A326 Support Biotechnologies: Cryopreservation and cryobiology, diagnosis through imaging, molecular biology and "omics"

Effect of dietary supplementation of Nelore heifers with polyunsaturated fatty acids on cryotolerance and membrane lipid profile of fresh and vitrified *in vitro*-produced embryos

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Dietary supplementation with polyunsaturated fatty acids (PUFA) for oocyte donor beef heifers can modulate the molecular mechanisms of lipid uptake in oocytes included in ovarian follicles, affecting the subsequent *in vitro* embryo development. Moreover, the success of embryo cryopreservation can be affected by membrane lipid composition, such as phosphatidylcholines (PC) and sphingomyelins (SM). The aim of this study was to evaluate the development and cryotolerance of IVP embryos from oocytes recovered from Nelore heifers (n = 16) fed with control diet (Control group) or fed with rumen-protected PUFA (Megalac-E®), for at least 60 days (Fat group). In addition, the membrane lipid profile of fresh and vitrified embryos from both treatments was evaluated using matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS). COCs (n = 491) recovered after 6 OPU sessions were IVM for 22 h. After IVF (D = 0) the zygotes were in IVC in SOF medium (5 mg/mL BSA and 2.5% FCS, 5% CO_2 in air) for 7 days. Cleavage and blastocyst (Bl) rates were evaluated in the D3 and 7, respectively. Bl were vitrified (Ingámed, Brazil) and embryo survival rates were measured at 24h after warming. Fresh and viable Bl after thawing were transferred to microtubes containing 200 µL of methanol HPLC 50% in aqueous solution, stored at -20°C and immediately transported for MS analysis. Each embryo was deposited at the center of the spots of MALDI-MS plate, under 1 µL of matrix (1.0 mol/12.5 dihydroxybenzoic acid (DHB) in methanol), at room temperature until its crystallization. Spectra were acquired in the mass range of *m/z* 700-1200, in the positive ion and reflectron modes using the Autoflex III (Bruker Daltonics, USA) mass spectrometer. The most intense ions of each spectrum were considered as starting point for determining the *m/z* ratios corresponding to membrane lipids and, only *m/z* clearly distinguished from noise in spectra were included in the partial least squares discriminant analysis (PLS-DA). The cleavage and Bl rates, and the relative abundances of the most relevant lipids that explained the variance of the data were subjected to ANOVA (GLIMMIX, SAS Institute), followed by Tukey's test. The re-expansion rates were evaluated by $_X2$ ($P < 0.05$). Data are presented as LSM ± SEM. The diet did not affected ($P > 0.05$) cleavage rate (average 65.0 ± 3.1%), Bl yield (average 43.2 ± 3.7%) and embryo survival after warming (average 79.3%). PUFA supplementation increased ($P < 0.05$) the relative abundance of only one lipid specie, assigned as PC ether (PCe) 38:2. Cryopreservation affected ($P < 0.05$) the relative abundance of 10 ions: PC 32:0, PC 34:1, SM 24:1, PC 40:6 or PC 42:9, PC plasmalogen (PCp) 44:10 or PC 42:7, triacylglycerol (TAG) 54:9 and a not assigned ion (*m/z* 833.2) were decreased ($P < 0.05$) in Bl that survived the vitrification process, compared with fresh Bl. However, the abundance of the ions PC 36:3 or PC 34:0, PCe 38:2 or PC 36:6 and PC 36:5 or PCe 38:1 were increased ($P < 0.05$) after vitrification. The results demonstrate that the mass spectrometry profiles of PC, SM and TAG species determined by MS differed significantly in fresh and vitrified–warmed bovine Bl. Due to the differences between the ions abundances, they can be used as potential markers of post cryopreservation embryonic survival.