

Vitrification of mouse immature oocytes using a straw for preventing the risk of liquid nitrogen contamination

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OBJECTIVE: Selection of a freezing method for oocytes is a prerequisite factor for developing an effective bank system. In previous study, we had examined the effect of vitrification with ethylene glycol (EG)-based cryoprotectants on mouse oocytes in grid, and found higher post-thawed survival and subsequent embryo development than conventional slow freezing methods. The disadvantage of vitrification by using grids is that oocytes with the vitrification solution directly contact with liquid nitrogen during cooling or storage. To protect the risk of LN₂ contamination, this study was conducted to develop effective vitrification method by using straws.

DESIGN: Intact immature oocytes were collected from superovulated ICR mouse and divided into three groups : group 1, no treatment (control) ; group 2, vitrified oocytes with grids, group 3, vitrified oocytes with straws. The morphological survival and spindle configuration were compared in mouse oocytes matured in vitro after vitrification with grids and straws at the germinal vesicle stage.

MATERIALS AND METHODS: Immature oocytes were pre-equilibrated in DPBS with 1.5 M EG for 2.5 min. Oocytes were then vitrified with DPBS with 5.5 M EG and 1.0 M sucrose for 30 sec. Oocytes were loaded onto electron microscope copper grids and plunged into LN₂. The straws were filled with 50 µl of vitrification medium (VM), air, 50 µl of VM containing oocytes, air, and 100 µl of VM. The straws were sealed and immersed directly into LN₂. Stored oocytes were thawed by a five-step method. Control and survived oocytes were cultured in TCM 199 media with 20% FBS for 16 h. In vitro matured oocytes in group 1 and oocytes that survived and matured after vitrification in group 2, 3 were used for immunostaining by using monoclonal anti-tubulin antibody and TRITC-conjugated second antibody.

RESULTS: Rates of survival were 87.3% in group 2 and 86.5% in group 3. Incidence of spindle abnormalities were 19.5%, 27.5%, and 26.0% in group 1, group 2, and group 3, respectively. There was no difference in the survival and spindle abnormalities between group 2 and group 3.

CONCLUSIONS: Vitrification method of oocytes using straws provides a simple, rapid and effective strategy for preventing the risk of LN₂ contamination during storage.