VITRIFICATION OF BOVINE IN VITRO MATURED AND PRONUCLEAR OOCYTES WITH DIFFERENT VITRIFICATION SOLUTIONS

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Vitrification of in vitro matured (IVM) and pronuclear (PN) oocytes was performed using three types of vitrification solutions (VS). IVM oocytes were vitrified with precooled (4°C) VS containing 40% ethylene glycol + 0.3M trehalose + 20% polyvinylpyrrolidone separately in dulbecco' s phosphate buffered saline negative (DPBS-), positive (DPBS+) and sodium chloride (NaCl) supplemented with 10% superovulated cow serum (SCS) and 0.3% bovine serum albumin (BSA).

The IVM oocytes were put in VSs for 1min, inserted into the 0.25ml plastic straws and plunged immediately in liquid nitrogen. After a week of storage the straws were warmed in 35°C water bath, the embryo content was poured into 0.3M trehalose in mPBS (containing 10% SCS, 0.6% BSA in DPBS+) for 5min in room temperature (22-25°C) and after 2-3 washing in mPBS and maturation medium, used for in vitro fertilization (IVF). On day 2 (day of insemination= day 0) cleavage rate was assessed. Similarly, PN oocytes (15h after IVF) were vitrified and cleavage rates were examined. The cleavage rates were 27% (26/96), 18% (20/114), 14% (13/90) for IVM oocytes and 23% (18/77), 21% (16/75), 13% (11/83) for PN oocytes when vitrified with VS in DPBS-, DPBS+ and NaCl, respectively. A significant difference ($P<0.05$, Chi-square test) of cleavage rate was observed between VS in DPBS- and NaCl when IVM oocytes were vitrified. The blastocyst formation was 3% (3/96), 4% (5/114), 3% (3/90) for IVM oocytes and 3% (2/77), 3% (2/75), 1% (1/83) for PN oocytes when vitrification was done using VS in DPBS-, DPBS+ and NaCl, respectively. These results did not differ significantly among each other.

There appears to be a higher tendency of cleavage and blastocyst rate when vitrification of bovine IVM and PN oocytes were performed with VS in DPBS- and DPBS+ than VS in NaCl.