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Survival of 7 day *in vitro* produced bovine embryos exposed to 0.5 M sucrose prior to slow cool freezing

M.E.B. Dode[1], C.B. Steinhauser[2], J.H. Pryor[2], T.V. Collares[1], J.C. Deschamps[1], C.R. Looney[2], D. Forrest[3]

[1]UFPEL, Centro de Desenvolvimento Tecnológico, Laboratório de Embriologia Molecular e Transgênese, Pelotas; [2]Ovagenix, Bryan; [3]Texas A&M University, Dept. of Animal Science, College Station.

Keywords: cryopreservation, direct transfer, ethylene glycol.

Embryo exposure to sucrose has been shown to reduce water within cellular structures thus lowering the incidence of ice crystal formation, which can lead to cellular damage during cryopreservation. This study evaluated the post-thaw survivability and viability of bovine in vitro produced (IVP) embryos exposed to 0.5 M sucrose solution prior to slow cool freezing. Three replicates of abattoir collected oocytes (n = 856) were matured in transit (DeSoto Biosciences Inc., Seymour, USA), fertilized (IVF), and cultured in vitro as previously described (Pryor et al, 2015, Reprod. Fert. and Dev. 28, 172). On Day 7 post-IVF, cleavage/development rates of grade 1 and 2 embryos (n = 488/241) were classified in accordance with IETS standards [compact morula (CM), early blastocyst (EB), blastocyst (BL), expanded BL (ExBL)] and randomly divided into 3 treatments: 2 min 0.5 M sucrose exposure - no freeze (S), no sucrose exposure - freeze in Vigro ethylene glycol freeze plus with sucrose (EG; Vetoquinol, Pullman, USA), and 2 min 0.5M sucrose exposure - freeze in Vigro ethylene glycol freeze plus with sucrose (SEG; Vetoquinol). Embryos were held in cryoprotectant 5-7 min before freezing in 0.25 ml straws at 0.5°C/min from –6°C to –32°C and plunging in liquid nitrogen. Frozen embryos were immediately air thawed (20-25°C) for 5 s and then immersed in 30°C water for 10 s before washing through warm (37°C) Vigro holding medium (Vetoquinol) for 15 m before being placed into culture medium for an additional 48 h (Day 9 post-IVF). Embryos advancing one stage were considered survived. Day 9 embryos were fixed in cold methanol, washed in PBS/0.1%Tween 20, mounted in 10 µg mL-1Hoechst 33342/glycerol and viewed under UV light to count cells. Data were analyzed using a one-way chi-square test comparing treatment groups overall and by stage with $P < 0.05$ as significant. There was no significant difference in survival and live cell counts at 48 h between S (72.8%; 119), EG (63.3%; 107), and SEG (51.9%; 123) for combined stages, respectively. Interestingly, survivability for EB and ExBL were significantly higher in S (85.7; 70.0%) when compared to EG (66.7; 55.6%) and SEG (58.6; 25.0%) with no difference for BL (81.3, 62.5, 68.8%), respectively. Oddly, CM stage embryos had a lower rate for S as compared to EG and SEG (47.0, 56.3 and 70.6%, respectively), but due to low numbers across treatments, statistics could not be verified. The results indicate that a 2 min exposure of embryos to sucrose prior to slow freezing in ethylene glycol may not be beneficial to embryo survival, and indeed, may even be detrimental to embryos at the EB and ExBL stage.

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Use of forskolin *in vitro* produced bovine embryos submitted to vitrification

D.M. Pereira[1], D.M. Pereira[1], M.G.C.R. Ferreira[1], M.B. Souza[2], W.A. Leite da Silva[1], C.J. Tavares Cardoso[3], H. Kischel[1], E.D.S. Arruda[1], A.C.B. de Lima[1], F.A. Melo Sterza[1]

[1]Universidade Estadual do Mato Grosso do Sul, Aquidauana; [2]Universidade Estadual de Londrina, Londrina; [3]Universidade Federal do Mato Grosso do Sul, Campo Grande.

Keywords: chemical lipolysis, cryopreservation, in vitro fertilization.

In vitro produced embryos have a larger amount of lipid droplets in their cytoplasm, which has been related to their lower cryotolerance compared to in vivo produced embryos. Forskolin is an activator of adenylatecyclase, which will activate the endogenous lipase through cAMP and protein kinase, causing lysis of intracellular triglycerides and consequently the formation of glycerol. In this sense, the aim of this study was to evaluate the effects of forskolin (Sigma-Aldrich®, Corporation, St. Louis, MO) added to the embryo culture medium on cryotolerance of in vitro produced bovine embryos. Therefore, bovine ovaries were collected at a local slaughterhouse and transported to the laboratory in 0.9% saline solution at 37°C, plus antibiotic. Follicles were aspirated and the Cumulus Oocyte Complexes (COCs) were tracked and sorted. Then, 1007 COCs were matured in vitro for 24 hours. In vitro fertilization (IVF) was performed with semen from a fertility proved Nelore Bull, previously prepared in Percoll® gradient. The IVF incubation period was 18-22 hours. The in vitro culture was held in SOF medium for 9 days. On D5 embryos were divided into two groups: control group (CG, group cultured in the absence of forskolin) and forskolin group (FG, group cultivated with 20 μM forskolin for 24 hours from D5). All stages of in vitro embryo production were performed in the incubator at 38.5°C, 5% CO2 and maximal humidity. On D7, grade I blastocysts were cryopreserved according to the protocol of Kuwayama et al. (2005). The warming was performed 1 month after embryo cryopreservation and they were cultured further for 3 to 24 hours. The variables evaluated were blastocyst rates on D7, the rate of ability to be cryopreserved embryos on D7 and re-expansion rate at 3 and 24 hours after warming. Data were submitted to analysis of variance (ANOVA) with 5% level of significance using the statistical program R. The blastocyst rate on D7 was not different ($P > 0.05$) between groups (FG 22.71% x CG 25.41%), however, the rate of ability to be cryopreserved embryos was higher in the group Forskolin (43.24% vs. 25%; $P < 0.05$).The re-expansion rate 3 hours after warming was increased in Forskolin group (45% vs 24%; $P < 0.05$), but after 24 hours in culture, this effect was no longer observed (Forskolin 55% vs. 56% control). Given this, it is suggested that the addition of 20 μM forskolin in the embryo culture medium improve quality and cryotolerance of blastocysts, reducing the time of re-expansion after warming.

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Development, cell counting and apoptosis in bovine embryos submitted to manual biopsy on day 7 after *in vitro* fertilization

C.C.R. Quintão, C.S. Oliveira, C. Freitas, E.D. de Souza, J.F. Souza, B.R.C. Alves, L.S.A. Camargo

EMBRAPA Gado de Leite, Juiz de Fora.

Keywords: biopsy, bovine embryo, IVF.

In the bovine species, the embryo biopsy technique (associated with in-vitro embryo production) is useful for genotyping as well as for the study of chromosomal alterations associated with the survival of the conceptus. In a previous study (Quintão, C.C.R. et al., Anim. Reprod., 2015), we observed that embryo biopsy performed on the sixtieth day (D6) after IVF does not affect embryo viability on D8 (when embryo viability were evaluated), however, there are no studies on the effect of biopsy on D7 blastocysts. The objective of this study is to evaluate the effect of manual biopsy, performed on D7, on development and viability of bovine embryos. Oocytes were obtained from slaughterhouse-harvested ovaries and submitted to in vitro maturation and fertilization. After the IVF, the putative zygotes were denuded and cultures in CR2aa media supplemented with 2.5% BFS at 38.5ºC with 5% CO2, 5% O2, 90% N2 and saturated humidity. At D7, embryos were distributed randomly in two groups: CON (control; n = 79) and (BIO biopsy; n = 78). The BIO embryos were manually sectioned with microblades (Bioniche, Canada) in 20 microliters drops containing TALP-HEPES added to 2% FBS under a stereoscope. Embryo from both groups were then transferred to individual 20 microliters drops of CR2aa, under the same cultured conditions cited above. On D8, the blastocoel re-expansion and embryo quality (IETS, 1998) were accessed. Further, CON (n = 18) and BIO (n = 20) blastocysts were selected randomly and fixed for counting the total number of cells (TOT) and the number of apoptotic cells (APO), according to the TUNEL technique (Promega, EUA). The apoptotic indexes were calculated by APO/TOT. Data related to embryonic development was evaluated by the Qui-square test. The values of TOT, APO and apoptotic indexes (indicated as average ± SEM) were submitted to ANOVA and the averages compared by the SNK test. Regarding embryo development, a lower proportion of blastocysts with expanded blastocoel ($P < 0.01$) was observed in BIO (67.9%) in comparison to CON (89.8%), however, the embryo quality did not differ ($P > 0.05$), given the proportions of grade 1 and 2 embryos: 66.2% (CON) and 56.6% (BIO) and grade 3 embryos: 33.8% (CON) and 43.4% (BIO). Differences were observed ($P < 0.05$) in TOT (149.9 ± 6.2 vs 112.8 ± 5.9), APO (9.6 ± 1.9 vs 16.7 ± 1.8) and in apoptotic indices (6% ± 1 vs 14% ± 1), between CON e BIO, respectively. In conclusion, the manual biopsy on D7 interferes with embryonic development in the 24-hour period after biopsy, because although not affect the embryonic morphological quality, it reduces the total number of cells and increasing the apoptotic index. Financial support: CNPQ, Fapemig and Embrapa.

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Analyze RNA-Seq data on a local galaxy server in a user-friendly, productive, and reproducible environment

J. Bick, S. Ulbrich, S. Bauersachs

Eth Zurich, Zurich.

Keywords: Galaxy platform, RNA-Seq analysis, NGS.

Since RNA-Sequencing generates huge amounts of data and more and more analysis tools are developed, it is important to find the most accurate and reliable tools with optimal settings. To face this challenge we use a local Galaxy server which provides us a productive and reproducible way to analyze huge amounts of data generated by functional genomics projects. Most of the published NGS analysis tools are command line tools running on Linux machines. For researchers with background in biology it is a big challenge to use complex tools and get used to the bioinformatics environment. Galaxy (Blankenberg et al., Current Protocols in Molecular Biology. ch 19: Unit 19.10.1-21, 2010) tools use these command line scripts and provide an easy-to-use graphical interface suitable for users without a computational background. Furthermore, Galaxy's histories keep track of each tool outcome to trace back the workflow and obtained analysis results. Galaxy is an open source web-based platform that has been developed and introduced in 2005 by Penn State University and John Hopkins University (Giardine et al., Genome Research. v.15, p.1451-5, 2005). It is designed to give easy access to research data (FastQ files, Fasta files, annotation data, large tables, etc.) and provides a bunch of starting tools for basic data analysis. To extend these basic tool packages, it is possible to install published tools from an App store (Galaxy Tool Shed - https://toolshed.g2.bx.psu.edu ~ 3700 different tools) which helps to setup tools related to a specific research area. Particularly, the field of RNA-Seq analysis has the biggest tool developer community. Since Galaxy is open source, in house tools are easy to implement and integrated into the Galaxy environment. Apart of the public Galaxy server https://usegalaxy.org, which is maintained by the Galaxy developer team, it is also possible to set up a locally installed Galaxy. This guarantees more functionality, flexibility, independency, and confidentiality if you deal with unpublished data. A basic analysis pipeline of RNA-Seq data includes quality checking, filtering, trimming, and adapter clipping followed by mapping to a reference genome or transcriptome. Finally, a read count table is generated based on a gene annotation file. Next steps could be statistical analysis with BioConductor R packages such as EdgeR or DESeq2. This pipeline can be translated into a Galaxy workflow. A workflow (analysis pipeline of tools running in a row) is a useful Galaxy feature which can be manually generated or extracted from existing histories and shared within Galaxy. Once the researcher has logged into the web interface it is possible to work in a personalized environment where basic tasks like viewing, converting or calculating datasets are easily accessible. Altogether, Galaxy is a practicable way to establish an efficient bioinformatics data analysis pipelines for large-scale data sets in a molecular biology laboratory.

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Long chain Acyl-CoA synthases modulators effect in the embryo development and cryosurvival

R.S. Valente[1], T.G. Almeida[2], M.F. Alves[2], D.R. Arnold[2], F.C. Landim-Alvarenga[3], J.H.F. Pontes[2], A.C. Basso[2], M.J. Sudano[1]

[1]Universidade Federal do Pampa, Uruguaiana; [2]In Vitro Brasil, Mogi Mirim; [3]Universidade Estadual Paulista, Botucatu.

Keywords: cryosurvival, embryo development, bovine embryo.

Long chain Acyl-CoA synthases (ACS) are a family of enzymes that activate long-chain fatty acids to generate long-chain acyl-CoA, essential substrate for the synthesis of various lipid species, including triacylglycerol, cholesterol ester, and phospholipid. The aim of the present study was to evaluate the effect of ACS modulators in the bovine embryo development and cryosurvival. Slaughterhouse ovaries were used to obtain oocytes, containing homogenous cytoplasm and more than three layers of cumulus cells, that were used for in vitro maturation (day -1) and fertilization (day 0). Presumptive zygotes were culture in aaSOF containing 2,5% of FCS. On day 5 of the culture, the medium was supplemented with the positive (ACS+) and negative (ACS-) ACS modulator. A preliminary study (1000 oocytes) was conducted to determine the better drug dosage among five concentrations (100x, 10x, 1x, 10^{-1}x e 10^{-2}x). The higher dosage that did not affect embryo development and quality at day 7 for each modulator was selected (ACS+: 10x and ACS-: 10^{-2}x). After that, 4105 oocytes were in vitro maturated, fertilized and cultured (aaSOF +2,5% FCS). On day 3, cleavage was checked and non-cleaved embryos were discarded. On day 5, embryos were randomly assigned for each treatment as follow: ACS+ (n = 579), ACS- (n = 594), association of both ACS+ and ACS- (ACS+/-; n = 581), and control (C; treated with PBS only, n = 600). At day 7, blastocyst production was recorded and expanded blastocysts grade I, II and III were vitrified (n = 293) by cryotop method. The data were analyzed by ANOVA using the PROC GLIMMIX of SAS. The total cleavage rate was 71.2 % (2921/4105). Blastocyst production was similar ($P > 0.05$) between ACS+ (24%; 199/829), ACS- (24%; 198/131), ACS+/- (23%; 190/815), and C (27%; 221/826) treatments. There was no difference ($P > 0.05$) in the re-expansion rate between treatments and blastocyst quality (ACS+: 91-100%; ACS-: 90-100%; ACS+/-: 91-95%; and C: 86-96%). The hatching rate of expanded blastocyst grade I (ACS+: 93.3%, 14/15; ACS-: 83.8%, 26/31; ACS+/-: 86.9%, 20/23; and C: 96.7%, 30/31) and grade II (ACS+: 91.3 %, 21/23; ACS-: 92.3%, 12/13; ACS+/-: 65.2%, 15/23; and C: 84.0%, 21/25) were similar ($P > 0.05$) between treatments. However, grade III expanded blastocysts derived from the ACS+ (78,2%, 18/23) had higher ($P < 0.05$) hatching rate compared with ACS- (53,5%, 15/28) and C (51.7%, 15/19), but similar ($P > 0.05$) with ACS+/- (58,6%, 17/29) treatment. Therefore, the findings of this study reveal the following: i) the use of positive and negative modulators of the ACS did not affect embryo development; ii) the negative modulation of the ACS did not improve cryosurvival; iii) the stimulation of the ACS increased post-cryopreservation survival of grade III expanded blastocysts even when associated with the negative modulator. Thus, it seems that the activation of fatty acids containing 12, 14 and 20 carbons favors cryosurvival of lower quality embryos.

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Study of JY-1 gene polymorphism and its association with *in vivo* and *in vitro* embryo in Holstein donors (*Bos taurus taurus*)

C.R.A. Silveira[1], J.C. Pierucci[1], C.A. Rodrigues[2], A. Castro Neto[3], G.M.F. de Camargo[4], D.J.A. dos Santos[1], A.C. de Freitas[1], P.S. Baruselli[5], H. Tonhati[1], L.U. Gimenes[1]

[1]FCAV - UNESP, Jaboticabal; [2]Clínica Veterinária SAMVET, São Carlos; [3]BIOEMBRYO, Bauru; [4]Universidade Tecnológica Federal do Paraná - UTFPR, Dois Vizinhos; [5]FMVZ - USP, São Paulo.

Keywords: SNP, molecular marker, dairy herd.

The JY-1 protein is oocyte-specific and has an important regulatory role in folliculogenesis, oocyte maturation and early embryonic development. The bovine gene that encodes this protein has three exons and two introns, with the coding region constituting parts of exons 2 and 3.The aim of this study was to verify the occurrence of polymorphism in the JY-1 gene and its association with the in vivo and in vitro embryo production in non-lactating Holstein donors. Retrospective data of in vivo (n = 572) and in vitro (n = 1,068) embryo production during the period of 2013 to 2015 were obtained from 144 non-lactating Holstein donors of embryos and oocytes. In order to identify JY-1 polymorphism, genomic DNA was extracted from hair follicles, and the exon 2 region was amplified and sequenced by the PCR-sequencing methods. Four SNPs were detected: two located in exon 2 (rs381676360; rs384600927) and two in intron 2 (rs378994837; rs211595914). The rs381676360 SNP characterized an amino acid not synonymous substitution (leucine / isoleucine) and rs384600927 a synonymous substitution of the amino acid valine. Regarding the Hardy-Weinberg equilibrium, one of the four genotypic frequencies was not in equilibrium (rs211595914; $P < 0.01$). Considering the linkage disequilibrium test (LD), higher r2 values (>0.33) were observed between the rs381676360, rs384600927 and rs378994837 SNPs (r2 = 0.767 to 0.973) and one SNP (rs211595914) showed lower r2 values (r2 = 0.016 to 0.025).The association of SNPs with the characteristics [number of in vivo transferable embryos (NET); in vivo transferable embryos rate (%ET); number of in vitro embryos (NE); cleavage rate (%CLIV) and in vitro embryo rate (%E)] was performed utilizing generalized linear models through the GLIMMIX procedure of statistical SAS 9.3® software. The Bonferroni correction for the number of SNPs studied was applied and those that obtained $P < 0.0125$ were considered SNPs associated. None of the SNPs alone was associated with the traits. However, when all SNPs were considered together for the characteristics association, significant values were found for NET ($P < 0.01$), NE ($P < 0.01$) and %E ($P < 0.01$). Further, the three SNPs in LD were associated with %E ($P = 0.012$). No SNP was associated with characteristic %ET. Thus, we conclude that in non-lactating Holstein cows JY-1 gene has polymorphism of the type SNP and they are associated with embryo production. The characterization of other regions of the gene needs to be emphasized in order to find new polymorphisms and new associations.

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Blastocoel collapse associated with melatonin in the culture media improves bovine embryo viability after vitrification

T.C. Marques[1], T.O. Diesel[1], C.G. da Silva[2], E.C.S. Santos[3], C.F. Martins[2], M.A.N. Dode[4], M.L. Gambarini[1]

[1]Universidade Federal de Goiás, Goiania; [2]EMBRAPA Cerrados/CTZL, Brasília; [3]National Institute Agrobiological Sciences, Tsukuba; [4]Embrapa/Cenargen, Brasília.

Keywords: cell apoptosis, antioxidant, cryotolerance.

Melatonin treatment (MEL) and blastocoel collapse (BC) have been suggested to be potential options to enhance embryo development and viability after cryopreservation of bovine embryos (Wang M et al, J Pineal Res, 56, 333-42, 2014; Wang F et al, PLoS One, 9, e93641, 2014; Min SH eta al, Cryobiology, 66, 195-9, 2013). We investigated the effect of BC performed before the vitrification of bovine embryos produced in vitro in a culture medium supplemented with MEL. Viable oocytes were in vitro matured (IVM) for 24 hours at 38.5°C at atmosphere of 5% CO2 and fertilized with conventional semen (Nellore, 1x106/mL). After 18 hours presumed zygotes were cultured without (Control) or with MEL 10-9 M (IVC+M10-9) for seven days under the same IVM conditions. On Day 3, feeding was done according to the treatment. On Day 7, half of the expanded blastocysts (Bx) Grade I and II of the IVC+M10-9 treatment was submitted to BC (IVC+M10-9 BC). In the sequence the embryos Grade I and II were vitrified (n = 618), thawed and cultured in the IVC medium for 72 hours. Re-expansion rate (RR) after 2 and 24 hours and hatching rates (HR) after 24, 48 and 72 hours were evaluated. Total number of cells (TC) and number of apoptotic cells (NAC) count was performed by TUNEL. TC and NAC presented normal distribution (Shapiro-Wilk test) and were compared by Tukey's test (ANOVA). RR e HR was performed by Chi square. MEL supplementation improved RR and HR at 24, 48 and 72 hours ($P < 0.05$), regardless the BC procedure. After 2 hs Control RR (n = 198), IVC+M10-9 (n = 215) and IVC+M10-9 BC (n = 205) were 29.3%, 86.7% and 89.3%, and after 24 hours were 78.8%, 89.7% and 87.7%, respectively. Control embryos required more time during re-culture (24 hs) for all expansion compared with MEL groups ($P < 0.05$). HR at 24 hs and 48 hs were 6.1% and 6.7%; 18.7% and 37.9%; 27.3% and 41.5%, respectively ($P > 0.05$). At 72 hs HR were 20.3%, 56.1% and 66.8% respectively for control, IVC+M10-9 and IVC+M10-9 BC ($P > 0.05$). HR was higher in IVC+M10-9 BC in all the evaluation moments ($P < 0.05$). Although Control and IVC+M10-9 showed the same HR at 24 hs ($P > 0.05$), MEL added in IVC enhances HR at 48hs and 72 hs ($P < 0.05$). The TC and NCA for fresh embryos were 135.6 ± 5.5 and 7.8 ± 0.6 (Control, n = 15) and 135.4 ± 5.6 and 3.4 ± 0.6 (IVC+M10-9, n = 15) and in the vitrified embryos were 139.8 ± 6.1 and 12.5 ± 0.6 (Control, n = 15), 136.1 ± 5.6 and 4.9 ± 0.6 (IVC+M10-9, n = 15), 140.1 ± 5.8 and 3.7 ± 0.6 (IVC+M10-9 BC, n = 15), respectively. There was no difference in the TC ($P > 0.05$). Embryos produced with MEL had similar NAC regardless of vitrification and BC ($P > 0.05$), but smaller compared with embryos cultured without MEL ($P < 0.05$). In conclusion, the addition of melatonin (10-9 M) in IVC improved embryo quality and viability, independently of BC.

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Effect of dietary supplementation of Nelore heifers with polyunsaturated fatty acids on cryotolerance and membrane lipid profile of fresh and vitrified *in vitro*-produced embryos

B.C.S. Leão[1], N.A.S. Rocha-Frigoni[1], E. Nogueira[2], E.C. Cabral[3], C.R. Ferreira[3], M.N. Eberlin[3], M.F. Accorsi[4], T.V. Neves[4], G.Z. Mingoti[4]

[1]Centro Universitário Católico Salesiano Auxilium - Unisalesiano, Araçatuba; [2]EMBRAPA Pantanal, Corumbá; [3]Laboratório Thomson de Espectrometria de Massas - UNICAMP, Campinas; [4]Laboratório de Fisiologia da Reprodução - FMVA/UNESP, Araçatuba.

Keywords: bovine, cryopreservation , mass spectrometry.

Dietary supplementation with polyunsaturated fatty acids (PUFA) for oocyte donor beef heifers can modulate the molecular mechanisms of lipid uptake in oocytes included in ovarian follicles, affecting the subsequent *in vitro* embryo development. Moreover, the success of embryo cryopreservation can be affected by membrane lipid composition, such as phosphatidylcholines (PC) and sphingomyelins (SM). The aim of this study was to evaluate the development and cryotolerance of IVP embryos from oocytes recovered from Nelore heifers (n = 16) fed with control diet (Control group) or fed with rumen-protected PUFA (Megalac-E®), for at least 60 days (Fat group). In addition, the membrane lipid profile of fresh and vitrified embryos from both treatments was evaluated using matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS). COCs (n = 491) recovered after 6 OPU sessions were IVM for 22 h. After IVF (D = 0) the zygotes were in IVC in SOF medium (5 mg/mL BSA and 2.5% FCS, 5% CO_2 in air) for 7 days. Cleavage and blastocyst (Bl) rates were evaluated in the D3 and 7, respectively. Bl were vitrified (Ingámed, Brazil) and embryo survival rates were measured at 24h after warming. Fresh and viable Bl after thawing were transferred to microtubes containing 200 µL of methanol HPLC 50% in aqueous solution, stored at -20°C and immediately transported for MS analysis. Each embryo was deposited at the center of the spots of MALDI-MS plate, under 1 µL of matrix (1.0 mol/12.5 dihydroxybenzoic acid (DHB) in methanol), at room temperature until its crystallization. Spectra were acquired in the mass range of *m/z* 700-1200, in the positive ion and reflectron modes using the Autoflex III (Bruker Daltonics, USA) mass spectrometer. The most intense ions of each spectrum were considered as starting point for determining the *m/z* ratios corresponding to membrane lipids and, only *m/z* clearly distinguished from noise in spectra were included in the partial least squares discriminant analysis (PLS-DA). The cleavage and Bl rates, and the relative abundances of the most relevant lipids that explained the variance of the data were subjected to ANOVA (GLIMMIX, SAS Institute), followed by Tukey's test. The re-expansion rates were evaluated by $_X2$ ($P < 0.05$). Data are presented as LSM ± SEM. The diet did not affected ($P > 0.05$) cleavage rate (average 65.0 ± 3.1%), Bl yield (average 43.2 ± 3.7%) and embryo survival after warming (average 79.3%). PUFA supplementation increased ($P < 0.05$) the relative abundance of only one lipid specie, assigned as PC ether (PCe) 38:2. Cryopreservation affected ($P < 0.05$) the relative abundance of 10 ions: PC 32:0, PC 34:1, SM 24:1, PC 40:6 or PC 42:9, PC plasmalogen (PCp) 44:10 or PC 42:7, triacylglycerol (TAG) 54:9 and a not assigned ion (*m/z* 833.2) were decreased ($P < 0.05$) in Bl that survived the vitrification process, compared with fresh Bl. However, the abundance of the ions PC 36:3 or PC 34:0, PCe 38:2 or PC 36:6 and PC 36:5 or PCe 38:1 were increased ($P < 0.05$) after vitrification. The results demonstrate that the mass spectrometry profiles of PC, SM and TAG species determined by MS differed significantly in fresh and vitrified–warmed bovine Bl. Due to the differences between the ions abundances, they can be used as potential markers of post cryopreservation embryonic survival.

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Cryotolerance and pregnancy rates after exposure of bovine *in vitro*-produced embryos to forskolin and linoleic acid before vitrification

M. Meneghel[1], P. Chediek Dall Acqua[1], M. Ambrogi[1], L.M. Gouvêa[2], M.A. Achilles[3], B.C.S. Leão[1], E. Nogueira[4], N.A.S. Rocha-Frigoni[1], G.Z. Mingoti[1]

[1]Universidade Estadual Paulista Júlio de Mesquita, Jaboticabal; [2]Concepção Reprodução Bovina, Araçatuba; [3]Achilles Genetics - Biotecnologia Em Reprodução Animal, Garça; [4]EMBRAPA Pantanal, Corumbá.

Keywords: Linoleic acid, forskolin, cryopreservation.

The objective of the present study was to evaluate the effects of supplementation of *in vitro* culture (IVC) medium with drugs that stimulates the lipolysis (Forskolin: Forsk) and inhibit the lipogenesis (Linoleic Acid LA) on the intracytoplasmic lipid content and cryotolerance of bovine embryos (Experiment 1), as well as to evaluate the effect of treatment of embryos with Forsk on the pregnancy rates after transfer to synchronized recipients (Experiment 2). In the experimente 1, the oocytes (n = 1242) were matured in vitro for 22h at 38.5°C and 5% CO2 in air in medium TCM199 with 10% FCS and hormones. After fertilization, presumptive zygotes were cultured in SOF medium (Control group) supplemented with: 100 μM LA throughout the culture period (LA group); or 5 μM Forsk from the 6th day to the end of the culture (Forsk group); or with the association of LA and Forsk, as described above (LA+Forsk group). The IVC was conducted at 38.5°C and 5% CO2 in air, for 7 days. Embryonic development was assessed on day 7 of culture (D7), when blastocysts were stained with 1% Sudan Black B to determine the intracytoplasmic lipid content. The embryos were evaluated by light microscopy and the images were analyzed by Q-Capture Pro Image software. The Control group was chosen as a calibrator and the measured value of each treatment was divided by the mean of the calibrator to generate the relative expression levels of pixels, expressed in arbitrary units. The expanded blastocysts were vitrified (Vitri Ingá®), warmed and cultured for 24h in SOF to evaluate the re-expansion rates. The data were analyzed by ANOVA followed by Tukey's test and rates of re-expansion by qui-square ($P < 0.05$). No differences were observed between treatments ($P > 0.05$) in blastocysts production rates (47.2 ± 3.9% to 51.6 ± 5.5%). Intracytoplasmic lipid content was decrease ($P < 0.05$) in embryos from Forsk group (0.86 ± 0.04) compared to Control (0.99 ± 0.02) and LA (1.02 ± 0.02); however, all these treatments were similar ($P > 0.05$) to LA+Forsk group (0.95 ± 0.03). There were no differences ($P > 0.05$) between treatments in the rates of re-expansion immediately after warming (0h: 30.8% to 41.9%); after 24h of culture post-warming the re-expansion rates were higher ($P < 0.05$) in Forsk group (71.4%) compared with Control (46.2%) and LA+Forsk (45.2%) groups, but there were no diferences between LA group (65,1%) and the other groups ($P > 0.05$). Based on these results, treatment with 5 μM Forsk was chosen for the Experiment 2: oocytes (n = 1947) were aspirated from 22 Nelore donnors and were matured and fertilized in vitro as described above. Presumptive zygotes were cultured according to the treatments Control and Forsk as described above, and in D7 the expanded blastocysts were vitrified and warmed, before being transfered to synchronized recipients. The data of pregnancy rates were analyzed using the command PROC GLIMMIX (SAS Inst. Inc.). The pregnancy rates after transfer of vitrified-warmed embryos were similar ($P > 0.05$) between Control (15.1 ± 3.6%) and Forsk (19.7 ± 4.0%) groups. According to the results, treatment with Forsk was effective to promote the reduction of intracytoplasmic lipids content in *in vitro*-produced bovine embryos and improves their cryotolerance. Nevertheless, it did not increase the pregnance rates.

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Evaluation of buffaloes cryopreserved in different thinners with use of fluorescent probes

A.S. Ramos[1], N.C. Barreto[1], S.T. Rolim Filho[1], H.F.L. Ribeiro[2], E.B. Araujo[1], O.M. Ohashi[1], H.D.M. Ayala[2], A.X. Santos[2], A.A. Gonçalves[2], V.C. Mileo[1]

[1]Ufra, Belem; [2]UFPA, Belem.

Keywords: buffalo, fluorescence microscope, resistance thermometer test.

The objective of this work was to compare the effect of thinners, Citrate (73mL of Sodium Citrate, 1g of D-fructose), Lactose (73mL of lactose to 11% and 1g of D-fructose), Tes-Tris (24, 5 of Tes, 5,3g of Tris, 1g of D-fructose, 11g of skimmed milk) and Tris(12,11g of Tris, 6, 8 g cítric acid, 2,05g of D-fructose and 2,05g of lactose), being added 7mL of glycerol, 20mL of egg yolk and1mg/mL of the antibiotic all four thinners in cryopreservation of buffaloes, with the purpose to increase the pregnancy rate anddisseminate reproductive biotech. The experiment was conducted in the Laboratory of Animal Reproduction Sector, UFRA, using two bulls of Murrah buffaloes. The seminal harvests were made through artificial vagina, totaling 10 ejaculates, which were cyopreserved in four different thinners and the preserved samples were subjected to analysis of motility, effective force, sperm plasma membrane integrity (IMP), hypo-osmotic swelling (HOS) and of Eosin and Nigrosin (EN). The resistance thermometer test (TTR) understood analysis of motility, effective force after three hours ofincubation. An epifluorescence microscopy was performed with fluorescent probes association: Propídio Iodide (PI), Hoechst 33342 (H342), Agglutination of Pisum sativum coupled with the fluorescein isothiocyanate (FITC-PSA), 5,5 ,6,6 -tetrachloro-1,1 3,3 -tetrathylbenzimidazolyl-carbocyanine iodide (JC-1) for analysis of the IMP, acrossomal membrane integrity and mitochondrial membrane potential. The analysis of variance (ANOVA) and Tukey test (5%) were used. In relation to motility, Tes-Tris (70.50 ± 10.00%) and Tris (78.50 ±10.81%) were better of Citrate (43.00 ± 23.59%) and Lactose (42.50 ± 25.52%). In relation effective force the Tes-Tris (2.80 ± 0.58%) and Tris (3.15 ± 0.91%) were superior to Lactose (1.85 ± 0.91%). In the tests for IMP(HOS and EN), in the four thinners did not differ statistically. In the TTR, motility in Tes-Tris (19.00 ± 20.25%) and Tris (20.00 ± 21.86%) differed statistically from Citrate (2.50 ± 6.35%) and Lactose (1.70 ± 2.36%). In relation to the Tes-Tris (1.30 ± 0.82) and Tris (1.35 ± 0.67) differed statistically of the Citrate (0.30 ± 0.67) and Lactose (0.40 ± 0.52). In an epifluorescence microscopy, demonstrated effectiveness in thinners cryopreservation, as did not differ statistically. However the percentage of sperm with intact plasma membrane, acrosome intact and with mitochondrial function was best preserved in thinner Tes-Tris (42.5 ± 12%). Fluorescent probes monitor the integrity of the structures sperm cells, which have the ability to bind to specific points of the cells, allowing the diagnostic practice and direct (CELEGHINI, Reprod Dom Anim, 42, 479–488, 2007). Therefore, the different thinners demonstrated satisfactory function of cryopreservation of buffaloes sperm, which they kept the IMP of the sperm and the Tes-Tris and Tris were the ones who presented the best results.

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Inhibition of HSP90 during maturation interferes on relative abundance of specific transcripts in the bovine oocyte

E.D. de Souza[1], N.C. Rabelo[2], R. Bernarde Silva Dias[3], M.M. Pereira[4], G. Torres de Souza[4], I. Drumond Louro[1], L.S.A. Camargo[5]

[1]Universidade Federal do Espirito Santo(UFES)/Renorbio, Vitoria; [2]Instituto Nacional de Saúde da Mulher, da Criança e do Adolescente Fernandes Figueira / Fiocruz (IFF, Rio de Janeiro; [3]Universidade Presidente Antonio Carlos (UNIPAC), Juiz de Fora; [4]Federal de Juiz de Fora (UFJF), Juiz de Fora; [5]EMBRAPA Gado de Leite, Juiz de Fora.

Keywords: genic expression, 17AAG, maturation.

The heat shock protein 90kDa (HSP90) is one of the most abundant and essential chaperone for cell survival. Its inhibition with 17-(allylamino)-17-demethoxygeldanamycin (17AAG, Sigma, St. Louis, USA) during IVM reduces oocyte competence, decreasing embryo production rate (Souza et al., 2013. AnimReprod, 10:515). In mice, HSP90 depletion or inhibition can cause several anomalies that impair meiosis progression in oocytes. The aim this study was to evaluate the effects of HSP90 inhibition by 17AAG during in vitro maturation on relative amount of transcripts of specific genes important to the development and maturation of bovine oocyte. Immature oocytes aspirated from ovaries obtained from slaughterhouse were selected and randomly allocated in three groups: 0 (control), 1 and 2µM 17AAG for the firsts 12h of IVM (24h) at 38.5ºC under 5% CO2 and saturated humidity. Three polls of ten oocytes were denuded in a solution of PBS plus 0,1% hialuronidase and then frozen in liquid nitrogen. The total RNA was extracted by RNeasy micro Kit® (Qiagen, São Paulo, Brazil) and then reverse transcribed by SuperScript® III Reverse Transcriptase (Thermo Fisher Scientific, Massachusetts, EUA). Relative quantification of MAPK, c-MOS, MATER, ZAR and HSP90 transcripts was performed by Real-Time PCR and results compared by the comparative Ct method, using the GAPDH gene as endogenous reference and control group as calibrator. Data was analyzed by the REST® software using the Pair Wise Fixed Reallocation Randomisation TEST®. Values are shown as mean ± SE. There was no difference ($P > 0.05$) between 1µM 17 AAG and control groups in the relative amount of MAPK (0,85 ± 0,17), c-MOS (0,86 ± 0,13), MATER (0,93 ± 0,14), ZAR (0,97 ± 0,15) and HSP90 (0,70 ± 0,11). However, the 2µM group displayed lower relative amount of MAPK, ZAR and HSP90 transcripts ($P < 0,05$; 0,63 ± 0,10; 0,68 ± 0,10; 0,60 ± 0,08, respectively) than the control group. In conclusion, inhibition of HSP90 by 2µM 17AAG for 12h of IVM interferes in the relative abundance of transcripts of genes related to oocyte maturation and further embryo development, which highlights the importance of HSP90 on oocyte maturation processes.

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Apoptosis rate after vitrification / rewarming of pure breed Gyr embryos produced *in vitro*

C.A.S. Monteiro[1], B.A.F. Barros[2], P.M.S. Rosa[3], G.R. Leal[1], A.J.R. Camargo[4], R.V. Serapião[4], C.S. Oliveira[5]

[1]Universidade Federal Fluminense, Niterói; [2]Fundação Educacional D. André Arcoverde-FAA, Valença; [3]Universidade Severino Sombra-USS, Vassouras; [4]PESAGRO Rio, Niterói; [5]EMBRAPA Gado de Leite, Valença.

Keywords: caspase, OPU, Bos indicus.

Gyr breed is the main representative Bos indicus in dairy systems in South America, especially in Brazil. Due to significant improvement of this breed regarding milk production, combined to its resistance to tropical conditions and ectoparasites, it has become one of the main IVP donors breed. Embryo cryopreservation is an important tool for IVP, and singularities among Bos Taurus and Bos indicus resistance to cryopreservation has been described. Despite the importance of Gyr breed in Brazil, little is known about their parameters for tolerance in cryopreservation. Thus, the objective of the study was to assess the apoptosis rate associated with cryotolerance of pure breed Gyr embryos produced in vitro. COCs recovery was performed by OPU in Gyr donors. Viable oocytes were matured, fertilized with sexed semen of Gyr bull, and presumptive zygotes were cultured for 7 days. An incubation system at 38.5º C, and 5% CO2 in atmospheric air and high humidity was used. MIV, FIV and CIV media were purchased from Bioklone® (Jaboticabal, Brazil). On day 7 of culture, embryos were vitrified using a protocol developed by Vajta (Vajta, Mol Reprod Dev, 51, 53-58, 2008) with minor modifications. The vitrified embryos were rewarmed and cultured for another 3 hours, when they were evaluated regarding the re-expansion of the blastocoele. Vitrified/warmed embryos presenting re-expanded blastocoele (V+) or not (V-) were stained for evaluation of the total number of cells (TNC) with Hoechst and for apoptosis evaluation by immunofluorescence detection of caspase-3. The comparison between the NTC and apoptosis rate between groups were performed using the Mann Whitney Test. Statistical analyzes were performed at INSTAT GraphPad software, with 5% significance level. A total of 38 vitrified / rewarmed embryos were obtained in 3 replicates (V + n = 10, V n = 28). There was no difference ($P > 0.05$) between groups, both for NTC analysis (V +: 82,4a ± 27.1; V-: 79,5a ± 26), and for the apoptosis rate analysis (V + 5, 9%: V: 15.4% a). It is noteworthy that, although this effect have not been significant, we observed in the group of embryos that did not show re-expansion of blastocoele, a greater number of embryos displaying more than 20% of apoptotic cells. However, the rate of apoptosis does not seem to be the cause of failure in re-expanding the blastocoele of the vitrified Gyr embryos that are not selected for transfer.

Acknowledgments: FAPERJ, FAPEMIG, CAPES, Embrapa Dairy Cattle.

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Use of Nelore clarifide molecular markers for predicting reproductive rates in heifers

J.R. Melo, A.J.G. Franco Filho, E.R. Carvalho, R. Carvalho, M. Meneguetti, F.A. Di Croce, O. Sá Filho, J.B. Osterstock

ZOETIS, São Paulo.

Keywords: clarifide, molecular, reproductive.

Studies indicate that the Molecular Value Prediction (MVP) for reproductive characteristics, such as Early Pregnancy Probability (EPP) and Age at First Calving (AFC), can be used as a tool to predict the future reproductive performance of cows (Evans et al., 1999; Doyle et al., 2000; Donoghue et al, 2004). The aim of this study was to evaluate correlations between MVP (EPP and AFC) and reproductive performance of Nellore beef heifers. A total of 908 heifers (263.0 ± 27.4 Kg, 17.6 ± 1.0 months), on Brachiaria decumbens pasture with protein supplementation (0.1% BW) were genomically evaluated (CLARIFIDE Nelore, Zoetis). All heifers were submitted to a protocol for cyclicity induction, followed by synchronization of ovulation and TAI. Heifers that did not become pregnant to TAI were resynchronized and then exposed to natural service for 90 days. Ultrasound evaluations were performed to evaluate the cyclicity induction protocol (CL presence at beginning of TAI protocol) and the pregnancy rate at the end of the breeding season. Statistical analyzes were conducted in SAS 9.3 (SAS Institute, Cary, NC, USA) using the PROC LOGISTIC to evaluate the binomial variables and the PROC GLM to evaluate continuous variables. There was an effect of EPP-MVP on the response to cyclicity induction protocol ($P = 0.005$), in which each percentage point increase in EPP-MVP improved by 5% the probability of cyclicity induction. Heifers with lower value for AFC-MVP tended ($P = 0.1$) to have better pregnancy rate at the end of the breeding season. The EPP-MVP and AFC-MVP provided by CLARIFIDE Nelore genomic tool allowed the identification of heifers with higher probability to respond to cyclicity induction protocol and that tended to have a higher probability of pregnancy at the end of breed season.

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Influence of cGMP pathway on lipid content in bovine oocytes matured *in vitro*

L. Schefer[1], K.L. Schwarz[2], D.M. Paschoal[2], H. Fernandes[2], F.C. Castro[2], C.L.V. Leal[2]

[1]Universidade Federal de São Carlos, Pirassununga; [2]Universidade de São Paulo, Pirassununga.

Keywords: cryopreservation, cGMP, oocytes.

Cryopreservation of oocytes and embryos is one of the limitations found in the in vitro production of bovine embryos (IVP). In vitro cultured oocytes and embryos feature higher accumulation of lipids in relation to those produced in vivo. This characteristic has been associated with lower embryo quality and increased sensitivity to cryopreservation. In human adipocytes lipolysis is mediated by the classical cAMP pathway, but the cGMP pathway also activates the process through cGMP-dependent protein kinase (PKG) activity. The objective of this study was to evaluate the influence of cGMP pathway on oocyte lipolysis. Pools of 25 CCOs were matured in vitro in TCM199 supplemented with 10% FBS and different modulators of cGMP pathway. Cyclic GMP synthesis was stimulated by 10-5 M Protoporphyrin IX and 10-6 M NPPA (atrial natriuretic peptide) and blocked by 10-4 M ODQ. Degradation by PDE5 was inhibited by sildenafil (10-5 M SDF) associated with 10-5 M KT5823 (PKG inhibitor). After 24h, oocytes were denuded and stained with Hoechst 33342 and Nile Red, and examined by epifluorescence microscopy to determine nuclear maturation stage and lipid content, respectively (emission 445-450nm and 475-490nm excitation and emission 590nm and 516-560nm excitation, respectively). Fluorescence intensity (FI) measured by ImageJ software. Data from 5 replicates/group were statistically analyzed by ANOVA followed by Tukey test with significance level of 5% (GraphPad Prism software). Nuclear maturation (MII%) was not influenced by any of cGMP pathway modulators (77.6%, $P > 0.05$). Protoporphyrin IX (21.5 ± 0.94 FI) and SDF associated with KT5823 (29.2 ± 1.15 FI) resulted in oocytes with lower lipid content in relation to other treatments and the control group (36.9I ± 2:12 FI $P < 0.05$). However, the stimulus generated by NPPA was not sufficient to reduce lipid content (34.1 ± 1:43 FI) that was similar to treatment with ODQ (32.6 ± 1.71 FI) and control ($P > 0.05$). The results suggest the interference of cGMP pathway on oocyte lipolysis and with different effects resulting from different stimuli. More studies are necessary for a better understanding of the role of cGMP pathway in oocyte lipolysis.

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Cloprostenol administration in the first week postpartum does not seem to change PTGFR expression in the endometrium in crossbred cows

A.S. Camargos[1], S. Wohlres-Viana[2], I.F. Costa[3], L.S.A. Camargo[2], J.C. Ferreira[3], A.A. Ramos[4], E. Oba[3]

[1]IF Goiano Campus Morrinhos, Morrinhos; [2]EMBRAPA Gado de Leite, Juiz de Fora; [3]Drarv FMVZ UNESP, Botucatu; [4]FMVZ UNESP, Botucatu.

Keywords: prostaglandin, bovine, uterus.

This study investigated if postpartum administration of the synthetic prostaglandin cloprostenol to crossbred cows affects PGF2α receptor expression in uterus. The study was conducted at Santa Fé farm, Brazil. Eight clinically healthy postpartum Holstein-Zebu crossbred cows, body condition score between 3.5 and 4 (0-5 point scale), were used. All cows had a normal delivery without retained placenta. After parturition (D0), cows were randomly assigned to one of two treatment: Control: 2 mL of saline i.m. (n = 4) or CLO: 530 μg of cloprostenol (n = 4) on D2 and D5 postpartum. Endometrial biopsies were performed at D2, D7 and D14, using a Yomann biopsy nipper (Hauptner®, Solingen, Germany). The endometrial samples were washed in PBS (LGC Biotecnology, SP, Brazil), put into micro tubes containing RNA later® (Life Technologies, CA, USA), kept at 5oC for 24 h and stored at -80ºC. Total RNA extraction was performed using the RNEasy Mini Kit (Qiagen, Hilden, Germany), according to manufacturer's instructions, and treated with DNAse. The RNA was quantified in a spectrophotometer (ND-100 Nanodrop, Wilmington, USA). Reverse transcription was performed using Superscript III First-Strand Synthesis Supermix kit (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's specifications. Relative quantification of Prostaglandin F2α receptor (PTGFR) transcripts was evaluated using ACTB and GAPDH as reference endogenous genes. Real time PCR was performed using the Power SYBR Green PCR Master Mix kit (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions. Data were analyzed by GLM ANOVA, using the SAS package (v. 9.2, SAS Institute, Cary, USA). Sources of variation were treatment (CONT and CLO); day (2, 7 and 14); and treatment vs day interaction. Means of relative gene expression were contrasted by SNK test. The expression of PTGFR was similar between treatments ($P > 0.05$) in all postpartum days evaluated. Nevertheless, we have noted a consistent decrease of PTGFR expression between day 2 and 14 for both groups, despite no significant differences among days postpartum. This decreasing could signalize that the uterine effect of PGF2α is reduced throughout postpartum period due to lower expression of its receptor. The absence of cloprostenol effect on endometrial expression of PTGFR in crossbred Holstein-Zebu cows contrasts to the finding reported in Nellore, where higher expression of PTGFR was detected in endometrium at day 14 postpartum in cows receiving cloprostenol at day 1 and 4 of postpartum (Moraes, Theriogenology, 83, 276, 2015). Such contrasting findings may due to the physiological differences in the puerperium between beef and dairy breeds (Carthy, Animal, 8, 675, 2014). In conclusion, cloprostenol administration on days 2 and 5 days postpartum does not change PTGR expression in the endometrium of crossbred Holstein-Zebu cows.

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The kinetic of early cleavages influence the DNA methylome status of bovine embryos

J. Ispada[1], K. Annes[2], C.B. de Lima[1], R.F. Leite[2], M. Sirard[3], M.P. Milazzotto[2]

[1]Universidade de Sao Paulo, Sao Paulo; [2]Universidade Federal do Abc, Santo Andre; [3]Universite Laval, Quebec.

Keywords: DNA methylation, embryo, epigenetic.

DNA methylation is an epigenetic control mechanism essential during embryonic development, directing the differentiation of cell lineages and preventing regression to an undifferentiated state. Based on the fact that embryos with different development kinetics present differences in gene expression, this study aimed to characterize the differences between fast and slow embryos regarding the methylome status. For that, COCs from slaughterhouse ovaries were subjected to IVM for 22-24 h, IVF using sexed semen (female) for 18 h and IVC for 7 days. After 40 hours of insemination, embryos were classified as fast (four or more cells) or slow (2 or 3 cells) remaining in culture until the blastocyst stage (40 blastocysts per group in 4 replicates). These embryos were analyzed by EmbryoGENE Methylation DNA Array (EDMA). Briefly, the DNA extracted from 10 embryos was fragmented with MseI restriction enzyme and the fragments were extracted using methylation-sensitive digestion and ligation-mediated amplification PCR- (LMA-PCR). Hybridization was performed according to the manufacturer's instructions (Agilent Technologies). It was considered as differentially methylated regions (DMRs) fragments with methylation differences with $P < 0.05$ and absolute log2 (fold-change) >1.5 between groups. From probes used for microarray, 9082 were expressed above background only in fast blastocysts (FBL), 20670 in slow blastocysts (SBL) and 47713 were observed in both groups. FBL presented 7976 DMRs and SBL presented 3608 DMRs. While fast embryos presented more hypermethylations in DMRs distributed throughout the genome, such as introns, exons, promoter and repeat elements, slow embryos presented more methylation of various densities (High - FBL: 17; SBL: 35; Intermediate - FBL: 81; SBL: 143 and Low - FBL: 31; SBL: 68) focused on CpG islands independent of length (Long - FBL: 14; SBL: 41; Intermediate - FBL: 79; SBL: 152 and Small - FBL: 36; SBL: 53). With Ingenuity Pathway Analysis 25 pathways from FBL and SBL were identified with differences in DNA methylation. These pathways include DNA damage, repair and replication, development disorder, cell morphology, gene expression, post-transcriptional modifications, metabolic diseases, lipid metabolism, post-translational modifications and embryonic development (33-35 genes observed in each pathway). Hypermethylation differences were also observed in genes related to pluripotency (NANOG, OCT4), epigenetic control (DNMT3A, HDAC8, MECP2) and embryonic development (EGFR, FGF8, IGFR1). In conclusion, the DNA methylation profile is different between embryos with fast or slow kinetics of development, thus influencing the phenotype and response to the environment.

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Effect of cumulus cells during vitrification of sheep oocytes

P.C. Claudino dos Santos Neto[1], M. Vilariño[1], M. Crispo[2], A. Menchaca[1]

[1]Fundación Irauy, Montevideo; [2]Institut Pasteur de Montevideo, Montevideo.

Keywords: vitrification, in vitro production, sheep oocytes.

The objective was to evaluate the effect of the presence or absence of cumulus cells during vitrification with Cryotop method of sheep oocytes on fertilization and *in vitro* development. Cumulus oocytes complexes were divided into five groups: a control group that was not subjected to vitrification (CG; N = 339), two groups of immature oocytes vitrified with (ImCOCs; N = 188) or without (Immature; N = 188) presence of cumulus cells, and other two groups of matured oocytes vitrified with (MatCOCs; N = 190) or without (Matured, N=195) these cumulus cells. The removal of cumulus cells could facilitate the achievement of vitrification in a smaller volume of solution, thus increasing cooling rates. Prior to vitrification, in those without COCs groups were denuded by gentle pippeting. Vitrification procedure was carried out by Cryotop method consisting of passing the oocytes by an equilibration solution (ES) for 10 minutes with 7.5% ethylene glycol (EG) and 7.5% dimethyl sulfoxide (DMSO) in a handling solution (HS: TCM199 medium Hepes and 20% fetal bovine serum). They were soon passed to a vitrification solution with 15% EG, 15% DMSO, HS and 0.5M sucrose for 90 seconds put on cryotops device and immediately immersed in liquid nitrogen. After vitrification were warmed at 37°C for 60 seconds, then passed to a dilution solution (0.5M sucrose) and subsequently were maintained in the same *in vitro* culture conditions of the control group until day 8 of embryonic development. For *in vitro* fertilization (Day 0) frozen semen was selected by swim up method with $1x10^6$ sperm in 100µl droplets (25-30 oocytes) in SOF-Fert medium supplemented with estrus ovine serum, heparin and hypotaurine maintained for 22 h in same conditions as the control group at 39°C in 5% CO_2 in humidified atmosphere. The zygotes were cultured in SOFaaBSA media with 5% CO_2, 5% O_2 and 90% N_2 at 39°C in a humidified atmosphere until Day 8. The survival rate (cleaved embryos/vitrified oocytes) at 48h, development rate on Day 6 (morula and blastocyst on Day 6/total oocytes), the blastocyst rate on Day 8 (blastocysts on Day 8/total oocytes) and their interactions were expressed as MEDIA ± SEM and compared by ANOVA with $P < 0.05$. The results show that the survival rate at 48h, development rate on Day 6 and blastocyst rate on Day 8 were higher in control group compared to groups which used vitrification of oocytes with the presence or absence of cumulus cells, both immature and matured oocytes (Control, 72.6 ± 7.1, 45.2 ± 5.1, 34.6 ± 5.2; ImCCOs, 15.7 ± 5.0, 8.3 ± 2.1, 8.1 ± 3.1; Immature, 6.6 ± 2.6, 3.0 ± 1.2, 2.9 ± 1.9; MatCCOs, 21.2 ± 4.9, 11.8 ± 4.5, 7.8 ± 3.1; Matured, 14.2 ± 5.2, 8.7 ± 4.4, 3.1 ± 2.2, $P < 0.05$), respectively. When the presence or absence of cells and the oocyte stage (Immature *vs.* Mature) were evaluated as main effect, no differences were found in all determined variables, P = NS. These results show that vitrification of oocytes affect negatively the cleavage rate and embryo development, and this effect was not influenced by the presence or absence of cumulus cells during vitrification.

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Influence of the bull on production, developmental kinetic and cryoresistance of bovine *in vitro* vitrified embryo by Cryotop

L.O. Leme[1], M.A.N. Dode[2]

[1]UNB, Brasilia; [2]EMBRAPA Recursos Genéticos e Biotecnologia, Brasilia.

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Numerous studies show that the bull´s choice in embryos IVF has great influence on the production and kinetics of its development. However, knowledge is limited about the impact of the male response on IVP embryos quality. This study aimed to evaluate the rate, the speed of development and the response to the cryopreservation of blastocysts derived from IVF with 5 Nellore bulls semen (here designated as T1, T2, T3, T4 and T5). In total, 7060 slaughterhouse oocytes, distributed in 23 repetitions, underwent for 24 hours IVM, were inseminated with 1 x 106 spermatozoids / mL concentration, co-incubated for 16-18 hours and placed in IVC for 7 days. Cleavage (D2) and blastocyst (D6, 144-156 hours post insemination) rates were evaluated. On D6, embryos in BL (blastocyst) stage were removed from IVC and divided into two groups: control (C), not exposed to cryopreservation; and vitrified (V), which were submitted to vitrification process by Cryotop method. The C and V embryos returned to IVC after warming and were evaluated 4 and 24 hours subsequently being in incubator again for re-expansion and development. Data were analyzed by Mann-Whitney (parametric data) or Tukey (non-parametric data) tests, considering ($P < 0.05$). The cleavage (56.8%) and blastocyst (18.8%) rates were lower for T2 (n = 616) than for the other bulls [T1 (n = 1832, 81.7% and 28.9%), T3 (n = 1779, 90.7% and 33.6%), T4 (n = 2172, 82.4% and 36.5%) and T5 (n = 661, 81.8% and 32.2 %)]. Though, the development speed was similar for all the animals. Cryotolerance assessment was observed that at 4h after warming, the degenerated embryos rate was similar in C and V for T1 and T2, while for the other bulls V showed a higher rate of degenerated embryos than C. At 24 hours post-warming, only for T2 and T5 degenerate rate was similar between C and V, while for the other animals V had a highest degeneration rate than C. In addition, the percentage of embryos that developed to the stage BX at 24 hours was similar between V (58.5%) and C (76.5%) to T2 and T5 (67.9% and 65.1%, respectively) and lower for V when comparing to C ones for T1, T3 and T4 bulls. These results suggest that bull's choice, besides affecting the embryo production and kinetics development, also affects the response to cryopreservation. Even though it similarly can be said that bulls which produce more cryoresistent embryos are not always those that produce higher IVP embryos rates.