDF9, BMP15, EGFR, AREG, GREM1* and *PDE3*. In addition, IVM-COCs followed in vitro fertilization and blastocysts were recovered seven days later. Pools of 3 blastocysts were used for the assessment of mRNA abundance of 44 genes related to embryo quality in a high throughput qPCR system (Biomark™ HD). Effects of Exo addition were tested by ANOVA ($P \leq 0.05$: significant difference; and $0.05 < P < 0.10$: considered tendency) and means were compared by Tukey-Kramer HSD test. Data were obtained from 5 replicates. Exo addition did not affect the percentage of oocytes in metaphase II, apoptosis rates or oocyte gene expression. However, the mRNA abundance of *GDF9* and *BMP15* in *cumulus* cells was higher in Exo/FSH when compared with Exo/NS and Exo/eCG. Exo addition did not affect blastocyst rates, but modulated the mRNA abundance of some target genes: *HAND1* expression was higher in Exo/eCG when compared to Exo/NS and control; *PFKP* expression was higher in Exo/FSH when compared to Exo/NS and control; *PKP2* was lower in Exo/FSH if compared with Exo/NS and Exo/eCG groups; and the expression of *SLC2A3* was higher in Exo/eCG when compared to Exo/FSH. Additionally, three genes tended to be different among groups: *ARO, CDH1* and *HSPA1A*. The *OCLN* gene expression tended to be higher in groups treated with Exo from superstimulated cows. In conclusion, whereas the precise mechanisms and molecules involved in this complex regulation remain unclear, our findings indicate that ovarian superstimulation in Nelore cows appeared to modify the Exo profile in the FF, which could modulate the gene expression of *cumulus* cells and blastocysts after added to the IVM medium.

A160 Folliculogenesis, Oogenesis and Superovulation

Mitofusin 1 is essential to oocyte fertility, whereas mitofusin 2 is required in oocytes to eliminate mutant mitochondrial DNA in mice

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Keywords: oocyte, mitochondria, mitofusin.

Mitochondria are known to play a major role in providing oocytes with developmental competence, but the mechanisms underlying this function are elusive. Compared to other cell types in an organism, the oocyte has the greatest amount of mitochondrial DNA (mtDNA) and mitochondria that distinguish by their small size and rounded shape. These unique characteristics are dependent on events of mitochondrial fusion and fission, which are determinant to organelle function and inheritance. Thus, to address the role of mitochondrial fusion in oocytes, the genes Mfn1 and Mfn2 were conditionally knocked out by Zp3-directed expression of Cre recombinase. Towards this aim, a heteroplasmic mouse lineage containing ~50% of mutant mtDNA (NZB) was used. Wild-type (WT) or knockout oocytes for Mfn1 (Mfn1-null), Mfn2 (Mfn2-null) or both (Mfn-dm) were compared regarding their developmental competence, mitochondrial function and inheritance of NZB mtDNA. Data were evaluated by one-way ANOVA followed by Duncan posthoc test. Differences with $P < 0.05$ were considered significant. Mating of females containing Mfn1-null oocytes with WT males did not result in birth. Infertility in this case was associated with a failure in ovulation, accumulation of secondary follicles (251 ± 36 vs. 54 ± 12 follicles) and arrest of meiotic progression when oocytes were in vitro cultured. Mfn1-null oocytes also presented smaller diameter (57.8 ± 0.73 vs. 85.7 ± 0.49 μm) and lower levels of mtDNA (39,263 ± 2,878 vs. 198,164 ± 18,611 copies), ATP (0.03 ± 0.016 vs. 0.46 ± 0.076 pmol) and mitochondrial membrane potential (ΔΨm; 0.71 ± 0.03 vs. 1.00 ± 0.03 a.u.). The lower abundance of Alix protein in Mfn1-null oocytes, a known marker of extracellular vesicles, suggests that secretion of oocyte-derived paracrine factors was deficient. In regards to Mfn2-null oocytes, in spite of the smaller diameter (78.6 ± 0.68 μm) and lower ovulation rate (10.3 ± 4.29 vs. 23.0 ± 3.82 oocytes), these oocytes were not affected based on the level of mtDNA, ATP and ΔΨm. As a result, the number of offspring born to Mfn2-null and WT oocytes did not differ. Moreover, the mice born to Mfn2-null oocytes grew normally, but had higher levels of blood glucose, both baseline (135 ± 3.9 vs. 115 ± 3.5 mg/dl) and fasted (172 ± 7.2 vs. 137 ± 4.1 mg/dl). Surprisingly, Mfn-dm oocytes presented a mild defect when compared to WT ones, characterized by slightly smaller diameter (76.7 ± 0.79 μm), intermediate level of mtDNA (104,215 ± 12,657 copies) and ΔΨm (0.81 ± 0.02 a.u.), and normal level of ATP, ADP and ATP/ADP. However, Mfn-dm oocytes contained smaller and fewer mitochondria, which aggregated with the endoplasmic reticulum. In spite of being ovulated, Mfn-dm showed a disrupted meiotic spindle and an incompetence to develop to term. In respect to the mitochondrial inheritance, the level of NZB mtDNA decreased in WT (-10.1 ± 1.25) and Mfn1-null (-10.3 ± 1.58) oocytes in comparison to their donor females, suggesting a selection in the germline against mutant mtDNA. However, this selection was less effective in Mfn2-null (-3.0 ± 1.50) and Mfn-dm (-4.4 ± 1.21) oocytes. In summary, this work gives evidence that Mfn1 is essential to provide oocytes with developmental competence whereas Mfn2 is involved in the elimination of mutant mtDNA. These findings support a key role of mitofusins in adapting mitochondria to meet oocyte's needs and prevent inheritance of mutant mtDNA.

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A161 Folliculogenesis, Oogenesis and Superovulation

Rutin can replace the use of other antioxidants in culture medium for sheep isolated secondary follicles

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Keywords: oocyte, antioxidant, GSH.

Rutin acts as a potent antioxidant in different cells types (KAUR and MUTHURAMAN, Life Sciences, 150, 89-94, 2016). However, there is no information about the effect of the antioxidant rutin on the in vitro culture of ovine secondary follicles. Thus, the aim of this study was to evaluate the effect of rutin as the only antioxidant added to the base medium on the in vitro culture of ovine isolated secondary follicles. Sheep (n = 90) secondary follicles (n = 55-61), approximately 200 µm, were individually cultured in 100 µL drops, during 12 days, at 39°C, in α-Minimal Essential Medium (α-MEM) supplemented with 3.0 mg/mL BSA, 10 ng/mL insulin, 2 mM glutamine and 2 mM hypoxanthine (antioxidant free-medium, called α-MEM-) or in this medium also added by 5.5 µg/mL transferrin, 5.0 ng/mL selenium and 50 µg/mL ascorbic acid (α-MEM+). To evaluate the effect of rutin, different concentrations of this antioxidant (0,1; 1 ou 10 µg) were added to the different base media, with or without antioxidant (α-MEM- or α-MEM+). All chemicals were purchased from Sigma Chemical Co. (St. Louis, USA). At each 6 days, normal follicles, antrum formation and follicular diameter were analysed. At the end of culture, the percentage of fully-grown oocytes (oocytes >110 µm) was evaluated. In treatments that had the best results of morphology, other parameters were analyzed, such as follicular viability through the fluorescent markers calcein-AM and ethidium homodimer 1, and intracellular levels of reactive oxygen species (ROS), glutathione (GSH) and active mitochondria. The rates of normal follicles, antrum formation, viability and oocytes >110 µm were compared by Qui-square test. Data from follicular diameter, growth rate, ROS, GSH and active mitochondria levels were submitted to Shapiro-Wilk followed by Kruskal-Wallis; data from diameter and growth rate were compared by Student Newman Keuls ($P < 0.05$). The results showed that after 12 days of culture, α-MEM+ alone (74.5%) and α-MEM- (without antioxidant) added by 0,1 µg rutin (81.6%) had higher percentages of normal follicles ($P < 0.05$) than other treatments (56.5%). There is no difference ($P > 0.05$) in the diameter and growth rate. Moreover, α-MEM+ alone and α-MEM- added by 0,1 µg rutin showed similar ($P > 0.05$) percentages of follicular viability, antrum formation, fully-grown oocytes, levels of ROS and active mitochondria. However, treatment α-MEM- added by 0,1 µg rutin showed higher GSH levels (157 pixels/oocyte) than α-MEM+ (110 pixels/oocyte). In conclusion, at a concentration of 0,1 µg, rutin can be used as the only antioxidant present in base medium for in vitro culture of ovine secondary follicles, maintaining follicular viability and increasing GSH levels.

A162 Folliculogenesis, Oogenesis and Superovulation

Oocyte lipids during folliculogenesis

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Keywords: lipids, folliculogenesis, oocyte

Oocyte maturation is associated with follicular growth, wherein the follicle size has an effect on embryo development capacity. Oocyte lipid content vary in mammals, is directly related to the ovulation process, and may contribute to oocyte and embryo developmental competence. The aim of this study was to characterize lipid content fluctuation during follicular development and associate with mRNA transcript levels of lipid-related genes. For this, slaughterhouse cow ovaries were used for the recovery of oocytes from follicles with ≤ 2 mm (n = 21), 3 to 5 mm (n = 34), 6 to 8 mm (n = 19) and > 8 mm (n = 13) in diameter, and assessment of lipid content by Sudan Black B staining, and mRNA levels of lipid-related genes (*ACSL3, ELOVL5, ELOVL6, LXRα* and *CYC-A* – endogenous control) by real time PCR – qPCR. The diameters of follicles were carefully determined with caliper device. Only oocytes with homogeneous cytoplasm and with more than three layers of cumulus cells were selected and denuded by repeated pipetting after trypsin treatment. For Sudan Black B staining, oocytes were prepared following a previously established protocol. ImageJ software was used to convert the Sudan Black B-stained oocyte images in gray scale and to determine the lipid content per oocyte expressed as arbitrary units (AU). The investigation of relative abundance of mRNA transcripts for *ACSL3*, *ELOVL5*, *ELOVL6* and *LXRα* genes (n = 4, total of 60 oocytes per follicle size) was conducted in a Stratagene Mx3005P Real Time PCR System (Agilent) using GoTaq qPCR Master Mix (Promega). The qPCR results are presented as fold change relative to the ≤ 2mm group, set as the reference group. The data were analyzed by ANOVA using the PROC GLIMMIX of SAS. Oocytes recovered from follicles > 8 mm had higher cytoplasmic lipid content when compared with the oocytes from other diameters (8.0 ± 2,7 AU; $P < 0.05$). The other groups showed no difference in lipid content (≤2 mm: 4.8 ± 0.6; 3-5 mm: 4.1 ± 0.8 e 6 to 8 mm: 4.2 ± 0.6 AU; $P > 0.05$). The mRNA abundance of *ELOVL6* was reduced ($P = 0.02$) in the oocytes recovered from 6 to 8 mm (5.3 x;) and > 8mm (5.6 x) follicles compared with the ≤ 2mm group; whereas the transcript level of *ELOVL5* was increased ($P = 0.02$) at 6-8 mm group compared with ≤2 mm (1.6 x) and 3-5 mm (1.3 x). The mRNA levels of *ACSL3* and *LXRα* were similar ($P > 0.05$) between groups. Therefore, the findings of this study reveal the following: i) oocytes recovered from follicles greater than 8 mm have higher lipid content; ii) the level of gene transcripts *ACSL3* and *LXRα* were not a fair marker of oocyte lipid fluctuation during folliculogenesis; and iii) the reduction of oocyte *ELOVL6* mRNA level during folliculogenesis suggests lower elongation reaction rate of fatty acids containing 12 to 16 carbons. Thus, the increase of follicle diameter favors oocyte lipid accumulation and it seems that the activation and elongation of fatty acids containing 12 to 16 carbons are not predominant during folliculogenesis.

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A163 Folliculogenesis, Oogenesis and Superovulation

Dynamics of follicular blood flow in FTAI protocols in Nelore cows

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Keywords: ovulation inductor, blood perfusion, Doppler.

The Color-Doppler ultrasound is a useful technique for reproduction by allowing real-time observation of blood flow. The objective of this study was to characterize the variation of follicular blood perfusion in Nelore cows submitted to three FTAI protocols. Were 21 cycling cows randomly divided into three FTAI protocols in a crossover model, with an interval of 30 days of rest were used. The BED9 protocol consisted of the insertion of an intravaginal first use implant P4(Sincrogest®, Ourofino, Cravinhos-SP) and IM application of 2 mg of estradiol benzoate (EB, HC Benzoate, Hertape Calier, Juatuba-MG) in D0, withdrawal of P4 and 150µg IM D-cloprostenol (PGF2a, Veteglan®, Hertape Calier, Juatuba-MG) in D8, and 1mg of EB IM in D9; BED8 the protocol consisted of insertion first use a implant P4 and 2mg BE D0, withdrawal of P4 and 150µg to 1mg PGF2a BE D8; and CED8 protocol of firts use an implant P4 and 2mg of BE in D0, withdrawal of P4, 150µg of PGF2a and 1mg of EC in D8. The cows were evaluated with ultrasound Color Doppler (MyLab ™ 30GoldVET, Italy) every 24 hours from D4 to D8 of the protocols, and after the withdrawal of P4 implant evaluations were made at intervals of 3 hours in the first three manipulations, and after 6 in 6 hours until ovulation occurs or a maximum of 90 hours after removal of the implant. For the evaluation of follicle vascularization used was a subjective rating of Grade 1 to 5, which considered the percentage of the wall movement assessed follicle. For statistical comparison of ovulation time and size of the pre-ovulatory follicle was used analysis of variance test (Tukey test) for ovulation rate the binomial test two proportions, and to characterize the vascularization descriptive analysis. Difference was observed ($P < 0.05$) in the ovulation time after the withdrawal of P4, where the BED8 (64.36 ± 10,42h) anticipated ovulation in the other two treatments (74.40 ± BED9 3,04h and CED8 72.00 ± 6,63h, $P > 0.05$). The average size of the preovulatory follicle (last evaluation time before ovulating) was the same in three protocols (BED9 12.53 ± 1,29mm; BED8 11.77 ± 1,54mm; CED8 12.77 ± 1,17mm; $p = 0.0708$). The ovulation rate was lower in BED8 protocol (52.38%), and equal in BED9 (80.95%) and CED8 (90.47%) ($P = 0.3778$). At the beginning of vascular assessment (D4) protocols, all cows that came to ovulation had a follicle in grade 1 with an average size of 5,89mm, and these ovulated on average 12 hours reached grade 5 with an average size of 12,25mm. BED9 protocol dominant follicles achieved a growth of 0, 25mm in passing grade 4 to grade 5 between 48 and 66 hours, in BED8 protocol, in the same passage of grade, achieved a growth of 0,52mm between 36 and 48 hours and CED8 protocol, the dominant follicles achieved in the same passage of grade a growth of 0,66mm between 42 and 66 hours. The Color Doppler tool lets you examine the vascular system in follicles, such as predicting the proximity of ovulation.

A164 Folliculogenesis, Oogenesis and Superovulation

Follicular viability and SOD activity in FOPA *Bos taurus* cattle subjected to different means and times transport

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Keywords: preantral follicles, *Bos taurus*, superoxide dismutase.

The manipulation of oocytes enclosed in preantral follicles (MOIFOPA) has been the aim of many studies in assisted reproduction, presenting satisfactory results in the *in vitro* culture of preantral follicles (FOPA), however, preservation of follicular viability during transport to the laboratory of the ovaries is an important aspect, whose protocols are not completely established. It is known that the accumulation of reactive oxygen species (ROS) generated during transport and cooling of the ovarian tissue may result in significant cellular damage, being the role of the enzyme superoxide dismutase (SOD) fundamental to neutralize these ROS. The aim of this study was to evaluate the efficiency of different medium and times to transport of bovine ovaries destined to MOIFOPA by assessing the viability follicular and SOD activity. Six pairs *Bos taurus* cows ovaries were used being the cortical layer fragmented and subjected to different treatments (3x2 factorial) accordance with the means or time used for the simulated transport at 4°C, these being: Control (immediate processing), T1 (TCM 199 for 6h); T2 (TCM 199 for 24 h); T3 (0.9% saline solution for 6 h); T4 (0.9% saline solution for 24 h), T5 (PBS for 6 h) or T6 (PBS for 24 h). For histological evaluation, the fragments were fixed in Carnoy and stained with PAS-hematoxylin, following being evaluated FOPA under optical microscopy in relation to their morphology and follicular viability according Celestino et al. (Celestino JJH, Brazilian Archives of Veterinary Medicine and Animal Science, v.59, 591-599.2007). The determination of SOD tissue was performed by the method described by Misra and Fridovich (HP Misra, The Journal of Biological Chemistry, v.247, p.3170-3175, 1972) based on their ability to inhibit autoxidation of adrenaline in adrenochrome. The statistical analysis was used two-way ANOVA. Evaluation of follicular viability observed that follicles of T1 (51.7%) did not differ from the control group (66.7; $P < 0.05$) while T2 treatments (33.3%), T3 (20%), T4 (16.7%), T5 (36.7%), and T6 (21.7%) reduced the percentage of viable FOPA ($P < 0.05$). The determination of SOD showed a lower activity ($P < 0.05$) of the enzyme in ovarian cortical tissue kept in T2 (7.59UI / mg) and T4 (6.36UI / mg) when compared to control (12.87UI / mg), T1 (11.46UI / mg), T3 (11.13UI / mg), T5 (10.19UI / mg) and T6 (9.35UI / mg). Therefore, it is suggested that bovine FOPA maintained *in situ* and 4ºC in 0.9% saline solution and PBS are not efficient in follicular preservation as well as the conservation of more than 6 hours in TCM 199. The TCM 199 medium at 4°C by 6 h can be efficiently used *in situ* to FOPA transport protocols for *Bos taurus* cattle.

A165 Folliculogenesis, Oogenesis and Superovulation

Variation in kisspeptin response for assessment to precocity in heifer

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Keywords: puberty, sexual maturation, Nelore.

The study aimed to compare the plasma luteinizing hormone (LH) concentration in response to exogenous kisspeptin in Nellore heifers from weaning until the breeding season beginning to assess the sexual maturation of the central nervous system. The tested hypotheses were: LH secretion in heifers increases with age and in the same age, heifers with greater weaning weight will secrete more LH in response to the Kisspeptin compared to heifers weaned lighter. The experiment was conducted at Santa Encarnação farm, located in Bataguassu-MS. At weaning of 184 Nellore heifers were segregated as 25 heaviest and 25 lightest group (increasing variability among animals). The 50 heifers were monthly challenged with 10 mg/kg (IM) of recombinant bovine kisspeptin (American Peptides, diluted in saline) from weaning (eight months) to 17 months and 20 minutes after drug application blood collection was performed. At 16 months, the heifers were exposed to bulls (25:1) and 30 days after the end of the breeding season (60 days) pregnancy will be assessed. LH quantification was performed by competitive Elisa, at the Endocrinology Laboratory of the Faculty of Veterinary Medicine of UNESP Araçatuba. After RM ANOVA it was observed that heifers LH secretion in response to kisspeptin increased according to age. Age in months where LH concentration was greater varied it happens at 14 months in the heavier group and at 15 months in the light group. At this month lighter group secreted 7.7 ng/mL while the mean LH concentration for the heavy animals was 5.1 ng/mL. It was observed that after this higher concentration at 14 or 15 months LH secretion in response to kisspetin decreased. There was time effect ($p = 0.0001$) and tendency interaction between time x weight ($p = 0.0780$) but the weight of the animals ($p = 0.3853$) did not affect the LH concentration. It was concluded that there was an increase on LH secretion in response to exogenous kisspeptin according to age. From the results of the pregnancy diagnosis, we will have an idea if it is possible to segregate heifers for potential precocity considering the LH concentration in response to kisspetin. We believe the decrease in response to kisspeptin after 14th or 15th month may be a consequence of the first ovulation, we intend to quantify progesterone from the samples to test this hypothesis.

A166 Folliculogenesis, Oogenesis and Superovulation

Insulin levels affect the *in vitro* survival and development of preantral follicles cultured in situ in the presence of goat bone morphogenetic protein 15 and / or growth and differentiation factor 9

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Keywords: goat, preantral follicles, in vitro culture, oocyte growth factors, insulin.

The objective of this study was to evaluate the effect of bone morphogenetic protein 15 (BMP-15) and growth and differentiation factor 9 (GDF-9) on the survival and development of goat primordial follicles in vitro cultured for 7 days when insulin was added at two different concentrations (low and high). Therefore, from each ovarian pair fragments were obtained, one was immediately fixed, constituting the non-cultured control, and the remaining fragments were cultured for 1 or 7 days in alpha-Minimum Essential Medium (alpha-MEM) alone (cultured control) or supplemented with 10 ng/mL (low) or 10 µg/mL (high) of insulin alone, or supplemented with 100 ng/mL BMP-15 and/or 200 ng/ml GDF-9. The ovarian tissue fragments non-cultured and cultured were processed by classic histology, being the follicles classified for survival (normal or degenerated) and follicular development (primordial, transition, primary or secondary). Data were analyzed by Chi-square test ($P < 0.05$). The results showed that after 7 days of culture, all treatments significantly reduced the proportion of normal follicles when compared to non-cultured control (87.37%). Regarding the medium supplemented with a low concentration of insulin (10 ng/mL), the percentage of normal follicles was equivalent to the treatment GDF-9 alone, significantly lower percentages were observed in the other treatments. However, when compared to the medium with high concentration of insulin (10 µg/mL), there was an effect of the addition of growth factors in alone and combined, since these treatments had significantly higher percentages of normal follicles rearding to the medium with high insulin . When comparing the treatments with low and high insulin supplementation after 7 days of culture, although the low insulin medium had shown significantly higher percentage of normal follicles than high insulin medium, the opposite happened when BMP- 15 was added alone or in combination with GDF-9. After 1 day of culture, in all treatments, except in the treatment with high insulin alone, the proportion of primordial follicles was significantly reduced compared to non-cultured control, with a concomitant increase in the proportion of developing follicles. A significant increase in the percentage of secondary follicles from day 1 to day 7 was observed in low insulin treatment alone or associated with GDF-9, and high insulin treatment with BMP-15 alone or in combination with GDF-9. Given the above, it can be concluded that the insulin concentration affects the effects of BMP-15 and GDF-9 on the in vitro survival and development of goat preantral follicles, and this is especially evident with BMP-15, which showed better effects in the presence of high insulin concentration (10 µg/mL).

A167 Folliculogenesis, Oogenesis and Superovulation

Meiotic arrest and changes on chromatin configuration of bovine oocytes submitted to pre-maturation with cilostamide and natriuretic peptide type C (NPPC)

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Keywords: oocyte competence, germinal vesicle, meiotic arrest.

The oocyte removal from follicular environment induces immediate and premature resumption of meiosis regardless of oocyte competency. Pre-Maturation Systems (PM) that delay or prevent spontaneous meiotic maturation and simultaneously provide cytoplasmic changes are alternatives to increase the oocytes competence. The acquisition of competence has been associated with changes in chromatin configuration, which can be considered as competence morphological markers. Our study aimed to evaluate the ability of two meiosis blockers, cilostamide and natriuretic peptide type C (NPPC), to promote meiotic arrest and cause changes in chromatin configuration, when used for 6 h of PM. Only grade 1 and 2 oocytes obtained from 3-8mm follicles of slaughterhouse's ovaries were used. In first experiment (n = 542), oocytes matured for 20h were compared to oocytes submitted to 6 hours of PM with 5 uM of cilostamide followed by 20 h of IVM. The same procedures were used for second experiment (n = 545) in which cilostamide were substituted for NPPC (100nM). Six replicate were performed for each experiment. All oocytes were fixed, stained with lacmóide and assessed for meiosis stage before (0h) and after (6h) PM and at 0, 6, 14 and 20 h of IVM. Oocytes at Germinal vesicle stage (GV) were classified as GV0, GV1, GV2 and GV3 (Lodde et al.; Mol Reprod Dev 74: 740-749, 2007). The results were analyzed by chi-square test ($P \leq 0.05$). After 6 h of PM with cilostamide the percentage of GV oocytes (87.3%) was similar ($P > 0.05$) to 0 h (95.2%). Although there was no difference on the rate metaphase II oocytes between PM (83.3%) and IVM (88.4%) groups, it was observed an acceleration on meiosis for group PM, which presented at 14 h of maturation some oocytes at MII stage (28,8 %). Regarding to chromatin configuration, no differences before and after PM were observed in the percentage of oocytes at GV0, GV1, GV2 and GV3 (7.4%, 18.5%, 38.3% and 35.8% x 9.5%; 15.9%; 25.4% and 49.2%, respectively). Similar results were observed when the PM was performed in the presence of 100 nM of NPPC. Most oocytes were arrested at GV after PM (82.9%), the maturation rate was similar between PM (82.5%) and IVM (82.9%) group and an acceleration on meiosis was observed when PM group was compared to the control after 14 hours of maturation. Retention with NPPC was also not able to change the chromatin configuration, being the percentage of oocytes at GV0, GV1, GV2 and GV3 similar before and after PM (2.1%, 29.5%, 40% and 28.4% vs 1.3%; 21.8%; 37.2% and 39.7% respectively). In conclusion, the PM for 6 h in the presence of cilostamide or NPPC inhibited spontaneous nuclear maturation, but did not change chromatin configuration, which suggested that it has not improved oocyte competence.

A168 Folliculogenesis, Oogenesis and Superovulation

Performance, thermal confort and ovarian phisiology of Canchim (*Bos indicus vs Bos taurus*) heifers grazing pastures with and without natural shade – preliminary results

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Keywords: animal comfort, performance, ovary.

Our objective was to verify the influence of natural shade in the pasture on thermal comfort and, ovarian physiology of Canchim heifers. Sixty-four prepubertal heifers were evaluated, during two years, while grazing pastures with shade provided by in a silvopastoral system (PRA; presence of eucalyptus trees with 15 x 2 m spacing) or pastures without shade (PR), at Embrapa Pecuária Sudeste. All pastures were intensively managed in a rotational system. On year 1: heifers (n = 32), aged 16 mo old and weighed 211 kg; on year 2: heifers (n = 32), aged 15.6 mo old and weighed 239 kg. Evaluations included: a) diameter of the largest follicle (LF) and second largest follicle (SLF); b) presence of corpus luteum, to determine age at first ovulation in months (AFO). Rectal temperature (RT) and respiratory frequencies were evaluated to obtain the Benezra comfort index (BCI). Heifers were weighted every month to evaluate average daily gain (ADG). For the statistical analysis of continuous variables and respective correlations it was used SAS®, considering in the model the effect of trees and year. Results are shown as least square means ± SE, and differences were considered when $P < 0.05$. Considering that animal comfort is indicated by a BCI index close to 2.0 (Benezra, 1954), heifers kept in PRA had better thermal comfort (2.99 ± 0.018) compared to those kept in PR (3.05 ± 0.018). There was a negative correlation between ADG and AFO ($r = -0.362$, $P < 0.0001$) and was interaction between year of evaluation and weight at first ovulation, heifers in PRA were heavier at first ovulation than those in PR (271.4 ± 7,2 vs. 249.2 ± 7,9 Kg), in the year two. Nonetheless, heifers in PRA and PR had similar diameter of LF (9.56 ± 0.112 vs. 9.58 ± 0.109 mm, $P = 0.89$) and SLF (6.57 ± 0.14 vs. 6.87 ± 0.14 mm, $P = 0.10$) and AFO (18.5 ± 0.27 vs. 18.6 ± 0.29 mo, $P = 0.91$). The presence of shade improved thermal comfort and weight at first ovulation but did not directly affect the ovarian variables studied. Nevertheless, that the positive effects observed were not sufficient to alter ovarian physiology in the animals.

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