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228 *IN VITRO* EMBRYO PRODUCTION AFTER EXPOSURE OF BOVINE OOCYTES TO DIFFERENT TRANSPORTATION MEDIA AND PERIODS: PRELIMINARY RESULTS

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This study was designed to evaluate the effect of different oocyte transportation media and time. Immature oocytes were recovered from slaughterhouse bovine ovaries. Oocytes ($n = 492$) of quality grades I to III were randomly allotted to one of the following transportation media based on TCM-199, either buffered with HEPES (control) or buffered with $NaHCO_3$ and added with FSH/LH (maturing). Both media were supplemented with pyruvate, penicillin G (10 000 IU), streptomycin (0.05 mg mL^{-1}), and 10% fetal bovine serum (FBS). In control medium, the oocytes were kept for 1 or 8 h at 37°C, and thereafter were transferred to maturing medium until the maturation period (24 h) was completed, under controlled atmosphere (5% CO_2) and temperature, in an incubator. The maturing oocytes were distributed in two types of equipment developed for oocyte and embryo transportation, with and without 5% CO_2, and kept for 1, 8, or 24 h before maturation or fecundation (IVF) procedures. The oocytes kept for 24 h in the transportation device were placed directly for IVF. All procedures used for *in vitro* maturation, IVF, and *in vitro* culture were the same as those adopted for commercial *in vitro* embryo production at Biotran LTDA (Alfenas, Minas Gerais, Brazil). The cleavage rate was evaluated on Day 3 post-insemination, and the blastocyst production was evaluated on Day 7. The statistical model included the main effects of treatment (control and maturing with or without 5% CO_2), time, replicate, and the interaction of media × time. Data (3 replicates) were analysed by ANOVA and differences were identified by Tukey's test. The time before *in vitro* maturation at the incubator negatively ($P < 0.007$) affected cleavage rates (76.4 ± 16.9 *v.* 58.8 ± 13.2 and 52.2 ± 18.5%, respectively, for 1, 8, and 24 h). However, treatment had no effects ($P = 0.3$) on cleavage (66.9 ± 15.0, 57.8 ± 19.7, and 67.7 ± 19.8% for the control and maturation with and without 5% CO_2, respectively). Similarly, blastocyst production rates differed ($P < 0.04$) between 1 h (33.4 ± 14.7) and 24 h (19.3 ± 17.0%), whereas blastocyst production at 8 h did not show significant effects (19.3 ± 17.0). Although it was not significant ($P > 0.05$), only 14.1% of the oocytes kept in medium 2 with a controlled atmosphere for 24 h became embryos. This difference was probably related to the trend in treatment effect ($P = 0.06$) and the lower rate of embryo production (22.2 ± 13.6%) in this treatment compared with the control (33.8 ± 18.1%) and maturation without 5% CO_2 (29.8 ± 17.3%). These results showed that 24 h of transportation is detrimental for the oocyte development potential and that the buffered medium used in this study with 5% CO_2 did not efficiently maintain embryo production.

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229 RAPID, UNTARGETED LIPID DETERMINATION IN INDIVIDUAL BOVINE OOCYTES AND PRE-IMPLANTATION EMBRYOS BY HIGH-RESOLUTION DESORPTION ELECTROSPRAY IONIZATION MASS SPECTROMETRY

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Lipid structural analysis in individual pre-implantation mammalian embryos is hampered by the small amount of biological material, such that most studies use staining methods or gas chromatography analysis generate information only on the fatty acyl residues. Recent developments in high-resolution desorption electrospray ionization mass spectrometry (DESI-MS) allow the analysis of free fatty acids (FA) and glycerophospholipids (PL) in individual bovine embryos. Here, we report on the use of DESI-MS for the sensitive analysis of triacylglycerol (TAG) species, profiles of FA and PL in individual bovine oocytes and embryos. Bovine oocytes ($n = 40$) and blastocysts ($n = 42$) were frozen in a minimal volume of PBS (2 to 5 µL). Samples were directly deposited on glass slides after thawing. After drying, a volume of 500 µL of methanol : water (1 : 1, vol/vol) was carefully deposited on the surface of the glass slide and removed by orienting the glass slide vertically to eliminate PBS salts. An Orbitrap mass spectrometer was used for the experiments. Parameters for the positive ion mode were as follows: acetonitrile (ACN) supplemented with 3 µL mL^{-1} of $AgNO_3$ at a 5 µL min^{-1} flow rate, injection time of 1000 ms, and a mass-to-charge range of m/z 400 to 1500. For the negative ion mode, the solvent combination used was acetonitrile + dimethylformamide (1 : 1, vol/vol) at a 1.0 µL min^{-1} flow rate, a maximum injection time of 1000 ms, and a mass-to-charge range of m/z 150 to 1000. Positive ion mode data for the detection of TAG species were acquired first, followed by acquisition of FA and PL in the negative ion mode. Detection of TAG by DESI, which is extremely useful for bovine embryo cryopreservation and metabolism research, has been performed by adding $AgNO_3$ in the DESI spray to obtain silver adducts, which are easily recognised by the characteristic 1 : 1 abundance ratio of the 107 : 109 Ag isotopes. The most abundant fatty acyl residues present in TAG species were palmitic (P), linoleic (L), oleic (O), and stearic (S) acids, such as TAG of m/z 937, PPL (50 : 2); m/z 965, POO (52 : 1); m/z 967, POS (52 : 2); m/z 989, OOL/LLS (54 : 4); and m/z 991, OOO, SOL (54 : 3). Free FA and PL profiles collected from the same samples in the negative ion mode were similar to those in our recent report (2012 J. Mass Spectrom. **47**, 29–33). Lipid attribution has been performed based on high-resolution mass analysis. Multivariate statistics from this data set will allow visualisation of differences observed in the lipid profiles among samples. In conclusion, we report the use of DESI-MS for the sensitive analysis of TAG in individual bovine oocytes and embryos and the creation of profiles of FA, PL, and TAG species in the same sample by DESI-MS.

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