

[O-293] THE DIFFERENCE OF SPINDLE RECOVERY DURING OOCYTE CRYOPRESERVATION: VITRIFICATION VS. SLOW FREEZING.

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OBJECTIVE: The aim of this study was to investigate the difference of oocyte spindle recovery between slow freezing and vitrification for oocyte cryopreservation.

DESIGN: Comparative study.

MATERIALS AND METHODS: Mouse oocytes (8-wk-old BDF1) were collected after 14 h of hCG and then cryopreserved by the vitrification method (15%Ethylene Glycol+15%DMSO+0.5M Sucrose) (Group A) or the slow freezing method (choline chloride substitution media with 1.5 M 1, 2 Propanediol + 0.3 M Sucrose) (Group B), respectively. To examine the spindle, the oocytes were fixed after freezing solution treatment, 0 min of thawing (thawing directly into fixative), and sequential time points after thawing (30 min, 1h, 2h, and 4 h), and stained with propidium iodide and FITC-conjugated anti- α -tubulin antibody by confocal microscopy. Results were analyzed by the Chi-square ($P<0.05$) statistical test.

RESULTS: A total of 330 oocytes were examined in the present study. After treatment with freezing solution, all the examined oocytes were still showing bipolar spindles (A: 23/23; B: 33/33; NS). Next; at 0 min after thawing, all oocytes (A: 28/28; B: 34/34) sustained a morphologically bipolar spindle, (NS). Disorganization of bipolar spindle was observed at 30 min after thawing in group B, but not in group A (A: 21/21; B: 0/29; $P<0.001$). In Group A, all oocytes maintained the bipolar spindle, while in Group B the spindle was recovered only in some oocytes examined at 1h (A: 19/19; B: 23/30; $P<0.05$), 2h (A: 13/13; B: 26/32; NS), and 4h (A: 28/28; B: 34/40; $P<0.05$) after warming/thawing.

CONCLUSIONS: This study demonstrated that slow freezing and thawing could cause spindle disruption, and some of the oocytes will not recover its spindle afterwards. However, the significant alteration of spindle was not found after the vitrification procedure. This study thus indicates that oocyte vitrification may cause less impact on spindle structure than slow freezing.

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