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Ovine oocytes fertilization followingICSI: effect of interspecific sperm injection, oocyte activation and sperm treatment

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Ovine oocytes fertilization following ICSI: effect of interspecific sperm injection, oocyte activation and sperm treatment Bogliolo, L; Fois, S; Ariu, F; Rosati, I; Zedda, MT; Pau, S; Ledda , S Department of Veterinary Pathology and Clinic, University of Sassari Introduction Intracytoplasmic sperm injection (ICSI) is a very powerful technique in domestic livestock species. In ovine species, although the birth of normal lambs has been reported, the efficiency of blastocyst production after ICSI is very low probably owing to defective activation of the oocyte. In the present study, we investigate the effect of interspecies sperm injection, oocyte artificial activation and sperm treatment on sheep oocyte fertilization following ICSI. Materials and methods Exp 1-Cumulus-oocyte complexes collected from sheep ovaries were matured in vitro for 24 h before being injected with: a) frozen-thawed ram semen (RS); b) frozen-thawed stallion semen (SS). Motile ram and stallion spermatozoa were selected by swim-up technique. Injected oocytes were thereafter cultured 18-20 h, fixed and stained with aceto: lacmoid to assess the presence of male and female pronuclei. Exp 2- On the basis of the results of Exp1, we tried to optimize sheep oocyte fertilization with the aid of activation of oocytes with ionomycin (5 µM, 5 min) and 6-DMAP (3h)and/ or ram sperm pre-treatment with 0.1% Triton X-100 (5 min). Fertilisation rate was evaluated by oocyte staining as previously described. Statistical analysis was done using the Chi-square test. Results Exp 1-A significantly ($P<0.01$) higher proportion of normal fertilization was obtained after injection of SS (84/116, 72.4%) compared to RS (48/104, 46.2%). Exp 2 –The treatment of injected oocytes with ionomycin and 6-DMAP resulted in increased pronuclei formation (69/ 98, 70.4%) compared to non-activated group (46. 2%). Fertilization rate after sperm pre-treatment was significantly lower in absence of oocyte activation (20/83, 24.1%, $P<0.01$) compared to activated group (38/79, 48.1 %) Conclusions Taken together these findings proves that stallion spermatozoa have a higher ability to activate sheep oocytes compared to ram semen and that oocyte artificial activation, but not sperm pre-treatment, enhances fertilization of sheep oocytes after ICSI.

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Analysis of gene expression of in vitro produced bovine blastocyst cultured for 72 hours post-vitrification

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Introduction In vitro produced (IVP) embryos frequently show low survival rates after cryopreservation. Among the available methods, vitrification has generally shown better results with IVP embryos. However, little is known about vitrification effects on genes expression. The aim of this study was to evaluate the expression of aquaporins encoding genes for (AQP) 3, AQP11 and ATPase α1 in vitrified/warming embryos cultured in vitro for 72h. AQPs are channels that facilitate water transportation across membranes, whereas ATPase α1 is a Na/K pump subunit. Both AQPs and Na/K pump may be involved in blastocoel expansion after cryopreservation.
Materials and methods In vitro produced bovine blastocyts (n=57) were vitrified using an open pulled straw (OPS) in a two steps protocol (10% DMSO + 10% ethylene glycol (EG) for 1 minute followed by 20% DMSO + 20% EG for 20 seconds). Warming was performed through OPS immersion on holding media with 0.26M of sucrose at 39ºC for 1 minute, followed by 0.26M and 0.16M for 5 minute each step. Warmed embryos were cultured in CR2aa with granulosa cells monolayer at 38,5°C under 5% CO2 in air for 72 hours. Non-vitrified embryos (control group; n=52) were cultured simultaneously. After 72 hours, viable embryos (expanded and hatched blastocysts) were frozen and stored in -80°C until mRNA extraction. RNA extraction was performed from pools of five expanded/hatched blastocysts equally distributed in each pool. RNA obtained from pool extraction was amplified and real time RT-PCR was performed in order to obtain quantitative data. Beta-actin gene expression was used as an internal control. Primers efficiency was assayed by LinRegPCR software. Calculations and statistical analysis of relative quantification were performed by REST® software. Data of embryos' hatching and survival rate were analyzed by Chi-square.
Results Control group presented higher ($P<0.05$) hatching and survival rates (78.8%; 84.6%, respectively) than vitrified group (17.5%; 57.9%). In contrast, relative expression of APQ3, AQP11 and ATPase α1 transcripts was not different between control group and vitrified embryos after co-culture for 72 hours (0.8, 0.3, and 0.9 fold difference relative to control group). These results suggest that, despite lower survival rates, vitrified IVP embryos that survived in vitro culture for 72h presented similarities to fresh IVP blastocysts. In conclusion, vitrification may result in expanded/hatched IVP blastocyts with no alterations on expression of genes associated with blastocoel expansion.

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Effect of melatonin on in vitro maturation of bovine cumulus-oocyte complexes and DNA damage of cumulus cells

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Introduction Melatonin, a major hormone of pineal gland, is known to be associated with the modulation of circadian rhythms and the regulation of seasonal reproductive function. In addition, its antioxidant properties as a scavenger have been also reported.
Objective The aim of this study was to examine the effect of melatonin on in vitro maturation (IVM) of bovine cumulus-germinal vesicle-stage oocyte complexes and DNA damage of cumulus cells.
Materials and Methods The bovine cumulus-oocyte complexes (COCs) aspirated from abattoir ovaries were cultured in Tissue Culture Medium 199 supplemented with 0.3 mM sodium pyruvate, and 10 µg/ml gentamicin sulfate (B199 medium) at 39°C in an atmosphere of 5% CO_2 in air. After 24 hours of culture in B199 medium with 0.5 µg/ml FSH and 5.0 µg/ml LH (control), in B199 medium with 1 µ/ml melatonin (MEL) or in B199 medium with 1 µ/ml melatonin, 0.5 µg/ml FSH and 5.0 µg/ml LH (MEL-FSH-LH) the oocytes were stained with Hoechst 33342 to evaluate the maturation rate. After in vitro maturation the DNA damage of the cumulus cells of each group was also measured by Comet assay.
Results Maturation rates were similar ($P > 0.05$) among groups (Control = 59.92±4.99; MEL = 60.96±4.99; MEL-FSH-LH = 55.08±4.99). The percentage of cumulus cells with no DNA damage was significantly superior in MEL group (37.77±1.59) than in Control (24.76±1.59) and MEL-FSH-LH groups (31.87±1.59). Additionally, the percentage of cumulus cells that exhibited a large migrating DNA fragment was lower ($P < 0.01$) in MEL group (16.78±1.62) than Control group (24.99±1.62) but not significantly different from MEL-FSH-LH (18.97±1.62).
Conclusion The results suggest that the addition of melatonin to the IVM medium protects the cumulus cells from DNA damage. However, additional studies are necessary to verify the role of melatonin on the regulation of oocyte maturation. Research supported by FAPESP.