POST-THAWING RESISTANCE OF BOVINE EMBRYOS IS IMPROVED BY *trans*-10, *cis*-12 CONJUGATED LINOLEIC ACID (CLA).

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INTRODUCTION

Excessive lipid content in embryo cells is suggested to increase their susceptibility to cryopreservation. The success in bovine embryo cryopreservation depends among other factors on the composition of media during embryo culture. Presence of serum in these media significantly increases fatty acids content of blastocysts (1, 2). CLA reduces the uptake of fatty acids by adipocytes, without increasing lipolysis (3). We intend to study the effect of different culture media, the presence of serum and CLA on the developmental competence of IVP bovine embryos.

MATERIALS AND METHODS

Follicles with 2-6 mm diameter were aspirated from slaughterhouse bovine ovaries. Selected cumulus oocyte complexes were matured in TCM199 with Earle's salts, L-glutamine, 25 mM Hepes, 10% superovulated oestrus cow serum (SOCS), 10 µg mL^{-1} FSH and antibiotics during 22-24 hours. For fertilization, frozen-thawed semen was submitted to swim-up and the final concentration adjusted to 10^6 spz mL^{-1}. Spermatozoa and 10 oocytes were placed in 40 µL droplets of TALP medium supplemented with 5.4 USP mL^{-1} heparin, 10 mM penicillamine, 20 mM hypotaurine and 0.25 mM epinephrine. Following co-incubation for 22 hours the presumptive zygotes (n=2931) were randomly placed in 100 µL droplets of: I) granulosa cell monolayer cultured with TCM199 + 10% SOCS + 100 µM GSH; II) granulosa cell monolayer cultured with TCM199 + 10% SOCS + 100 µM CLA+ 100 µM GSH; III) modified SOF (4) + 100 µM GSH; under paraffin oil, where embryo culture proceeded for 8 days. Atmospherical conditions for IVM-IVF and embryo co-culture were 39º C and 5% CO2 in air with humidified atmosphere. In SOF, 39º C and 5% CO2, 5% O2 and 90% N2 with high humidity were used. Cleavage was assessed 48 hours after fertilization and embryos were morphologically evaluated on day 7 and 8 p.i. Fair quality blastocysts (5) were frozen in PBS + 10% glycerol + 15% FCS and thawed to assess embryo survival. Data representing 12 replicates were analyzed using ANOVA and LSD except for post-thawing embryo survival where ÷2 was used.

RESULTS AND DISCUSSION

Cleavage rates or D7/D8 embryo quality did not vary among treatments. D7/D8 embryo rate was significantly ($P<0.004$) lower in SOF (III- mean ± sem 20.1±1.9%) than in co-culture conditions (I- 34.0±3.0%; II- 31.0±2.5%). Under Normarsky microscopy the presence of embryo cytoplasmic "lipid like granules" appears to be lower in SOF (III), higher with CLA (II) and the highest in TCM+serum+cells (I) alone. Post-thawing intact blastocyst rates where 59.4%, 100% and 85.2% respectively from 91 frozen and thawed embryos (I: n=32, II: n=32, III: n=27; I×II $P<0.0001$, I×III $P=0.03$, II×III $P=0.02$). Post-thawing reexpanding rate was significantly lower ($P<0.05$) when embryos were cultured in TCM+serum+cells alone (I- 40.6%) than with CLA (II- 65.6%). SOF embryos reexpanding rate (III- 51.9%) was not significantly different ($P>0.05$) between treatments. Results show for the first time that CLA can improve post-thawing embryo survival without affecting embryo producing rates even when cultured with serum. SOF post-thawing results although better than those with traditional media containing sera presented worse embryo rates. The role of CLA in early bovine embryo development and their protection against cryoinjury requires further investigation.

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