W56 - Alpha-linoleic acid enhances mitochondrial activity in cumulus cells and improves developmental capacity of bovine oocytes matured under lipotoxic conditions

Authors and co-authors:

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Dietary omega-3 (n-3) fatty acids (FAs) can enhance fertility in human and farm animals. We have shown that supplementation of cumulus oocyte complexes (COCs) with alpha-linoleic acid (ALA), the parent n-3 FA, reduces oxidative stress, supports mitochondrial activity in bovine oocytes, and induces a MAPK-mediated improvement in nuclear oocyte maturation and subsequent in vitro embryo development. In contrast, saturated (SFAs) and mono-unsaturated FAs (MUFAs), which are typically elevated in certain metabolic lipolytic conditions, exert lipotoxic effects on oocytes. This involves oxidative stress, increased apoptosis, and hampered development. In the present study, we hypothesized that these lipotoxic effects can be attenuated or alleviated by ALA. Bovine COCs were supplemented in vitro during maturation with either: 1) ALA (50 µM), 2) a mixture of the most predominant lipotoxic SFAs and MUFAs in the follicular fluid (palmitic, stearic and oleic acids, PSO, 425µM), or 3) a combination of both (PSO+ALA). 4) A standard FA-free control and 5) a solvent control (SCONT) were included. In Exp. 1, treated COCs (n=198, 3 repeats) were partially denuded at 22h of maturation, stained with JC1 and examined under a confocal microscope to determine mitochondrial activity (intensity of J-aggregates) in oocytes and surrounding cumulus cells (CCs) separately. In Exp. 2, treated COCs (1529 COCs, 5 repeats) were fertilized and cultured in FA-free and serum-free conditions until day 8 post-fertilization to examine embryo development and quality. Compared with the controls, ALA significantly enhanced mitochondrial activity only in CCs ($P<0.05$) but not in the oocytes. PSO-FAs reduced mitochondrial activity in both CCs and oocytes ($P<0.05$), an effect that was alleviated by ALA only in the CCs. Compared with SCONT, PSO-FAs resulted in higher fragmentation rates (16.8% vs. 9.5%, $P<0.05$) and lower blastocyst rates on day 7 ($P<0.05$), either expressed as a proportion from the total number of fertilized oocytes (15.6% vs. 22.8%) or from cleaved embryos (20.4% vs. 30.6%). Moreover, hatched and expanded blastocysts produced from PSO-exposed oocytes had higher apoptotic cell indices. In contrast, these negative effects were alleviated by ALA supplementation. In the PSO+ALA group, fragmentation (6.9%), blastocyst rate on day 7 (21.4% from total fertilized oocytes and 28.7% from cleaved embryos), and apoptotic cell index were similar to the SCONT. In addition, PSO+ALA group had significantly higher total cell numbers in expanded and normal blastocysts compared with those from PSO group. In conclusion, ALA supplementation enhanced mitochondrial activity in CCs during maturation under lipotoxic conditions, which was associated with a significant improvement in developmental potential of the oocytes. These results may have clinical implications to improve fertility through dietary interventions in animals and humans suffering from metabolic disorders associated with lipolysis.

PW57 - Antioxidant effects of IGF-I on bovine oocytes subjected to heat shock

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The objectives of this study were to evaluate the effect of different concentrations of IGF-I added to the in vitro maturation (IVM) medium on mitochondrial activity and reactive oxygen species (ROS) production of oocytes subjected to heat shock. Immature oocytes aspirated from ovaries obtained from a slaughterhouse were selected and randomly allocated in a factorial experiment design 3x2. Three concentrations of IGF-I (0, 25 and 100 ng/mL - Sigma, BR) were added to the medium and two IVM incubation conditions were tested

(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.

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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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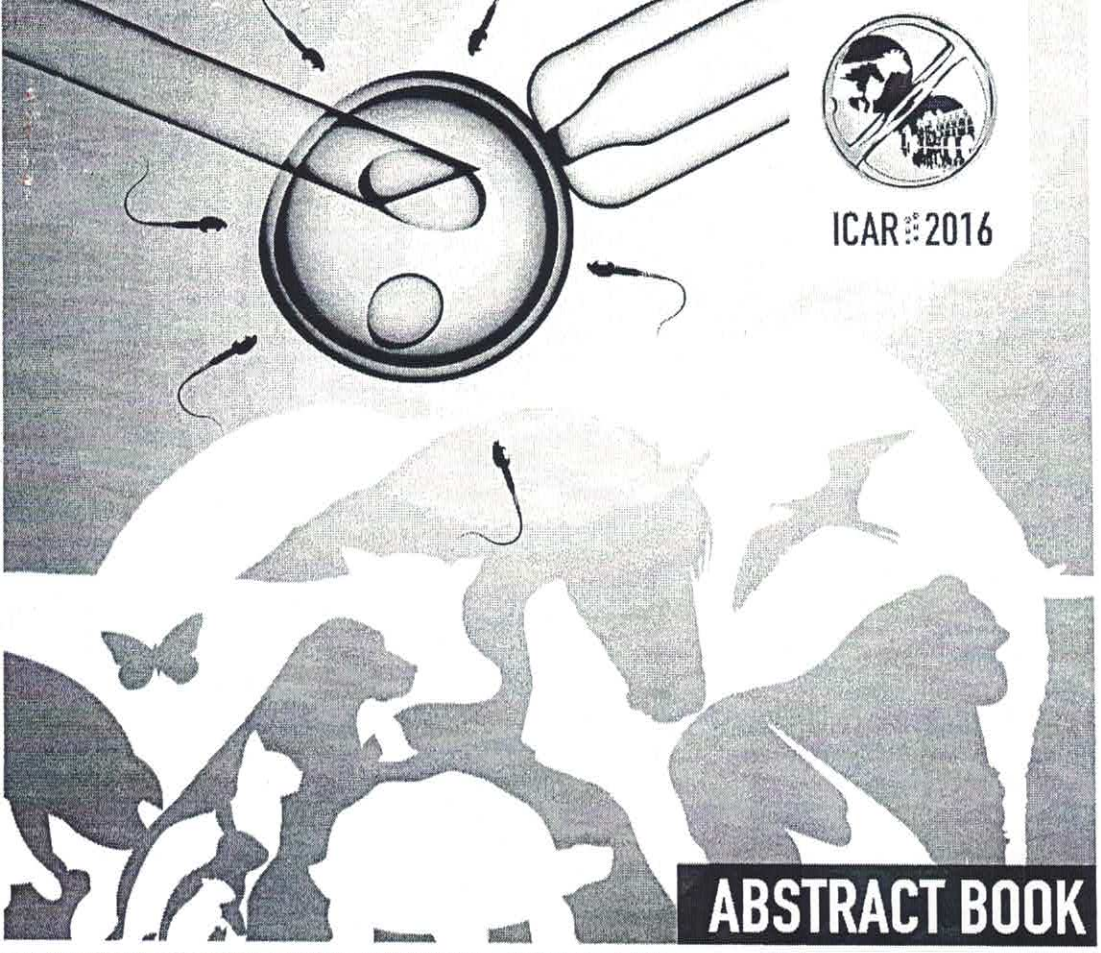
The aim of this study was to evaluate the effect of different concentrations of insulin-like growth factor-I (IGF-I) added to the IVM medium of oocytes subjected to heat shock on embryo quality. Immature oocytes aspirated from ovaries obtained from slaughterhouse were selected and randomly allocated 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



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