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Vitrification of Bovine Immature Oocytes using Microdrop Method

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Successful cryopreservation of mammalian oocytes would provide a source of materials for *in vitro* embryo production. This study was conducted to determine vitrification conditions for bovine immature oocytes using micro-drop method and, to examine maturation, fertilization and development of vitrified bovine immature oocytes.

In experiment 1, cumulus cells oocytes complexes (COCs) were equilibrated in HEPES buffered TCM 199 + 20% FBS (H-TCM) containing 10% ethylene glycol (EG) and 10% dimethylsulphoxide (DMSO) (VS1) for 0, 1, 3 or 5 min and then immerged to H-TCM containing 20% EG, 20% DMSO and 0.5M sucrose (VS2) for 0.5 min. In experiment 2, COCs were equilibrated to VS1 for 3 min and then transferred to VS2 for 0.5, 1 or 2 min. The oocytes in VS2 droplet were dropped down to liquid nitrogen using micropipette. In experiment 1, the maturation rates of vitrified oocytes in 3 min group (41.1%) of the first equilibration time was higher than that of 0 (21.4%), 1 (33.9%) and 5 min group (27.4%). And normality of microtubule and chromosomes was not significantly differ among 0, 1, 3 and 5 min group. In experiment 2, the maturation rates of vitrified oocytes in 1 min (44.4%) of the second equilibration time was significantly higher than that of 0.5 (28.6%) and 2 min group (21.4%), but lower than that of fresh oocytes (60.5%). And normality of microtubule and chromosomes in 1 min group (52.8%) was significantly ($P<0.05$) higher than those of 0.5 (16.7%) and 2 min group (12.3%) but significantly lower than that of fresh group (100%). This result suggests that vitrification using micro-drop method can be use to cryopreservation of bovine immature oocytes.

Key words: ***Bovine immature oocytes, Vitrification, Micro-drop method, Microtubule, Chromosomes***