

## Cryosurvival of bovine blastocysts is enhanced by culture with *trans*-10 *cis*-12 conjugated linoleic acid (10*t*,12*c* CLA)

R.M. Pereira<sup>a,\*</sup>, M.C. Baptista<sup>a</sup>, M.I. Vasques<sup>a</sup>, A.E.M. Horta<sup>a</sup>,  
P.V. Portugal<sup>a</sup>, R.J.B. Bessa<sup>a</sup>, J. Chagas e Silva<sup>b</sup>,  
M. Silva Pereira<sup>a</sup>, C.C. Marques<sup>a</sup>

<sup>a</sup> Estação Zootécnica Nacional – INIAP, 2005-048 Vale de Santarém, Portugal

<sup>b</sup> Divisão de Seleção e Reprodução Animal, DGV, Venda Nova, 2704-507 Amadora, Portugal

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### Abstract

An excessive lipid content in embryo cells is a consequence of embryo culture in the presence of serum which is suggested to be responsible for their high susceptibility to cryopreservation. The objective of the present study was to examine the effects of supplementing serum-containing culture media with *trans*-10 *cis*-12 conjugated linoleic acid (10*t*,12*c* CLA) on embryo lipid accumulation and its subsequent cryopreservation. Abattoir-derived oocytes were matured and fertilized in vitro (IVF= day 0). On day 1, presumptive zygotes ( $n=3390$ ) were randomly placed in: (I) (MS), granulosa cell monolayer cultured with M199 and 10% serum; (II) (SCLA), granulosa cell monolayer cultured with M199, 10% serum and 100  $\mu$ M 10*t*,12*c* CLA and (III) (SOF), modified synthetic oviduct fluid, where embryo culture proceeded for 8 days. Cleavage rates or D7/D8 embryo quality did not vary among treatments. D7/D8 embryo production rate was significantly ( $P<0.001$ ) lower in SOF ( $17.9 \pm 1.6\%$ ) than in groups MS ( $29.8 \pm 2.5\%$ ) and SCLA ( $27.8 \pm 2.0\%$ ). After cytoplasmic lipid droplets observation under Nomarski microscopy, classified embryos were the leanest when cultured in SOF, intermediate in SCLA and the fattest in MS ( $P<0.02$ ). Post-thawing intact blastocyst rates were significantly higher in the SCLA group ( $84.7 \pm 4.1\%$ ) than in SOCS ( $50.3 \pm 4.8\%$ ,  $P=0.0007$ ) or SOF ( $65.3 \pm 6.9\%$ ,  $P=0.03$ ) groups. Post-thawing re-expanding rates were significantly lower when embryos were cultured in MS ( $34.7 \pm 3.7\%$ ) than in SCLA ( $63.7 \pm 5.3\%$ ,  $P=0.0006$ ) or SOF ( $49.0 \pm 4.6\%$ ,  $P=0.04$ ). Moreover, re-expanding rates were lower ( $P=0.05$ ) in SOF than in SCLA cultured embryos. These results clearly show that addition of CLA to serum-containing

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\* Corresponding author. Tel.: +351 243 767 316; fax: +351 243 767 307.

E-mail address: rosaln@hotmail.com (R.M. Pereira).

media reduced lipid accumulation during in vitro culture and significantly improved cryopreservation survival.

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