(conventional: 24 hours at 38.5°C and 5% CO2; or heat shock: 12 hours at 41°C followed by 12 hours at 38.5°C and 5% CO2). The IVM was performed in Nunc plate containing 400 µL of TCM-199 (Tissue Culture Medium 199 - Invitrogen, USA) supplemented with 20 µg/mL of FSH (Pluset®, Calier Laboratories, Spain) and 10% of estrus cow serum. After the maturation period, the cumulus-oocytes complex were denuded in a solution of 0.1% hyaluronidase in PBS (Sigma, USA) by vortexing for 5 minutes and washed twice in PBS containing 0.1% PVP. For mitochondrial activity analysis, denuded oocytes (n = 352) were incubated in 200 uL of PBS supplemented with 0.1% PVA and 50 nM MitoTracker Red CMX-Ros (Invitrogen, USA) for 30 minutes at 38.5 °C. Additionally, the oocytes were washed in PBS-PVA. To evaluate the ROS production, some oocytes used for mitochondrial activity analysis (n = 332) were incubated in drops of PBS containing 10 mM of diacetate 2',7'-dichlorofluorescein (DCFDA, Sigma, BR) for 15 minutes at 38°C. Oocytes were washed three times in PBS-PVA droplets. To assay fluorescent emission, oocytes were mounted on slides. The intensity emitted was quantified using the software Image J. Data were analyzed by Proc Mixed of SAS software (version 9.3, SAS Inst., Inc., USA) and oocyte was considered as a random effect. Were considered the effects of IGF-I concentration, incubation condition, replicate and interaction between IGF-I and incubation condition. Values are shown as the lsmean ± s.e.m. There was no interaction (P > 0.05) between IGF-I concentration and incubation condition on mitochondrial activity and ROS production. The mitochondrial activity was increased (P < 0.01) by IGF-I (15015 ± 757 a; 21448 ± 994 b and 21425 ± 1042 b with 0, 25 and 100 ng/mL IGF-I, respectively) and was reduced (P < 0.01) by heat shock (conventional: 21274 ± 899 vs heat shock: 17387 ± 652). ROS production was increased (P < 0.05) by IGF-I (9170 ± 457 a; 14869 ± 727 b; and 15205 ± 723 b with 0, 25 and 100 ng/mL IGF-I, respectively) and heat shock (conventional: 11590 ± 509 vs heat shock: 15025 ± 622). The results suggests that IGF-I may reduce the harrmful effect of heat shock by the increasing oocyte mitochondrial activity , but its thermoprotective effect was not related to antioxidant activity.

Acknowledgments: Fapemig, CNPq and Embrapa.

Key words: mitochondrial activity, reactive oxygen species, heat shock.

PW58 - Effect of insulin-like growth factor-I on embryo quality of bovine oocytes subjected to heat shock

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The aim of this study was to evaluate the effect of different concentrations of insulin-like growth factor-I (IGF-I) added to the IVM medium of oocytes subjected to heat shock on embryo quality. Immature oocytes aspirated from ovaries obtained from slaughterhouse were selected and randomly allocated in factorial experiment design 3x2. Three concentrations of IGF-I (0, 25 and 100 ng/mL; Sigma, BR) were added to the IVM medium and two incubation conditions (conventional: 24 hours at 38.5°C and 5% CO2; or heat shock: 12 hours at 41°C followed by 12 hours at 38.5°C and 5% CO2) were tested. The IVM was performed in Nunc plate (Thermo Fisher Inc., DNK) containing TCM-199 (Invitrogen, USA) supplemented with 20 µg/mL of FSH (Calier Laboratories, ES) and 10% of estrus cow serum. Oocytes were in vitro fertilized (IVF) in Fert-Talp medium for 20-22h and incubated at 38.5°C and 5% CO2. After IVF, the presumptive zygotes were denuded and cultured in CR2aa medium supplemented with 2.5% fetal calf serum (Nutricell, BR) in an incubator at 38.5°C for 8 days. Fifteen blastocysts with 8 days post-IVF from each combination of the factors IGF concentration and incubation conditions were fixed and evaluated by TUNEL assay to evaluate embryonic quality. Data were analyzed by generalized linear mixed models considering the Poisson distribution using the Proc Glimmix (SAS®, version 9.3). The embryo was considered as a random effect. Were considered the effects of IGF-I concentration, incubation condition, replicate and interaction between IGF-I and incubation condition. Values are shown as lsmean ± s.e.m. The interaction between IGF-I concentration and incubation condition was not significant (P > 0.05). The total cell number of blastocysts (91.4 ± 3.8; 98.3 ± 3.6 and 100.2 ± 4.1 with 0, 25 and

100 ng/mL IGF-I, respectively) and the inner cell mass (ICM) index (30.5 ± 2.3%; 33.8 ± 2.4% and 36.9 ± 2.8% with 0, 25 and 100 ng/mL IGF-I, respectively) were not affected (P > 0.05) by IGF-I. However, IGF-I reduced (P < 0.05) the apoptosis index of blastocysts (5.5 ± 0.8% a; 2.2 ± 0.4% b and 2.7 ± 0.5% b with 0, 25 and 100 ng/mL IGF-I, respectively) and apoptosis index of the ICM (13.6 ± 3.2% a; 5.1 ± 2.2% b and 8.5 ± 2.5% b with 0, 25 and 100 ng/mL IGF-I, respectively). Heat shock reduced (P < 0.05) the total cell number of blastocysts (conventional = 104.3 ± 3.5 a; heat shock = 88.9 ± 2.3 b) and ICM index (conventional = 40.1 ± 2.1% a; heat shock = 27.2 ± 1.8% b), increased (P < 0.05) the apoptosis index of the ICM (conventional = 6.2 ± 1.4% a; heat shock = 12.0 ± 2.7% b) and had no effect on the apoptosis index of blastocysts (conventional = 3.5 ± 0.6%; heat shock = 3.4 ± 0.5%). In conclusion, the addition of IGF-I to IVM medium, even at low doses, reduced the effects of heat shock on bovine oocytes, resulting on lower embryo apoptosis and improved embryo quality.

Acknowledgments: Fapemig, CNPq and Embrapa.

Key words: apoptotic index; inner cell mass; cell number of blastocys

PW59 - Cellular and molecular markers of oocyte quality: differences between immature, in vivo and in vitro-maturation in the rabbit model

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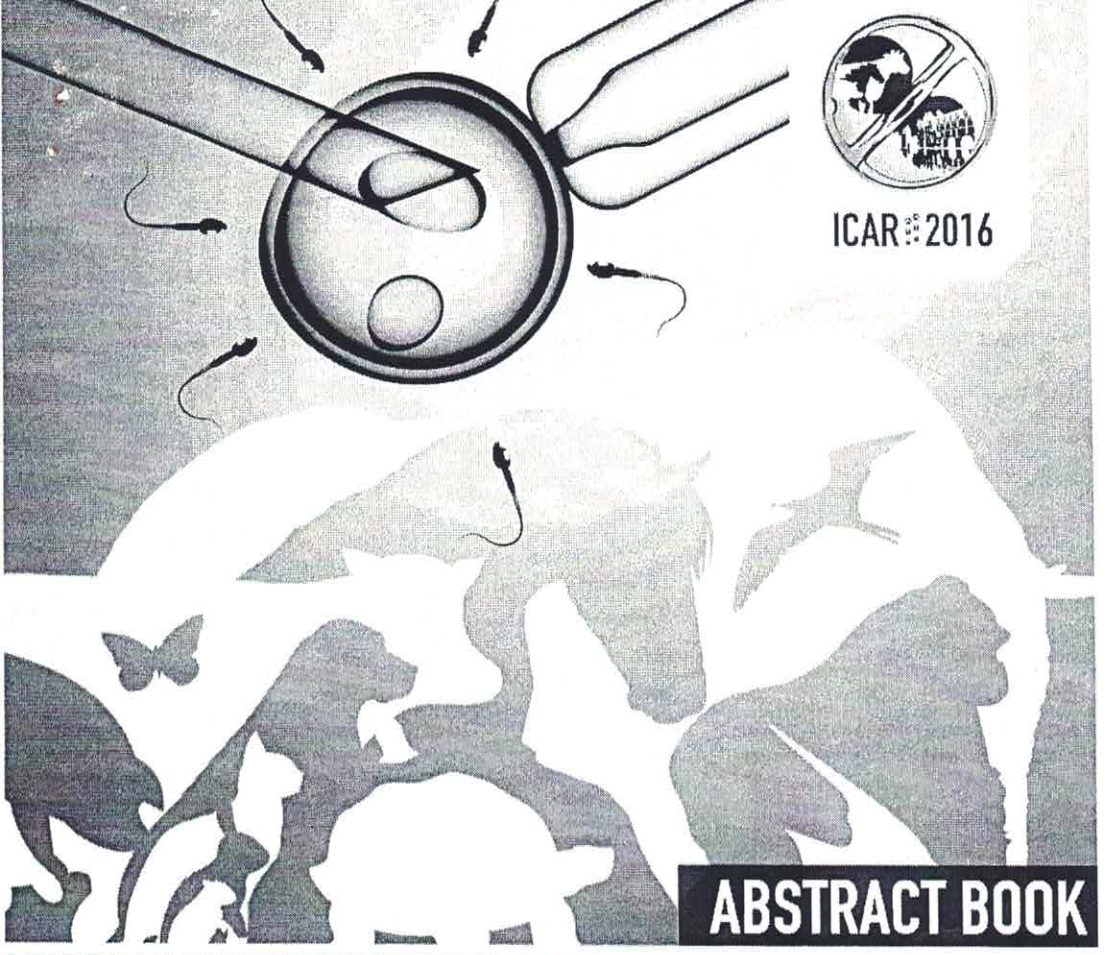
The aim of this study was to establish some cellular and molecular indicators of oocyte developmental competence during oocyte maturation in the rabbit model. The apoptosis rate, mitochondria patterns and mRNA transcripts in oocytes and cumulus cells (CC) from immature, in vivo- and in vitro-matured oocytes were determined. Cumulus oocyte complexes (COC) from follicles >1mm were recovered before in vitro maturation (IVM) or in vitro-matured for 16h (38ºC, 5% CO2) in TCM-199 serum free medium plus 10 ng mL-1 Epidermal Growth Factor. In vivo-matured COC were recovered by flushing 15h after ovulation induced by i.m. administration of 20 µg GnRH analogue (Inducel, Ovejero Lab.). The mRNA transcripts were quantified in 132 oocytes and their respective CC (n=45, n=42, n=45, respectively) by qRT-PCR to contrast relative levels of histone H2Az and genes related with cumulus expansion (GJA1), apoptosis (Tp53, CASP3) and oxidative stress (SOD2). Apoptotic index was assessed by TUNEL technique (n=14, n=10, n=13 COC, respectively) and mitochondria staining in denuded oocytes (n=20, n=27, n=46, respectively) with 200 µM MitoTracker Orange probes (Invitrogen). ANOVA or a Kruscal-Wallis for non-parametrical samples and chi-square tests were performed. In the CC, there were not found significant differences in the GJA1, Tp53, CASP3 and SOD2 transcripts before and after IVM, but GAJ1 was significantly decreased (P<0.05) and SOD2 slightly up-regulated (P=0.06) in CC from in vivo-matured COC compared to those IVM. In oocytes, Tp53, CASP3 and SOD2 mRNA content was significantly down regulated after in vivo maturation compared to those IVM and immature (P<0.05). Only SOD2 poly (A) mRNA content significantly increased in oocytes after IVM compared to immature oocytes (P<0.05). Mitochondrial pattern was different between the three groups showing a diffuse pattern in most of the immature oocytes (80%); a migrated homogeneous pattern in in vivo-matured oocytes (85.5%) and migrated pattern but with frequent clusters in IVM oocytes (54.3%). In addition, apoptosis rate was significantly lower in COC's in vivo-matured than those IVM or immature (6.0±1.0 vs 10.9±1.7 and 10.9±2.0%, respectively; P<0.05). This study corroborates that gene-expression profile studied of in vivo-matured rabbit COC is improved from those IVM. These findings are accompanied by stage-specific mitochondria remodelling in oocytes and lower apoptosis rate in in vivo-matured COC, indicating that oocyte quality is compromised during IVM. We acknowledge UCM, CM and MICINN for funding.



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