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Use of lipolysis supplement during IVM reduces lipid content of bovine oocytes but do not affect blastocyst cryosurvival

Viviane Luzia da Silva Feuchard[1], Sheila Costa De Souza Marques[2,4], Raquel Varella Serapião[1], Naiara Zoccal Saraiva[2], Marina Ragagnin de Lima[3], Joaquim Mansano Garcia[3], Clara Slade Oliveira[2]

[1]PESAGRO-RIO - PESAGRO RIO, Niterói, Rio de Janeiro, Brasil; [2]EMBRAPA GADO DE LEITE - Empresa Brasileira de Pesquisa Agropecuária, Juiz de Fora, MG, Brasil; [3]UNESP - UNESP, Jaboticabal, SP, Brasil; [4]UBM - Centro Universitário Barra Mansa, Barra Mansa, RJ, Brasil.

In vitro produced embryos have high lipid content and this characteristic is related to cryotolerance. Since lipid accumulation first occurs during in vitro maturation (IVM), our hypothesis was that the use of lipolysis regulators during IVM would reduce lipid content of the oocytes and consequently of the embryos and improve cryosurvival. We evaluated the effect of a lipolysis supplement (L-carnitine, linoleic acid and forskolin) during IVM on total lipid content of oocytes and in vitro produced blastocysts and their influence on cleavage, blastocyst and survival rates after vitrification. CCOs were obtained from slaughterhouse ovaries, in 6 replicates, and randomly distributed in two groups: Control (C, TCM 199 + 10% SFB) and Lipolysis supplement (L, TCM 199 + 10% SFB and lipolysis supplement (2,5 mM L-carnitine, 150 µM linoleic acid and 15 µM forskolin) for 24 h IVM. After IVM, part of matured oocytes was denuded with Tryple Express (Gibco, Grand Island, NY) solution and fixed for Oil Red O staining and most followed IVF and IVC for 7 days. All steps were performed at 38.5°C, 5% CO2 and maximum humidity. Samples of denuded oocytes and day 7 blastocysts from C and L groups were fixed in 4% paraformaldehyde and stained with Oil Red solution for 30 min. Lipid content was estimated by stained cytoplasm area fraction (µm²) and staining levels (pixels) using ImageJ® software. Bl and Bx grade 1 were vitrified in vitrification solution 1 (HSOF + 7.5% ethylene glycol [EG] + 7.5% dimethyl sulfoxide [DMSO]) for 3 minutes and solution 2 (HSOF + 15 % ET + 15% DMSO) for up to 30 seconds. Subsequently, the embryos were transferred to vitrification forks and submerged in liquid nitrogen. The lipolysis supplement reduced lipid content in L oocytes compared to Control group considering staining levels (C- 49.90 ± 1.59, L - 45.00 ± 1.86, P <0.046, T test, n = 192,104-88 per group) and area fraction (C - 127.25 ± 4.06, L – 114.77 ± 4.76, P<0.046, T test, n = 192,104-88 per group). In blastocysts, there was no difference between groups (C - 62.38 ± 2.68, L- 66.78 ± 2.61, P = 0.27, T Test, n = 40, 25 -15 per group). There was no difference in developmental rates or survival rates after vitrification. The mean rate of cleavage (C - 74.88 ± 5.52, L - 78.21 ± 5.76, P = 0.45, T Test, n = 152, 74-78 per group) and blastocyst (C - 42.24 ± 5.75, L - 31.46 ± 3.10, P = 0.09, T Test), as well as the reexpansion rate (C - 64.36 ± 8.58a, L - 54.78 ± 7.73a, P = 0.39, Test T, n = 196, 108-88 per group) and hatching (C - 23.42 ± 5.66a, L - 24.51 ± 6.87a, P = 0.93, Test T, n = 72, 36 per group) of vitrified blastocysts 48 hours after warming did not differ between groups. We conclude lipolysis supplement use during IVM was efficient in reducing the total lipid content of oocytes but levels were similar to control in blastocysts. No effect was detected in developmental rates or survival after vitrification. Acknowledgments: FAPERJ, EMBRAPA, Pesagro-Rio.