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

S. Saha, A. Boediono, C. Sumantri, M. Murakami, Y. Kikkawa and T. Suzuki
United Graduate School of Veterinary Science
Yamaguchi University, Yamaguchi 753, Japan.

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 deulbecco’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.