Animal Reproduction

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

Supporting biotechnologies Cryopreservation and cryobiology, diagnostic imaging, molecular biology and "omics"

# In vitro effect of C-type natriuretic peptide supplementation on the cryotolerance of bovine blastocysts

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## Resumo

Modulations of cAMP and cGMP concentrations have already been associated with an increase or decrease in lipid content in bovine oocytes and embryos. Thus, the use of C-type natriuretic peptide (CNP) - a modulator of intracellular concentrations of cAMP and cGMP - can be used to modulate the lipid profile of embryonic cells in the in vitro culture (IVC) of bovine embryos. This study aimed to evaluate the effect of the addition of CNP during the IVC of bovine embryos (Bos taurus indicus), on the blastocyst rate and the re-expansion of blastocyst after cryopreservation with OPS (Open Pulled Straw). Two concentrations of CNP were used (100 nM - CNP-100 group and 400 nM - CNP-400 group) besides fresh Control and cryopreservation Control groups, in the IVC with high oxygen tension (20%). Eight replicates were performed with approximately 1,000 presumptive zygotes/group. On days 7 and 8, only expanded and grade I blastocysts (according to the IETS criterium) were submitted to vitrification by the OPS technique, with a total of 142 vitrified. Data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk test. Normally distributed data were submitted to analysis of variance by one-way. (significance was considered when P≤0.05). On day 7, the blastocyst rate for fresh Control, cryopreservation Control, CNP-100, and CNP-400 groups were, respectively, 32.0 ± 2.1; 26.1±7.8; 24.8±7.6 and, 31.5 ±10.7 (mean± standard deviation of percentage). The re-expansion rate at 12 hours after warming was 51.2% ± 1.6; 50.0% ± 1.8 and 45.4% ± 2.1, respectively for cryopreservation Control, CNP-100 and CNP-400. Respectively, the hatching rate was evaluated at the following times: 12 hours [9.5 ± 0.4; 4.3 ± 0.3 and, 4 ± 0.3], 24 hours [39.1 ± 0.7; 39.1 ± 0.9 and, 32.0 ± 0.9] and, 48 hours after warming [28.5 ± 0.7; 17.3 ± 1.0 and, 40.0 ± 1.1]. Finally, there was no difference in the total hatching [76.1% ± 0.9; 60.8% ± 1.9 and, 76.0% ± 1.6, respectively (P≤0.05)]. The results indicate that the use of CNP in IVC was not able to change the embryonic response to the cryopreservation technique when re-expansion and hatching were the endpoints evaluated. Although, other studies from our research group suggest that the use of CNP changes the lipid content, however, more studies are needed to better investigate molecular changes.

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Animal Reproduction

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

Supporting biotechnologies Cryopreservation and cryobiology, diagnostic imaging, molecular biology and "omics"

# Phenazine ethosulfate added to maturation medium does not reduce lipid concentration in blastocyst stage embryos

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## Resumo

The objective was to determine the effect of different doses of the phenazine ethosulfate (PES) added to the in vitro maturation medium (IVM) on lipid concentrations (triglycerides, phospholipids, and cholesterol) in oocytes, and embryos from slaughterhouse cows. Oocytes (n =2,232) grades 1, and 2 were randomly submitted to IVM with different concentrations of PES: 0 µM (Control); 0.16 µM (PES0.16); 0.4 µM (PES0.4); 1.0 µM (PES1); and 2.5 µM (PES2.5). Matured oocytes were fertilized, and cultivated in vitro using commercial media (Botupharma, Botucatu, SP). Sub-samples of oocytes (n =171), and blastocysts (n =180) were randomly selected for fluorescence optical microscope analysis – fixed in 4% paraformaldehyde, and stained with Nile Red (NR; Sigma-Aldrich) – measured in arbitrary units (a.u.). Lipid content was obtained by fluorescence using ImajeJ. Data on mean fluorescence intensity of oocytes and embryos were submitted to distribution analysis and their distribution was verified as Poisson. For oocytes, the treatment effect was analyzed using the generalized linear models procedure and the means compared using the Student method. The PES 2.5 µM dose was toxic and did not yield any blastocyst. The lipid concentration in oocytes was lower (P <0.01) in the treated groups in comparison to the Control. In embryos, the triglycerides concentrations of PES1 (494,2 a.u.) and PES0.4 (552,6 a.u.) did not differ (P >0.05) from control during culture, and was higher (P =0.01) in PES0.16 (610,8 a.u.) compared to the Control (463,2 a.u.). The phospholipid and cholesterol concentrations (combined) of PES0.4 (746,2 a.u.), and PES0.16 (926,1 a.u.) were higher (P <0.01) than in the Control (674,0 a.u.), and PES1 (713,4 a.u.). Triglycerides from the treated groups were more abundant (>494,2 a.u) than in the Control (463,2 a.u.; P =0.03). In the PES0.16 and PES0.4 groups, triglycerides were 1.4 and 1.1 times, respectively, more abundant than for PES1 (P =0.05). The concentrations of phospholipids and cholesterol were higher in the treated groups (>713,4 a.u.; P =0.01) than in Control (674,0 a.u.), and PES0.4 embryos had lower concentrations (746,2 a.u.) of phospholipids and cholesterol compared to PES0.16 (926,1 a.u.; P <0,001). Surprisingly, the concentration of triglycerides in control embryos was lower when compared to the groups treated with PES (P =0.03). The treatments PES0,16, and PES0.4 resulted in embryos with higher (P =0.05) lipid concentration than PES1 (610,8 a.u., 552,6 a.u., and 494,2 a.u., respectively). The use of PES in this study reduced the lipid concentration in oocytes. This effect and the metabolic change induced by it were not sustained when evalueted in the blastocists stage. A compensatory effect of PES was observed, characterized by an increase in lipids after in vitro culture. The PES in IVM reduced lipids in oocytes but was not able to reduce it in the embryos under the current conditions.

Animal Reproduction

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

Supporting biotechnologies Cryopreservation and cryobiology, diagnostic imaging, molecular biology and "omics"

# Metabolic screening of inner cell mass of bovine blastocysts correlates with its epigenetic and molecular status

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## Resumo

The metabolic dynamics of the preimplantation embryo is a consistent matter when focusing on its viability and adequate developmental status. Nevertheless, recently, it has been intelligible the relationship between the metabolism and the transcriptional profile of the embryo as well as the mechanisms that build up this scenery, such as chromatin availability and the distribution of epigenetic marks. We demonstrate this connection by matching the metabolic screening of inner cell mass (ICM) of bovine blastocysts by Raman spectroscopy (Bruker), with their epigenetic and molecular status. Bovine embryos were cultured into 3 experimental groups according to culture medium [synthetic oviductal fluid with amino acids (SOFaa) + 4% bovine serum albumin]: Control (no additional supplementation), sodium dichloroacetate (DCA; 2 mM; acetyl-CoA conversion stimulator) and sodium iodoacetate (IA; 2 µM; glycolysis inhibitor). Blastocysts were collected on Day 7 and their ICM analyzed for mitochondrial membrane potential (4 blastocysts/rep./group; 4 rep. - fluorescence), H3K9 and H3K27 acetylation and H3K27 trimethylation [Nucleus=experimental unit (min. 100, max. 360/ antibody/group) – immunostaining] and transcriptional profiling by RNA sequencing [3 ICM/group/rep. (3 rep. – Illumina RNA-Seq)]. Data were submitted to normality test and treatment groups were compared to control using t-test or Mann–Whitney test for non-parametric data (mean ± s.e.) considering $P < 0.05$. RNA-Seq data were analyzed by DESEq 2 and transcripts with Padj <0.05 were submitted to gene ontology by DAVID. ICM metabolomics showed DCA group with lower levels of fructose-6-P, phosphoenolpyruvate and an unexpected decrease in acetyl-CoA levels, suggesting a higher influx to the pentose phosphate pathway, which may represent an adaptive response to DCA. The lower levels of acetyl-CoA in blastocysts were followed by lower mitochondrial membrane potential. Still in DCA group, higher levels for H3K27ac were found together with lower levels of H3K9ac, suggesting that the presence of acetyl-CoA may be decisive for H3K9ac. IA-derived blastocysts also presented lower acetyl-CoA levels when compared to control, as expected by the metabolic impairment proposed on the glycolytic pathway. Raman spectroscopy indicated increase in fatty acids, suggesting an attempt of the embryo to maintain the energy production by mobilizing fatty acids through beta-oxidation. Furthermore, blastocysts from the IA group, showed lower levels of H3K27ac together with higher levels of H3K27me3, indicating the competitive profile regarding these two modifications. Transcriptome analysis indicates that those metabolic and epigenetic alterations resulted in molecular differences mainly associated to metabolic processes, establishment of epigenetic marks, control of gene expression and cell cycle, outlining the complex and close relationship composing the metaboloepigenetics boundaries in the preimplantation bovine blastocyst.

Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)
Supporting biotechnologies Cryopreservation and cryobiology, diagnostic imaging, molecular biology and "omics"

# COMPARISON BETWEEN GENETIC SEQUENCING METHODS (ILLUMINA X PACBIO) IN THE INVESTIGATION OF THE VAGINAL MICROBIOTA OF DAIRY COWS AND BACTERIAL IDENTIFICATION AT THE SPECIES LEVEL

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## Resumo

The aim of this work is to characterize the vaginal microbiota of dairy cows by sequencing long DNA reads (PacBio sequencing) and comparing the data with the short reads technique (Illumina sequencing). To that end, vaginal swabs were collected from Holstein Black and White cows (HPB, n = 13), multiparous females (5 ± 1.3 years). Next-generation sequencing (SNG) of the V4 region of the 16S rRNA gene by the Illumina and PacBio platform. The sequence data was processed in the Mothur software (Illumina) and the DADA2 and SBanalyzer 2.4 software (PacBio). The statistical analysis were performed comparing the average of the phyla found in the different platforms (PacBio and Illumina) and the correlation between the indices (Chao, Simpson, Shannon) using the Minitab 18 software ($p \leq 0.05$). We obtained 366,509 readings of the vaginal microbiota from the PacBio platform, consisting of 27 phyla, 677 genera and 677 species identified. Five species represented about 51% of the vaginal microbiota, namely UCG-005_unclassified, UCG-010_ge_unclassified, Ureaplasma unclassified, Rikenellaceae_RC9_gut_group_unclassified and Bacteroides_unclassified. On the other hand, the Illumina platform identified 28 phyla and 662 genera, with 631,586 readings. The heterogeneity of community composition among individuals was confirmed. When comparing the averages of the two technologies for the main phyla, only one showed a great variation ($p<0.0001$), a set of bacteria without identification called 'unclassified bacteria', which was observed in greater quantity in the Illumina. It was not possible to directly compare the genera sequenced by PacBio with Illumina since the most abundant bacteria did not show similarities. The alpha diversity showed a strong and significant correlation ($r=0.758$, $p=0.003$) between the number of species and genera among the different techniques. Furthermore, when comparing PacBio and Illumina, sequencing long reads of the 16S rRNA gene provided Results similar and even more accurate than standard short-read sequencing platforms.

Animal Reproduction

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

Supporting biotechnologies Cryopreservation and cryobiology, diagnostic imaging, molecular biology and "omics"

# Characterization of histone lysine β-hydroxybutyrylation in bovine tissues, cells, and cumulus-oocyte complexes.

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## Resumo

In addition to their canonical roles as energy sources, short-chain fatty acids act as metabolic regulators of gene expression through histone posttranslational modifications. Ketone body β-hydroxybutyrate (BHB) causes a novel type of epigenetic modification, histone lysine β-hydroxybutyrylation (Kbhb), which is associated with genes upregulated in starvation-responsive metabolic pathways. Dairy cows increase BHB in early lactation, and the effects of this increase on cellular epigenomes are unknown. On this basis, there seems to be an intriguing and complex connection between bovine metabolism and epigenetics that can be further understood by studying Kbhb biology. To achieve this goal, we carried out a series of experiments aimed at (1) determining whether histone lysine β-hydroxybutyrylation (Kbhb) is physiologically present in several tissues/organs in dairy cows; (2) examining whether supplementation with BHB in vitro increases Kbhb in a dose-dependent manner in bovine and human fibroblast cultures; (3) exposing cumulus-oocyte complexes (COCs) during in vitro maturation (IVM) to investigate whether BHB affects Kbhb levels in cumulus cells and oocytes; (4) determining whether the exposure of COCs to different concentrations of BHB during IVM alters the oocyte's ability to complete meiotic maturation and develop to the blastocyst stage following parthenogenetic activation or in vitro fertilization (IVF); and (5) characterizing the alterations in cumulus cell gene expression patterns (RNA-seq) in COCs exposed to ketone body β-hydroxybutyrate during IVM. As a result, we identified that Kbhb is present in bovine tissues in vivo and confirmed that this epigenetic mark is responsive to BHB in bovine and human fibroblasts cultured in vitro in a dose-dependent manner. Maturation of cumulus-oocyte complexes with high concentrations of BHB did not affect the competence to complete meiotic maturation or to develop until the blastocyst stage. IVF blastocysts derived from oocytes treated with BHB during IVM increased the expression of NANOG compared with the control group. BHB treatment strongly induced H3K9bhb in cumulus cells, but faintly in oocytes. RNA-seq analysis in cumulus cells indicated that BHB treatment altered the expression of 345 genes. The downregulated genes were mainly involved in glycolysis and ribosome assembly pathways, while the upregulated genes were involved in mitochondrial metabolism and oocyte development. Our data showed that BHB is a strong epigenetic modifier in bovines. The full consequences of the transcriptional alterations on the pregnancy establishment and fetal development need to be investigated. The genes and pathways altered by BHB will provide entry points to carry out functional experiments aiming to mitigate metabolic disorders and improve fertility in cattle. Grant Numbers: Research was supported by São Paulo Research Foundation (FAPESP) grant #2016/13416-9 and #2018/09552-0 (JRS); #2013/08135-2 (FVM).

Animal Reproduction

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

Supporting biotechnologies Cryopreservation and cryobiology, diagnostic imaging, molecular biology and "omics"

# The effect of resveratrol during immature oocyte vitrification on the mitochondrial activity in feline

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## Resumo

The use of a domestic cat as an experimental model for wild felids has provided advances in the investigation of reproductive physiology and gamete cryopreservation. One of the main challenges of oocyte vitrification is to reduce the cooling damage, such as mitochondrial dysfunction, caused by low mitochondrial membrane potential. This study aimed to evaluate the effect of resveratrol, as an antioxidant, and to identify the best moment for its exposure during vitrification of immature feline oocytes on mitochondrial activity. For this, oocytes were recovered from feline ovaries obtained in elective ovariosalpingohisterectomy surgeries. Oocytes presenting homogeneous cytoplasm and surrounded by, at least one layer of cumulus cells, were selected and vitrified in eight replicates, according to the groups: control (CONT), vitrified oocytes without resveratrol exposure; pre-exposure (PRE), oocytes subjected to resveratrol exposure before vitrification; or post-exposure (POST), oocytes subjected to resveratrol exposure after vitrification. Exposure was carried out for 90 min in TCM 199 supplemented with 1 mmol/L of pyruvate, 4 mg/mL of BSA, 100 uL/mL of penicillin-streptomycin, and 1 uM of resveratrol, at 38.5 °C, 5% CO2 in atmospheric air and maximum humidity. Oocytes from CONT remained for the same time and medium (without resveratrol) after warming. In addition, two experimental groups containing fresh oocytes were also assessed: exposed (FRESH-R) or not (FRESH) to resveratrol. After vitrification, oocytes were warmed, denuded, and incubated with 0.5 nM Mitotracker Green to assess mitochondrial activity. During the evaluation under the fluorescence microscope, pictures of each oocyte were taken and the fluorescence intensity was measured using the ZEN 3.5 Blue Edition software (Carl Zeiss Microscopy, Jena, Germany). The mitochondrial activity was obtained from the ratio of the fluorescence intensity and the total area in each oocyte. The data obtained were normalized and submitted to ANOVA. The SNK test was used for comparison among groups, at a significance level of 5%. Both vitrified (n=62) and fresh (n=39) oocytes were evaluated. The CONT showed higher ($P<0.05$) mitochondrial activity (0.537a) when compared to the other groups. Regarding the vitrified groups treated with resveratrol, there was no difference ($P>0.05$) between PRE (0.369b) and POST (0.300b,c). The groups FRESH-R (0.192c,d) and FRESH (0.180d) did not differ ($P>0.05$), as well as, POST and FRESH-R. In conclusion, resveratrol reduced the mitochondrial activity of vitrified feline immature oocytes when compared to the control group. However, it did not affect the mitochondrial activity of fresh oocytes, suggesting that appropriate culture conditions lead to lower mitochondrial activity.

**Keywords:** Cryopreservation, mitochondria, antioxidant

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Animal Reproduction

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

Supporting biotechnologies Cryopreservation and cryobiology, diagnostic imaging, molecular biology and "omics"

# Assessment of seminal cell-free DNA as a potential marker for bovine sperm cryoresistance

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## Resumo

Sperm cryopreservation is a great impact technique used in the fertility preservation and animal production. Although it is well established in some species, the response to cryopreservation is varies depending on individual. Thus, the identification of a marker that can indicate whether a semen sample is of high or low freezeability, prior to freezing, would be of great value to the semen industry. Them, searching for a new assessment that can predict the post-thawed sperm quality, this study evaluated the quantity of cell-free DNA (cfDNA) and mtDNA copy number in seminal plasma of Nelore bulls. Semen from nine bulls (1/bull) was collected by electroejaculation, half of the ejaculate was used for fresh semen evaluation and molecular analyses, the other half was cryopreserved. Evaluation of movement parameters by CASA (IVOS 12.3/Hamilton-Thorne, EUA) and of plasm membrane integrity (PMI), acrosome integrity (AI), plasm membrane stability (PMS) and apoptosis (Apo) by flow cytometry (AMNIS FlowSight - Amnis Corp., EUA) were performed in fresh semen and frozen/thawed semen at 0, 3, 6 and 12h post thawing. Seminal plasma was used for cfDNA isolation and quantification. The mtDNA copy number was quantified by qPCR. The data were analyzed by ANOVA and Tukey test. The concentration of cfDNA present in seminal plasma ranged from 15.23 ng/µL to 519.71 ng/µL. The median was calculated (58.95), and two groups were defined according to the cfDNA concentration: low-cfDNA (<58.95 ng/µL (n=5) and high-cfDNA (>58.95 ng/µL (n=4). All parameteres were compare between the two groups. The cfDNA average was 38.09±53.49 for low-cfDNA and 273.70±59.80 for high-cfDNA group (P=0.02). The mtDNA copy number was similar (P>0.05) between groups (low=9.14±3.42; high=7.91±3.82). When fresh semen was evaluated, the percentage of cells with membrane stability was higher (P<0.05) on low (84.24%) compared to high (52.72%) group. The effect of group according to cfDNA concentration was evaluated over time on thawed semen. Analysis was performed as repeated measure whitin individual, using the SAS PROC GLIMMIX. Model tested group fixed effect (lowcfDNA vs high cfDNA), time after thawing (0, 3, 6 and 12 h) and the group and time interaction. The results showed no differences for all parameters evaluated over 12 hours between the low-cfDNA and high-cfDNA groups. In conclusion, cfDNA in seminal plasm may have some implication on sperm quality, but after freezing process this effect disappears, therefore cfDNA in plasm cannot predict sperm freezability.

Animal Reproduction

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

Supporting biotechnologies Cryopreservation and cryobiology, diagnostic imaging, molecular biology and "omics"

# EVALUATION IN THE CASA SYSTEM OF CRYOPRESERVED BUFFALO SEMEN IN FARM FOR FTAI

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## Resumo

The aim of this study was to evaluate the motility and sperm membrane integrity of cryopreserved buffalo semen on the farm for use in FTAI. The property is located in Oliveira/MG, Brazil Latitude 20°41'45" South and Longitude 44°49'37" West. Thirteen Murrah and Mediterranean breeders were used, with ages ranging from 42 to 124 months, with 1 post-thawed straw being evaluated for each bull, totaling 13 straws. The ejaculates were collected by artificial vagina, diluted and frozen in a Botu-Bov® extender, packaged in 0.5 mL straws, at a total concentration of 50 million SPTZ/mL and processed for stabilization and freezing (Almeida, J., UFMG, Thesis, 2018, 214p.). Sperm motility was evaluated by CASA (Sperm Class Analyzer - SCA® v.4.0) and sperm membrane integrity by hypoosmotic test (HOST). The STATA 12.0 Statistical Analysis Software (Statacorp, 2012) package was used to analyze the evaluations. The average results found for progressive motility ($p>0.05$) and HOST ($p>0.05$) for the 13 sires were respectively: A = 71,5±6,1a and 57,9±1,1a%; B = 75,0±1,5a and 62,1±5,1a%; C = 55,0±0,7b and 48,3±4,3c%; D = 75,6±6,2a and 63,9±5,2a%; E = 52,5±0,6c and 45,7±7,4c%; F = 59,6±1,1b and 51,5±0,5b%; G = 51,7±0,7c and 44,3±7,9c%; H = 51,3±0,6c and 46,2±9,7c%; I = 51,1±7,2c and 52,8±3,6b%; J = 63,0±4,2b and 59,2±1,3a%; K = 48,9±7,6c and 46,0±5,8c%; L = 66,0±5,7a and 62,7±6,6a%; and M = 51,7±5,5c and 49,0±5,4b%. The results indicate that even with lower resistance or tolerance to freezing of buffalo semen, when compared to bovine species, they have presented satisfactory results after adequate conditioning of the breeders, even when freezing is carried out on the property. As only one straw per bull was thawed, it is not possible to carry out more detailed analyzes of the results obtained. In experiments conducted in the last 7 years, even though there is no indication in the manufacturer's package insert for the use of the Botu-Bov® extender for the specie, good results have been obtained for refrigeration and cryopreservation of buffalo semen. It is concluded that it is feasible to obtain frozen semen from buffaloes on farms for use in the FTAI with quality, which meets the technical recommendations (CBRA 2013), especially at a time when there is a shortage of semen in the national market and with few centers having buffalo breeders in a collection and freezing system.

**Keywords:** Breeders, cryopreservation, semen quality.

Animal Reproduction

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

Supporting biotechnologies Cryopreservation and cryobiology, diagnostic imaging, molecular biology and "omics"

# The influence of ovarian follicular niche on oocyte development

João Vitor Puttini Paixão [1],
Gabriella Mamede Andrade [1], Juliano Coelho Da Silveira [1], Ricardo Perecin Nociti [1], Dewison Ricardo Ambrizi [1], Amanda Nespolo Silva [2], Valério Valdetar Marques Portela Júnior [3], Gustavo Zamberlam [4,3], Paulo Bayard Dias Gonçalves [3], Flávio Vieira Meirelles [1,2]

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## Resumo

The follicular environment is essential for the development of a gamete. Extracellular vesicles (EVs) present in the follicular fluid (FF) act in intercellular communication, carrying bioactive contents, such as miRNAs and mRNA, contributing to the oocyte maturation. The first aim of this work was to evaluate through RNAseq the RNA contents of small EVs from the FF and its relation with the oocyte competence to reach the blastocyst stage. Therefore, the small EVs from the FF were collected from 3 to 6 mm diameter follicles using a retrospective model previously described. To track its competence, FF and oocyte were individually recovered from follicle dissection of slaughterhouses ovaries collected without selection. Then, were matured, parthenogenetically activated and cultured individually for 7 days. Oocytes and their respective follicular fluids were classified and separated in three groups, accordingly to developmental competence: IM Group (Incompetent Mature): Oocytes matured in vitro but did not cleave after parthenogenetic activation; IC Group (Incompetent Cleaved): Parthenotes cleaved after parthenogenetic activation, but blocked the development at 3rd or 4th cell cycle; BL Group (Blastocysts): Oocytes which developed to the blastocyst stage. Differential gene expression, exclusive and HUB genes analyses of the transcriptomic data were performed, and transcriptional alterations among the more competent (BL) and less competent (IC and IM) groups ($q<0.1$) were observed. Interestingly, several gene transcripts found significantly altered among these groups were related to signaling pathways associated with cell proliferation and meiosis modulation such as WNT, PI3K-Akt and Hippo signaling pathways. Due to its key function in all pathways, GSK3 (Glycogen Synthase Kinase 3) was selected as a candidate gene potentially regulating these signaling pathways and the oocyte competence. To test this, we matured the oocytes for 8 hours in maturation medium depleted of FSH/LH under 3 conditions: 1) CTL (control); 2) DKK1 treatment that indirectly stimulates GSK3B through DKK-1 (Dickkopf related protein 1) inhibition of the WNT signaling pathway; 3) CHIR treatment that directly inhibits GSK3B through the chemical probe CHIR99021. After treatment, oocytes were washed and matured for 16 hours in regular maturation medium. Maturation rates were observed, then mature oocytes were parthenogenetically activated and in vitro cultured. Thus far, 6 replicates were performed, and the blastocysts rates, CTL (41±4.1%; N=264), DKK1 (47.9±4.2%; N=249) and CHIR (45.2±4.1%; N=260) were similar among the groups ($p<0.05$). The retrospective model identified crucial genes and pathways associate with oocyte competence. Initial attempts to modulate these pathways failed in enhance the oocyte competence. Currently we are testing different periods of treatment and molecularly investigating the alterations caused in the oocyte following GSK3 modulation.

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# Metaboloepigenetic modulation the during early development promotes alterations in chromatin accessibility and transcription in bovine embryos.

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## Resumo

Embryos are interesting models to study metaboloepigenetics, since they undergo broad metabolic changes and widespread epigenetic remodeling. The modulation of α-Ketoglutarate (AKG) and Succinate (SUC) ratio was capable to alter the levels of 5-methylcytosine (5mC) in embryos (Ispada, 2020). In the present work, the transcription profile on inner cell mass (ICM) of these embryos was accessed. After that, gene accessibility of the most affected transcripts was checked. For this, Bovine embryos were in vitro produced using standard protocols and cultured as control (CO) or treated from day 0 of cleavage until day 4 with analogs for AKG or SUC. Embryos were collected at day 7 and the ICMs were removed by microimmunosurgery and polled (3 ICMs per treatment for RNAseq and 15 ICMs per treatment for ATACseq) for later analysis. The RNAseq resulted in a total of 174 differently expressed genes (DEGs) between CO and AKG, with 119 being downregulated and 55 upregulated in embryos from the AKG. The comparison between CO and SUC reveled 356 DEGs in total, with 241 downregulated and 115 upregulated in embryos from the SUC group. SUC and AKG groups had the greatest differences in DEGs number, with 274 being upregulated in AKG and 263 upregulated in SUC embryos. The 10 DEGs with lowest adjusted p-values between comparisons were selected, with NEB and TNRC18 upregulated and MAP3K4, EIF2B5, FER, CLPB, RPF2, PTK2, KIAA1217 and KMT2B downregulated in AKG, when compared to CO. For SUC, FAT1 and ACAT2 upregulated and AHNAK, ATXN2L, RESTB, SSR2, PEG3, FLNA, CEP68 and MED16 downregulated in relation to the CO. The comparison between AKG and SUC resulted in TBL1XR1, SCHIP1, MELK, RNF112, WIPI1, PLD2 and CDC40 upregulated in SUC, while SOCS7, SMARCC2 and SPRED2 were upregulated in AKG. The chromatin accessibility revealed that 277 peaks were obtained for the CO, with CASK, SORCS3, NAA35, METTL25, LRRFIP1, ALCAM, C2H2orf76, CSMD3, ASCC1, CFAP4, CACNA2D3 as the 10 most significative ones. For AKG, the genes CSMD3, SORCS3, CASK, ALCAM, IMMT, NAA35, PKIA, METTL25, CFAP43 and CACNA2D3 were most accessible, from the total of 1564 peaks. On SUC, the total of 168 peaks were identified, with SIMC1, NAA35, METTL25, CASK, C2H2orf76, CSMD3, SORCS3, RYR2, IMMT and CACNA2D3 as the 10 most significative genes. So far, the preliminary analysis of ICM from embryos treated with AKG or SUC resulted in chromatin and transcription alterations. Considering the changes promoted by the metaboloepigenetic modulators, it is possible to assume that the perturbation/stimulation of DNA demethylation in bovine embryos promote strong effects and physiological and molecular alterations up to blastocyst stage.