Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Folliculogenesis, oogenesis and superovulation

The role of autophagy on cytoplasmic maturation of oocytes exposed to hyperglycemia

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Resumo

Diabetes mellitus (DM) is a worldwide disease characterized by hyperglycemia which can trigger oocyte dysfunctions. For example, hyperglycemia increases oocyte reactive oxygen species (ROS), apoptosis, mitochondrial dysfunction and delay nuclear maturation. It has been demonstrated that stress conditions can activate survival mechanisms such as autophagy. Autophagy is a physiological process that recycles cellular damaged compounds providing building blocks to the cell. Therefore, the objective of this study was to determine the effects of hyperglycemia and the inhibitor of autophagy (3-methyladenine: 3-MA) during in vitro maturation (IVM) of bovine oocytes on mitochondrial activity and cumulus cell expansion. Slaughterhouse-derived cumulus-oocyte complexes (COCs) were in vitro matured in TCM-199 containing 5.5 (physiological glucose group) or 20 mM glucose (hyperglycemic group) in the presence of 0 or 10 mM 3-MA during 21-24h IVM. Cumulus cell expansion area was determined by image acquisition immediately after oocyte collection (0h) and after IVM followed by image analysis using ImageJ 1.53.n (N= 7 replicates using 108-134 COCs/treatment). After IVM, COCs were denuded to determine mitochondrial activity using MitoTracker Red CMX-Ros (Invitrogen M7512). Oocyte fluorescence pixel intensity was quantified by ImageJ 1.53.n (N= 6 replicates using 29-81 COCs/treatment). Nonparametric data was analyzed using Wilcoxon test of SAS. Autophagy inhibition caused a drastic reduction in cumulus cell expansion (P= 0.0035) mostly in the hyperglycemic group (P= 0.0051). COCs matured under physiological glucose with 3-MA had a reduction on cumulus cell expansion compared with hyperglycemic group without 3-MA (P= 0.0294). Autophagy inhibition also reduced mitochondrial activity, mostly for oocytes matured under hyperglycemic conditions (P= 0.0330). In conclusion, autophagy activity may exert a protective role during cytoplasmic maturation of bovine oocytes under hyperglycemic conditions.

Keywords: Autophagy; Mitochondria; Cumulus cells.

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Folliculogenesis, oogenesis and superovulation

In vivo embryo production in donors with low and high antral follicle counts superovulated with low and high FSH doses

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Resumo

This study evaluated embryo production in Nelore donors with low and high antral follicle count (AFC) subjected to a MOET program using low and high FSH doses for SOV. On D-30, 16 donors cows were underwent a pre-synchronization protocol with an intravaginal P4 device (P4, 1g, ReproNeo®, Globalgen, Jaboticabal, Brazil), 2mg EB (Bioestrogen®, Biogénesis Bagó, Curitiba, Brazil) and 75µg PGF2α (Croniben®, Biogénesis Bagó). On D-22, P4 device was removed and was applied 75µg of PGF2α, 300IU of eCG (Ecegon®, Biogénesis Bagó) and 1mg EC (Croni-Cip®, Biogénesis Bagó). On D-26, the AFC (> 2 mm) of each donor was evaluated by ultrasound and divided in 2 groups, low AFC (n=8; ≤ 15 follicles; mean=10±0.91) and high AFC (n=8; ≥ 25 follicles; mean=36±6.05). They received two SOV programs using a dose of 150 and 300 IU of FSH (Pluset®, Biogénesis Bagó). The SOV protocol started with an intravaginal P4 device and 2mg EB on D0. On D4, all cows received FSH distributed in decreasing doses twice a day, D4 40%; D5 30% and D6 20% of FSH plus 150µg of PGF2α. On D7, P4 device was removed and 10% of FSH was divided in two applications. On the morning of D8, 10.5 mg of buserelin acetate (Gonaxal®, Biogénesis Bagó) was applied and the inseminations were performed 12h and 24h later. On D15, uterine flushing was performed and the embryos were recovered, identified, and classified with a stereomicroscope according to the IETS criteria. Embryos with grades of I and II were frozen and stored at -196°C. Data were analyzed by two models employing ANOVA and Tukey's test in a procedure for an adjusted mixed effect model (P≤0.05). The first model contemplated a split-plot scheme (AFC, FSH dose and interaction) and second model considered the treatment effect (low-150, low-300, high-150 and high-300). In the first model, AFC showed effect (P<0.05) for the number of CL (low 10.7±1.6a vs high 19.2±3.1b), but total structures, viable and freezable embryos were similar (P>0.1) between low and high AFC. The 300 IU of FSH resulted greater (P<0.01) numbers of CL (20.5±2.4b vs 9.4±2.3a), total structures (12.2±1.9b vs 6.2±1.7a) and viable (9.1±1.5b vs 4.9±1.4a) and freezable embryos (7.8±1.3b vs 3.0±0.9a) than the 150 IU dose. There was no interaction of AFC*FSH (P>0.05). In the second model, a treatment effect (P<0.05) generally showed better MOET performance for high-300 group in relation to low-150, high-150 and low-300 to numbers of CL (26.0±3.4b vs 6.4±1.5a, 12.4±4.1a and 15±1.9a), total structures (14.0±2.8c vs 4.6±2.1a, 7.9±2.7ab and 10.4±2.4b), viable (10.9±2.1b vs 4.2±2.2a, 5.6±1.9a and 7.2±2.2ab) and freezable embryos (9.2±1.7b vs 2.4±1.5a, 3.6±1.3ab and 6.4±1.9b), respective groups. In conclusion, the embryo yield was not influenced by the AFC category but was influenced by the FSH dose. Furthermore, high-AFC donors superovulated with the highest FSH dose presented the best performance in terms of embryo yield.

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Folliculogenesis, oogenesis and superovulation

DISORDERS OF PYRUVATE METABOLISM ALTER THE METABOLIC AND THE TRANSCRIPTIONAL PROFILE OF BOVINE OOCYTES

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Resumo

Previous research from our group has shown the importance of pyruvate metabolism in the epigenetic reprogramming of bovine oocytes during maturation. More specifically, oocytes matured in the presence of sodium dichloroacetate [DCA, a stimulator of pyruvate oxidation in acetyl-CoA] or sodium iodoacetate [IA, a glycolysis inhibitor] showed increased mitochondrial membrane potential (MMP) and changes in the dynamic of lysine 9 histone 3 (H3K9) deacetylation. In the present work, we characterize the metabolic pathways involved in this mechanism and the molecular consequences of the modulation of pyruvate metabolism during in vitro maturation (IVM). Oocytes were IVM for 24h in three experimental groups: Control [IVM medium], DCA [IVM medium supplemented with 1.5 mM DCA], or IA [IVM medium supplemented with 5 µM IA]. The metabolic profile of single oocytes (at least 5 oocytes/3 replicates/ group) was analyzed at the end of IVM (metabolome, Raman spectroscopy; lipid droplets, Nile Red, Sigma; and reactive oxygen species (ROS), CellRox Green®, ThermoFisher Scientific). The images were acquired using a fluorescence microscope and analyzed by Fiji software. Raman spectra were processed and analyzed using the Spectrograpy 1.2.15 software. Peak attributions were done according to previous references. Results were compared by Student's t-test (treatment vs. control) considering $P<0.05$. Changes in transcript content were assessed by RNASeq analysis (5 oocytes/3 replicates/per group). Differentially expressed genes (DEGs) were assessed using the DESeq2 R package considering a $P<0.05$ and absolute log2 fold change >1 and the enrichment analysis was done by submitting the lists of DEGs to ClusterProfiller. The oocytes from DCA and IA groups had a decrease in lipid droplets (DCA: $P=0.003$; IA: $P=0.004$) and an increase in the intensity of Raman bands attributed to fatty acids (DCA: $P=0.0001$ and $P=0.0005$; IA: $P=0.0285$ and $P<0.0001$), suggesting that beta-oxidation may be responsible for the higher MMP previously identified. This was followed by higher levels of ROS content in treated groups (DCA: $P=0.0036$; IA: $P<0.0001$). A total of 148 and 356 DEGs were identified in DCA and IA groups, respectively. The enrichment analysis revealed that the control group presented more transcripts related to the ROS pathway than the DCA group while mRNA surveillance and oxidative phosphorylation pathways were enriched in the control oocytes compared to the IA group. In conclusion, disorders in pyruvate metabolism during maturation alter the lipid and the mitochondrial metabolism, with consequences for the mRNA content of bovine oocytes.

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Folliculogenesis, oogenesis and superovulation

Oocyte quality and antral follicle count of Nelore heifers (Bos indicus) in grazing receiving different levels of concentrated supplementation

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Resumo

Concentrated supplementation of heifers is an interesting strategy to maximize the productivity of beef cattle. It makes possible to reduce the age at puberty, of heifers with genetic potential for precocity, and the finishing time for slaughter of those with lower reproductive efficiency. However, there is a gap regarding the most appropriate supplementation levels to a maximum reproductive efficiency. The aim of this study was to evaluate the influence of different levels of concentrated supplementation on antral follicle count (AFC) and on oocyte quality of grazing heifers. We used 40 nulliparous Nellore heifers (age of 12 ± 0.71 months, weight of 262 kg ± 18.85 and body condition score (BCS) of 2.33 ± 0.37), grazing B. brizantha pastures, divided into 4 groups (n=10), receiving different concentrate supplementation level in initial (80d) and finishing (60d) phases: Trat 05-1 (0.5% of animal live weight (LW) of concentrate supplement in the initial and 1.0% LW in the finishing phase), Trat 05-2 (0,5% LW in the initial and 2.0% LW in the finishing phase), Trat 1-1 (1.0% LW in the initial and 1.0% LW in the finishing phase), and Trat 1-2 (1.0% LW in the initial and 2.0% LW in the finishing phase). After 140 days, the heifers were slaughtered, and the ovaries were collected and sent for counting antral follicles (AFC) followed by follicular aspiration, recovery and classification of cumulus oocyte complexes (COCs). Data were submitted to a statistical analysis, according to a completely randomized design, using PROC GLIMMIX of the SAS On Demand (Sas Institute Inc., Cary, CA, USA). A significance level of 0.05 was considered. The total (17,2 ± 3,18) and grade 1 (2,71 ± 0,902), 2 (1,97 ± 0,668) and 3 (1,79 ± 0,669) COCs and AFC of heifers in group 05-1 were lower than those of the other groups (P<0.001). In addition, the number of degenerate COCs was higher (8,31 ± 2,98) and the COC quality (52,1 ± 6,00) rate was lower (P<0.05) in heifers in group 1-2. The use of intermediate concentrated supplementation levels (Trat 1-1) or the use of high level of supplements just in the finishing phase (Trat 05-2) can positively affect the antral follicle count and oocyte quality of Nelore heifers.

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Folliculogenesis, oogenesis and superovulation

Effect of palmitic acid on the miRNA biogenesis from bovine cumulus cells

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Resumo

The postpartum negative energy balance (NEB) is an important risk factor in the establishment of reproductive failure in high-producing dairy cows. In this period an excessive mobilization of body reserves increase serum and follicular fluid concentrations of non-esterified fatty acids (NEFAs), such as palmitic acid (PA). Such increased plasma NEFAs concentrations induce changes in the microenvironment of the ovarian follicle, which may alter the pattern of epigenetic marks in cumulus cells, oocytes, and embryos. However, the complete mechanism has not been fully elucidated. MicroRNAs (miRNAs) are a class of non-coding RNAs that play important roles in regulating gene expression. MiRNAs biogenesis is regulated at multiple levels, including at the level of miRNA pre- and posttranscriptional processing and their dysregulation is associated with many diseases. The aim of the study was to investigate the effect of PA on miRNAs biogenesis in bovine cumulus cells. Bovine ovaries were obtained from a local abattoir and the cumulus-oocyte complexes (COCs) were aspirated and classified according to the morphology of oocyte and cumulus cells. Grade 1 and 2 COCs were matured in groups of 15 - 20 in a 500 µL serum-free maturation medium (containing TCM 199 supplemented with human recombinant FSH (hrFSH), pyruvate, gentamicin, BSA, AREG, IGF-1, progesterone and estradiol) in four-well plates for 24 h in humidified air with 5% CO2 at 38.5o C. COCs were exposed during IVM to the following conditions: Control (physiological PA concentration – 23 µM) and High PA (PA equivalent to that measured in follicular fluid during NEB – 150 µM). The PA was dissolved in ethanol according to the recommended solubility and the concentrations used in this study are based on bovine in vivo studies in follicular fluid during a period of NEB (Leroy et al., Reproduction, 4:485-95, 2005). Cumulus cells were submitted to total RNA extraction using QIAzol, followed by DNase treatment and cDNA synthesis using High Capacity cDNA Reverse Transcription Kit. The relative gene expression of DGCR8, DROSHA, XPO5, DICER1, TARBP2, PRKRA, and AGO2 were determined using three genes (RPL15, PPIA, and YWHAZ) as references. Expression levels were calculated using the 2-ΔCt method and differences in continuous data between treatments were assessed by Student's t-test. A level of 5% significance was used. Maturation rates were similar between groups. High PA concentrations during IVM significantly increased DICER1 relative expression compared to the control. DICER1 cleaves pre-miRNA into miRNA in the cytoplasm; transforming precursor in mature miRNA and dysregulation of this enzyme can affect miRNA functionality. In conclusion, exposure to high PA concentration during maturation can affect miRNAs due to altered DICER1 levels in bovine cumulus cells, suggesting that miRNAs are involved in NEB response.

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Folliculogenesis, oogenesis and superovulation

Increased progesterone concentration in follicular fluid due to corpus luteum proximity: Consequences in miRNA biogenesis pathway in bovine granulosa cells

Paola Maria da Silva Rosa [1], Marina Port Lamellas [1], Giuliana de Ávila Ferronao [1], Natália Marins Bastos [1], Alessandra Bridi [1], Felipe Perecin [1], Flavio Vieira Meirelles [1], Juliano Coelho da Silveira [1]

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Resumo

Follicular fluid (FF) is modulated by dynamic changes in progesterone (P4) levels during the different stages of the estrus cycle. Interactions between microRNA (miRNA) expression and changes in P4 levels are reported in several tissues in female reproductive physiology. Nevertheless, the P4 effects on the follicular microenvironment, especially in the miRNA-biogenesis pathway of follicular cells, are poorly understood. Thus, this study hypothesizes that elevated intrafollicular P4 levels caused by corpus luteum proximity alter the miRNA-biogenesis-related genes in granulosa cells. Ovaries (n=3/replicate; 6 replicates) from a local slaughterhouse were collected in pairs and classified as stage 3 of the estrous cycle (according to corpus luteum morphology, related to the middle diestrus; Ireland et al. 1980. J Dairy Sci. 63:155–160) to obtain groups modulated by high or low intrafollicular P4. Small follicles (3-6mm) were aspirated (n~15/ovarie) from ipsilateral or contralateral ovaries to the corpus luteum. The FF was analyzed for P4 concentration (6 replicates; intra and interassay coefficients variations were 9.77% and 21.47%, respectively) and granulosa cells (n~5 pools/replicate; 6 replicates) were collected for mRNA analysis. Total RNA was isolated (TRIzol®; Invitrogen) with an RNA co-precipitant (GlycoBlue®; ThermoFisher Scientific) and treated with DNase (DNaseI, Invitrogen). The cDNA was synthetized (High Capacity; ThermoFisher) and analyzed by RT-qPCR (GoTaqR qPCR Master Mix; Promega) analysis. Seven transcripts related to the miRNA-biogenesis pathway (DROSHA, DICER1, AGO2, DGCR8, XPO5, PRKRA, and TARBP2) were analyzed and normalized by the geometric mean of two endogenous genes (PPIA and ACTB). The relative expression (ipsilateral n=6; contralateral n=4) was calculated using the ΔCt method, and the normalized data were transformed by 2−ΔCt for representation of the relative expression. P4 concentration and relative gene expression data (mean ± SEM) were tested for outliers' presence, normality (Shapiro-Wilk test) and were analyzed by Student's t-test (GraphPad Prism; Software), considering a significance level of 5%. The intrafollicular P4 concentration was higher in follicles localized ipsilateral (high P4 - 348.38 ± 60.26 ng/mL) to the corpus luteum compared to contralateral (low P4 - 91.06 ± 3.99 ng/mL; p=0.0018) group. The results demonstrated that AGO2 transcript was up-regulated in ipsilateral (0.01252 ± 0.00193) when compared to contralateral (0.00633 ± 0.00129) granulosa cells (p=0.0465). In this sense, altered miRNAs-biogenesis machinery is a possible pathway affected by P4, which could impact transcripts and protein levels in granulosa cells. Further analyses are necessary to understand the effects of high and or low intrafollicular progesterone concentration on AGO2 protein levels and granulosa cells as well as miRNAs levels.

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Folliculogenesis, oogenesis and superovulation

SHORT OR LONG-TERM PROGESTERONE PROTOCOLS ASSOCIATED WITH DIFFERENT FSH PRESENTATIONS FOR SUPEROVULATION IN ACYCLIC TOGGENBURG GOATS

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Resumo

This study aimed to evaluate short (six days, G6, n=28) or long-term (l7 days, G17, n=28) ovulation induction protocols using intravaginal controlled internal drug release devices (0.33 g of P4, CIDR®, Zoetis, São Paulo, Brazil) followed by superovulation (SOV) with 133 mg (G6A and G17A; n=14 each one) (Folltropin®-V, Vetoquinol, São Paulo, Brazil) or 250 IU (G6B and G17B; n=14 each one) (Pluset®, Hertape Calier, Barcelona, Spain) of pFSH, administrated in six decreasing doses (25, 25, 15, 15, 10 and 10%), every 12 h. The G6 protocol started with CIDR insertion simultaneously to the administration of 37.5 µg d-cloprostenol (Prolise®, Agener União, São Paulo, Brazil) i.m. (D0) and SOV started 48 h before CIDR removal. At G17 protocol, 37.5µg d-cloprostenol i.m. was administered at D11, SOV started at D15 and one injection of 250 IU hCG (Vetecor 5000®, Ceva, Juatuba, Brazil) at D19. In all animals, both estrus detection and mating started after the last dose of FSH and finished 60 h later and three doses of 75 mg flunixin meglumine (Flumax®, JA Saúde Animal, Patrocínio Paulista, Brazil) i.m. were administered at 84, 98 and 122 h after estrus onset. Transrectal ultrasound evaluations were performed on the day of the first FSH administration, after CIDR removal and six days later. Non-surgical embryo recovery (NSER) and embryo evaluation were performed on the sixth to seven days after first mating. Six hours before NSER, 37.5µg d-cloprostenol was administered for cervical dilation. Parametric variables were analyzed by ANOVA and t Student test, followed by the Tukey test; non-parametric variables were analyzed by Mann-Whitney test or Kruskal-Wallis test, followed by the Dunn test, chi-squared test or Fisher exact test. Goats that received 133 mg of pFSH presented a higher ($P<0.05$) estrus interval and duration (46.2±2.3 and 38.2±1.9 h) compared to 250 IU (35.3±2.5 and 31.3±3 h), although there was no difference ($P>0.05$) between groups when comparing only the length of the treatment (short or long). Similar ($P>0.05$) results were obtained for G6A, G6B, G17A and G17B regarding the mean CL number / goat (5.3±1.1; 5.9±0.9; 4.1±1 and 4.2±0.7) and rate of responsive goats [≥3 CL, 70 (710), 90 (910), 70 (710) and 73% (811)], respectively. There was also no difference ($P>0.05$) in the mean number of structures recovered between G6A (4.0±1.6), G6B (4.7±1.4), G17A (3.0±0.8) and G17B (5.0±1.6). The rate of viable structures was higher ($P<0.05$) in G6A (52.5%; 21/40) and G17B (54.5%; 30/55) than in G6B (10.6%; 5/47) and G17A (23.3%; 7/30) groups. We concluded that a short-treatment with 133 mg of FSH is an excellent option for SOV in goats as it presents a shorter exposure to P4, lesser time of CIDR use and higher number of viable structures in a shorter period.

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Folliculogenesis, oogenesis and superovulation

Heat stress during mouse oocyte growth uncouples DNA methylation reprogramming from developmental competence

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Resumo

Oocytes are susceptible to heat stress (HS) but its impact on epigenetic reprogramming remains elusive. Oocyte-specific DNA methylation occurs synchronously during oocyte growth in newborn mice, which allows exploring the effect of environmental challenges. We determined the impact of HS during oocyte growth on DNA methylation and developmental competence. Swiss mice with F0 litters were weighted on postnatal day 9 (P9) and randomly allocated to control (CTL) (21°C/24h) or HS (35°C/12h/light and 21°C/12h/dark) from P10 to weaning on P21. F0 females remained under 21ºC until puberty on P35. F0 females received 10 IU eCG to collect fully-grown (FG) oocytes 46h later for whole genome bisulfite sequencing (WGBS) or were subject to natural mating with fertile males. Superovulation of F0 females was with 10 IU eCG and 10 IU hCG 44-48h apart to collected mature oocytes 12-14h post-hCG for parthenogenetic activation (PA) or mated for *in vivo* embryo production and their collection was 94h post-hCG. PA was in calcium-free M16 medium with 10 mM $SrCl_2$, 5.0 µg/mL cytochalasin B, and 0.1 mg/mL PVA under 5% CO_2 at 37 ºC for 5h. Activated oocytes underwent *in vitro* culture in KSOMaa and embryonic development recorded at 24h (cleavage) and 96h (morulae + blastocysts) post-activation. Data was subject to ANOVA using general linear model of SAS. FG oocytes were subjected to next-generation sequencing of DNA converted by sodium bisulfite and differently methylated regions (DMRs) located by the DSS method. Functional analysis relied on g:Profiler. Exposure to HS did not affect survival of lactating females (P = 0.95) or offspring (P = 0.95). However, HS reduced body weight at P21 and weight gain for both lactating females (P = 0.0002) and offspring (P < 0.0001). Groups did not differ for mean number of ovulated oocytes (P = 0.86) and oocyte viability (P = 0.85). Exposure of F0 females to HS during oocyte growth did not affect cleavage rates (P = 0.12) after PA. However, HS tended to reduce (P= 0.06) embryonic development. F0 females has similar litter sizes after natural mating (P = 0.94) and *in vivo* embryo production (P = 0.35), which had similar percentage of viable embryos (P = 0.77). WGBS indicated more hypo-methylation in differentially methylated regions (DMRs) from HS oocyte than in the CTL counterparts. Enrichment analysis of DMRs found binding sites of transcription factors linked to HS response (Hsf4), genome activation (Klf4/6), trophectoderm development (Tfap2a/c), Wnt signaling/histone deacetylation (Kaiso), TGF-β signaling (Smad3/Rreb1), nuclear receptor signaling (Vdr), and uncharacterized factors (Pax4/5, Sall1). Nitric oxide and calcium signaling (Cacna1d-Kcnn4 complex) were novel processes affected by HS. In conclusion, HS during oocyte growth compromises genome-wide DNA methylation without affecting developmental competence. These results uncouple these biological processes and suggests that oogenesis tolerates substantial epigenetic noise.

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Folliculogenesis, oogenesis and superovulation

Follicular fluid progesterone variation due the proximity to the corpus luteum can affect bovine cumulus cells molecular pattern

Marina Port Lamellas [1], Paola Maria da Silva Rosa [1], Giuliana de Ávila Ferronato [1], Natália Marins Bastos [1], Alessandra Bridi [1], Gislaine dos Santos [1], Juliana Germano Ferst [1], Felipe Perecin [1], Flávio Vieira Meirelles [1], Juliano Coelho da Silveira [1]

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Resumo

Progesterone (P4) is a well-known hormone due to its association with oocyte functions and its role in establishment and the maintenance of pregnancy. In this study, we hypothesized that the follicular environment is exposed to different P4 levels, according to the proximity of the corpus luteum (CL), leading to molecular differences within the intrafollicular environment. To test this hypothesis, ovaries from local slaughterhouse were collected in pairs and separated in groups ipsilateral and contralateral to the CL, according to morphological characteristics associated with stage 3 of the estrous cycle (Ireland et al. 1980. J Dairy Sci. 63:155–160). Small follicles (3-6 mm in diameter) were aspirated from each group and cumulus oocyte complexes (COCs) were collected. Additionally, follicular fluid was used to measure the P4 levels by immune assay. Next, the cumulus cells (CCs) from immature COCs were collected and frozen (-80°C) for gene expression analysis. The CCs were submitted to total RNA extraction according to Trizol® (Thermo Fisher Scientific) protocol, with an RNA co-precipitant (GlycoBlue®; Thermo Fisher Scientific), treated with DNase Amplification Grade (Invitrogen, Brazil) followed by the reverse transcription using the High-Capacity Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA). The RT-qPCR analysis of FSHR, LHR, CYP17A1, 3b-HSD, CYP19A1, ADAMTS1, PGR, PAQR8, PAQR5, PGRMC1, PGRMC2, ESR1, ESR2, GPER1, BAX and BCL2 transcripts were performed using the Power SYBR Green PCR Master Mix kit (Thermo Fisher Scientific), according to the manufacturer's instructions. The relative expression of progesterone-related genes involved in oocyte maturation (PGR, PAQR8, PAQR5, PGRMC1, PGRMC2, ESR1, ESR2, and GPER1), steroidogenesis (FSHR, LHR, CYP17A1, 3b-HSD, CYP19A1 and ADAMTS1) and apoptosis (BAX and BCL2) on CCs from ipsilateral and contralateral follicles were analyzed in 6 and 4 replicates/group, respectively. Expression levels were calculated using the 2ˆ-ΔCt method, and normalized by the geometric mean of PPIA and YWHAZ as reference genes. P4 concentration and relative gene expression data (mean ± SEM) were tested for outliers' presence, normality (Shapiro-Wilk test) and were analyzed by Student's t-test ($p<0.05$) (GraphPad Prism; Software). The intrafollicular P4 concentration in ipsilateral and contralateral was 348.38 ng/mL and 91.06 ng/mL, respectively ($p=0.0018$). The intra and inter-assay were 13.42 ng/mL and 30.02 ng/mL for progesterone, respectively. The results demonstrated that ADAMTS1 relative expression increased in CC from ipsilateral follicles compared to the contralateral ($p=0.01$). ADAMTS-1 is a secreted protease that is involved in several biological functions important for oocyte competence and ovulation. In conclusion, different P4 levels, due corpus luteum proximity, modulates CCs at molecular levels which can impact oocyte quality.

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Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Folliculogenesis, oogenesis and superovulation

Meiotic and developmental competence of bovine oocytes recovered from early antral follicles

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Resumo

The aim of this study was to evaluate the effect of the meiotic inhibitor C-type natriuretic peptide precursor (NPPC) during a prematuration (PM) culture of incompetent bovine oocytes derived from follicles smaller than 2 mm on progress of nuclear maturation and acquisition of competence for in vitro embryo development. COCs were recovered from early antral follicles measuring <2 mm in diameter and selected COCs (n=621) were PM for 8h or 22h in 100 µL of TCM199 with 25 mM NaHCO3, 0.2 mM sodium pyruvate 0.3% fatty acid-free BSA, 100 µM cysteamine, 75 µg/mL amikacin, 1x10-4 IU/mL FSH and 100 nM NPPC [group rFSH(10-4)]. Medium could also be supplemented with 1% ITS and 100 ng/mL IGF [group rFSH(10-4)+ITS+IGF]. Next, the COCs were washed to remove NPPC and cultured for 22h in in vitro maturation medium (IVM; TCM199 with hormones, antibiotic and 10% FCS). Control oocytes were cultured in IVM medium for 22h, in the absence of NPPC. All cultures were performed under mineral oil, at 38.5°C under 5% CO2 in humidified air. Immature oocytes were evaluated immediately after removal from the follicular environment. At the end of each specific moment of the evaluation, the diameter of oocytes was assessed and they were then stained with Hoechst 33342 to assess the chromatin remodeling: immature and blocked oocytes were evaluated for germinal vesicle (GV) rates [graded from GV0 (fully incompetent) to GV3 (fully competent)] and meiosis progression to the metaphase II phase (MII) was evaluated in oocytes submited to IVM. Matured oocytes were also fertilized and cultured until day 7 to assess blastocyst rates. Data were analyzed by ANOVA followed by Tukey's test (P<0.05). The diameter of immature oocytes was 73.6±1.1 µm. No difference in diameter (P>0.05) was observed between groups after 8h preIVM (77.1 µm on average) and after 22h preIVM (76.1 µm on average). However, at the end of IVM, oocytes from rFSH(10-4)+ITS+IGF group were bigger than those from control (82.7±1.5 vs 78.7±1.3 µm; P<0.05), and both were similar to rFSH(10-4) group (83.4±1.1 µm; P>0.05). Most immature oocytes were at GV0 stage (77.2±6.2%) and after 8h preIVM (72.7% on average; P>0.05). After 22h preIVM, most oocytes were still in GV0, however, lower GV0 rate was observed in rFSH(10-4)+ITS+IGF group compared to control (35.8±3.8% vs 75.0±8.4%, P<0.05), and both were similar to rFSH(10-4) group (55.6±14.9%; P>0.05). At the end of IVM, GV2 rate was higher (P<0.05) in rFSH(10-4) (34.0±6.4%) than in control (12.8±2.4%) and rFSH(10-4)+ITS+IGF (18.4±1.7). Few oocytes progressed to metaphase I (3.1% on average) and no metaphase II was observed. Few oocytes cleaved after IVF (4.1±2.2 to 7.9±2.2; P<0.05) and few zygotes developed to the blastocyst stage (0.0% to 2.6±1.0%; P<0.05). We conclude that the PM system proposed in the present study was not sufficient to allow the completion of the growth process and acquisition of competence of oocytes derived from early antral follicles.

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Folliculogenesis, oogenesis and superovulation

Increased intrafollicular concentration of β-hydroxybutyrate (BHBA) affects follicular growth but does not compromise the ovulatory cascade in cattle

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Resumo

Metabolic stress conditions caused by negative energy balance (NEB) have been associated with reduced fertility in cows. β-hydroxybutyrate (BHBA) is the main circulating ketone body during NEB, which accumulates within follicular fluid.The aim of this study was to evaluate the effects of BHBA on follicle growth and ovulatory mechanisms in cattle. Two experiments were performed. In Experiment 1, to assess the effect of increased BHBA concentration on follicular development, cows had the emergence of a new follicular wave induced by using a P4-releasing intravaginal device (IVD; Primer, Tecnopec, Brazil; 1 g P4) and an intramuscular injection of 2 mg EB (Gonadiol, Zoetis, Brazil; D0). In D4, P4 device was removed and PGF2α analog (500 µg cloprostenol, Estron, Agener União Saúde Animal, Brazil) was administered intramuscularly. The ovaries were monitored daily using transrectal ultrasonography procedures. When the follicles reached a diameter of 8-9 mm, the animals were intrafollicularly injected with BHBA (15 mM; n = 8) or PBS (control; n = 8). Follicular growth was monitored for 72 h, ovulation rate and follicle diameter at ovulation were monitored for 120 h. In Experiment 2, the effect of high concentration of BHBA on the granulosa cells of preovulatory follicle was assessed to study the ovulatory mechanism. Cows had the emergence of a new follicular wave induced as described above. On D9, the follicles with diameter ≥ 12 mm were injected intrafollicularly with BHBA (15 mM; n = 4) or PBS (n = 5). The cows were ovariectomized after 6 h of BHBA injection. After ovariectomy, granulosa cell was collected for evaluation of relative abundance of mRNA transcripts. Differences between follicular sizes were compared between groups by mixed models for repeated data. The effect of treatments on ovulation rate was analyzed by chi-square test. Differences in the relative abundance of mRNA transcripts were analyzed by one-way ANOVA. At 72 h after intrafollicular injection, there was a decrease in follicular diameter in BHBA group (7.7 ± 1.6 mm) compared to the control (11.5 ± 0.6 mm, P = 0.02). Furthermore, follicle growth rate was reduced post-treatment with BHBA in comparison to the control group (P < 0.03). There was no difference between groups in ovulation rate, however, the diameter of the follicles that ovulated were 12.2 ± 0.46 mm and 10.4 ± 0.30 mm for the control and BHBA groups, respectively (P < 0.01). However, the BHBA intrafollicular injection in follicles with ≥ 12 mm did not affect the relative abundance of genes involved in the ovulatory cascade (ADAM 17, AREG, EREG, PTGS2) and steroidogenesis (CYP19A1, 3BHSD, STAR) between groups. In conclusion, the increase in intrafollicular concentrations of BHBA affects follicular growth but it does not seem to compromise the ovulatory cascade in bovine granulosa cells.

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Folliculogenesis, oogenesis and superovulation

Inhibition of the autophagic pathway during maturation of bovine oocytes exposed to hyperglycemia

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Resumo

Diabetes mellitus (DM) is a metabolic disease that affects about 6% of the population worldwide. Hyperglycemia induced during DM causes critical toxicity to the reproductive system reducing fertility. Oocytes are more prone to metabolic imbalances or environmental damage during maturation, hence oocytes are excellent models to investigate the effects of hyperglycemia. It has already been shown that glucose concentrations above ~10 mM during in vitro maturation (IVM) increased oocyte reactive oxygen species (ROS) and activated apoptosis. Moreover, increased ROS production has been shown to induce autophagy, a lysosomal-mediated recycling mechanism that acts as a survival strategy under stress conditions. However, little is known about the deleterious effects of autophagy inhibition in germ cells exposed to hyperglycemia. Thus, this project used the bovine IVM system as a model to determine the role of autophagy during maturation of oocytes exposed to hyperglycemia. Cumulus-oocyte complexes (COCs) were incubated in TCM-199 containing 0 or 10 mM of the autophagy inhibitor 3-methyladenine (3-MA) under 5.5 (control group) or 20 mM (hyperglycemic group) of glucose for 21 hours of IVM. The concentration of 3-MA used was defined according to previous studies of our laboratory (Latorraca, et al., Sci Rep. 2020, 10:13711). For the first experiment, COCs matured under the conditions described above were denuded, subjected to the TUNEL assay and counterstained with 5 µg/mL Hoechst 33342 to determine the percentage of apoptotic oocytes and meiotic progression to metaphase II (MII), respectively. Hyperglycemia and inhibition of autophagy did not affect the percentage of apoptotic cells. However, the presence of 3-MA during IVM reduced the percentage of MII oocytes in the groups with 5.5 (P= 0,0312) and 20 mM (P= 0,01410) of glucose. In the second series of experiment, denuded oocytes were incubated in 5mM Cell ROX Green to determine the amount of reactive oxygen species (ROS). Oocyte ROS concentration increased in the group hyperglycemic without 3-MA when compared to the hyperglycemic supplemented with 3-MA (P= 0,047) and control groups (P= 0,0265). These results indicated that oocytes were negatively affected by hyperglycemia and the inhibition of autophagy leading to increased oxidative stress and reduced nuclear maturation. It is plausible that these cellular damages compromise the oocyte developmental competence.

Keywords: Female gamete; Hyperglycemia; Autophagy; Cell death; Oxidative stress.

Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Folliculogenesis, oogenesis and superovulation

EFFECT OF PREPUBERAL HEAT STRESS ON MICE EMBRYO PRODUCTION

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Resumo

It has been widely demonstrated that heat stress triggers a series of cellular and molecular changes in the mammalian oocytes reducing oocyte developmental competence. However, little is known about the effects of heat stress in prepubertal animals. Thus, the objective of this experiment was to determine the effect of heat stress during the oocyte growth phase of prepubertal females on subsequent in vivo and in vitro embryo production. Swiss mice were mated for production of offspring. At postnatal day 10 of development (P10), offspring with lactating females were placed in environmental chambers under control (21°C/24h) or heat stress (35°C/12h during the light period and 21°C/12h during the dark period) for 11 days. All animals were weighed before (P9) and after (P21) exposure to treatments, followed by weekly weighing until P35. Upon puberty, females from the litter were subjected to hormonal stimulation. For experiment 1, females (control: N = 6; heat stress: N = 12) were superovulated with 10 I.U. of eCG and 10 I.U. of hCG within a 48-hour interval. Females were euthanized 14-15h after hCG administration to collect mature oocytes. Oocytes were submitted to parthenogenetic activation with strontium chloride and cytochalasin B to assess cleavage rates and embryonic development (morula and blastocyst) at 12 or 96h post-activation, respectively. For experiment 2, females (control: N = 14; heat stress: N = 19) were superovulated with 5 I.U. of eCG and hCG for in vivo embryo production. Females were allocated with fertile males and euthanized 94h after hCG administration for embryo collection and morphological classification according to the stage of development. Data were subjected to ANOVA using SAS. Heat stress reduced body mass of lactating females at P21 (P=0.0053) and body mass variation (Δ body mass = final mass – initial mass; P= 0.0011) in relation to control. There was no effect of heat stress on the body mass of offspring females. Exposure of prepubertal females to heat stress during oocyte growth phase did not affect cleavage rate after parthenogenetic activation. However, heat stress reduced embryonic development (morula and blastocyst) from 23.74 ± 4.12% in the control group to 0% in the stressed group (P=0.0003). There was no effect of heat stress on developmental competency and viability of in vivo produced embryos. Therefore, exposure of prepubertal females to moderate heat stress reduced oocyte developmental competence for oocytes that were activated in vitro, but not for oocytes in vivo fertilized, suggesting that the paternal genome and/or the reproductive tract environment rescued the deleterious effects of heat stress during the oocyte growth phase in mice.

Keywords: oocyte competence, oocyte growth, prepuberal, in vivo embryo, in vitro embryo