

## Development of Effective Cryopreservation Method for Mouse Oocytes

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**Objective:** The purpose of this study was to evaluate the efficacy and effect of various cryopreservation method on the survival and the cytoskeletal stability of metaphase II mouse oocyte.

**Methods:** Mouse ovulated oocytes were collected and cryopreserved by a modified slow-freezing method with 1.5 M 1,2-propanediol (PrOH)+0.1 M sucrose or by vitrification using cryo loop and EM grid with 40% ethylene glycol+0.6 M sucrose. Four hours after thawing, intact oocytes were fixed and stained with fluorescein isothiocyanate (FITC)-conjugated monoclonal anti- $\alpha$ -tubulin antibody to visualize spindle and propidium iodide (PI) to visualize chromosome. Spindle morphology was classified as follows: normal (barrel-shaped), slightly and absolute abnormal (multipolar or absent).

**Results:** Survival rate of the frozen-thawed oocytes in vitrification group was significantly higher than that of slow-freezing group (62.7% vs. 24.4%,  $p < 0.01$ ). Vitrification with cryo loop showed significantly higher survival rate than that with EM grid (67.7% vs. 53.5%,  $p < 0.05$ ). On the other hand, proportion of normal spindle and chromosome configurations of the frozen-thawed oocytes between two vitrification group was not significantly different.

**Conclusion:** For mouse ovulated oocytes, vitrification with cryo loop may be a preferable procedure compared to slow-freezing method. Further study should be needed to investigate developmental competency of frozen-thawed mouse oocytes.

**Key Words:** Mouse oocyte, Vitrification, Slow-freezing, Spindle, Chromosome

freezing) (modified slow- (vitrification) 48 5 IU human chorionic gonadotropin (hCG, Sigma) hCG 14 Hya- luronidase (Sigma) 1 (first polar body)가 , EM grid, cryo loop, open closed pulled straw (OPS or CPS) 4-10 2. 1) (Modified slow-freezing) 20% fetal bovine serum (FBS) Dulbecco's phosphate-buffered saline (dPBS, GIBCO BRL) , 1.5 M 1,2-propanediol (PROH, Sigma) 0.1 M sucrose (Sigma) 37 5 , 1.5 M PROH 10 , 1.5 M PROH + 0.1 M sucrose 10 0.25 ml straw sealing powder loading 20 -7 1 2 -7 1 forcep (seeding) 5 가 microtubule repolymerization 9 , microtubule 17 digyny (chromosome) spindle 14,17,18 30 0.3 , -30 -150 30 -150 straw straw 40 , 37 1 1.0 M PROH + 0.2 M sucrose, 0.5 M PROH + 0.2 M sucrose 5 , 0.2 M sucrose dPBS 10 37 G-FERT (Vitrolife, Sweden) 2) 10% FBS-dPBS 10% (v/v) ethylene glycol (EG, Sigma) 5 (dehydration equilibration) 40% (v/v) EG + 0.6 M sucrose 5 IU pregnant mare's serum gonadotropin (PMSG, Sigma)

electron microscope grid (EM grid, 400 mesh; Gilder, USA) cryo loop (Hampton, USA) EM grid (barrel-shaped), or absent shaped) . Spindle (multipolar)

60 EM grid 4.

cryo loop 0.5 M sucrose , 0.25 ABstat (rel 6.54, Anderson-Bell Co.) chi-square test , p 0.05

G-FERT

3. (chromosome) 1.

4 permeability modified buffer M (25% glycerol, 50 mM KCl, 0.5 mM MgCl<sub>2</sub>, 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM -mercaptoethanol, 50 mM Imidazol, pH 6.7, 3% Triton X-100, and 25 mM phenylmethylsulfonyl fluoride) <sup>19</sup> 10 (24.4%) (62.7%) (p<0.01). cryo loop EM grid cryo loop 가 (67.7% vs 53.5%, p<0.05) (Table 1). 2. spindle

0.1 mM glycine, 0.01% Triton X-100, 1% powdered milk, 0.5% BSA, 0.02% sodium azide가 가 PBS Cryo loop EM grid spindle (69.3% vs. 60.6%) . cryo loop (65.3% vs. 30 5 µg/ml propidium iodide (PI, Sigma, P-4170) 90 . PBS

**Table 1.** Survival rates of the mouse mature oocytes by different freezing methods

	No. of experiments	No. of oocytes	No. of survival
Slow-freezing	8	234	57 (24.4%) <sup>a</sup>
Vitrification	8	244	153 (62.7%) <sup>b</sup>
Cryo loop		158	107 (67.7%) <sup>c</sup>
EM grid		86	46 (53.5%) <sup>d</sup>

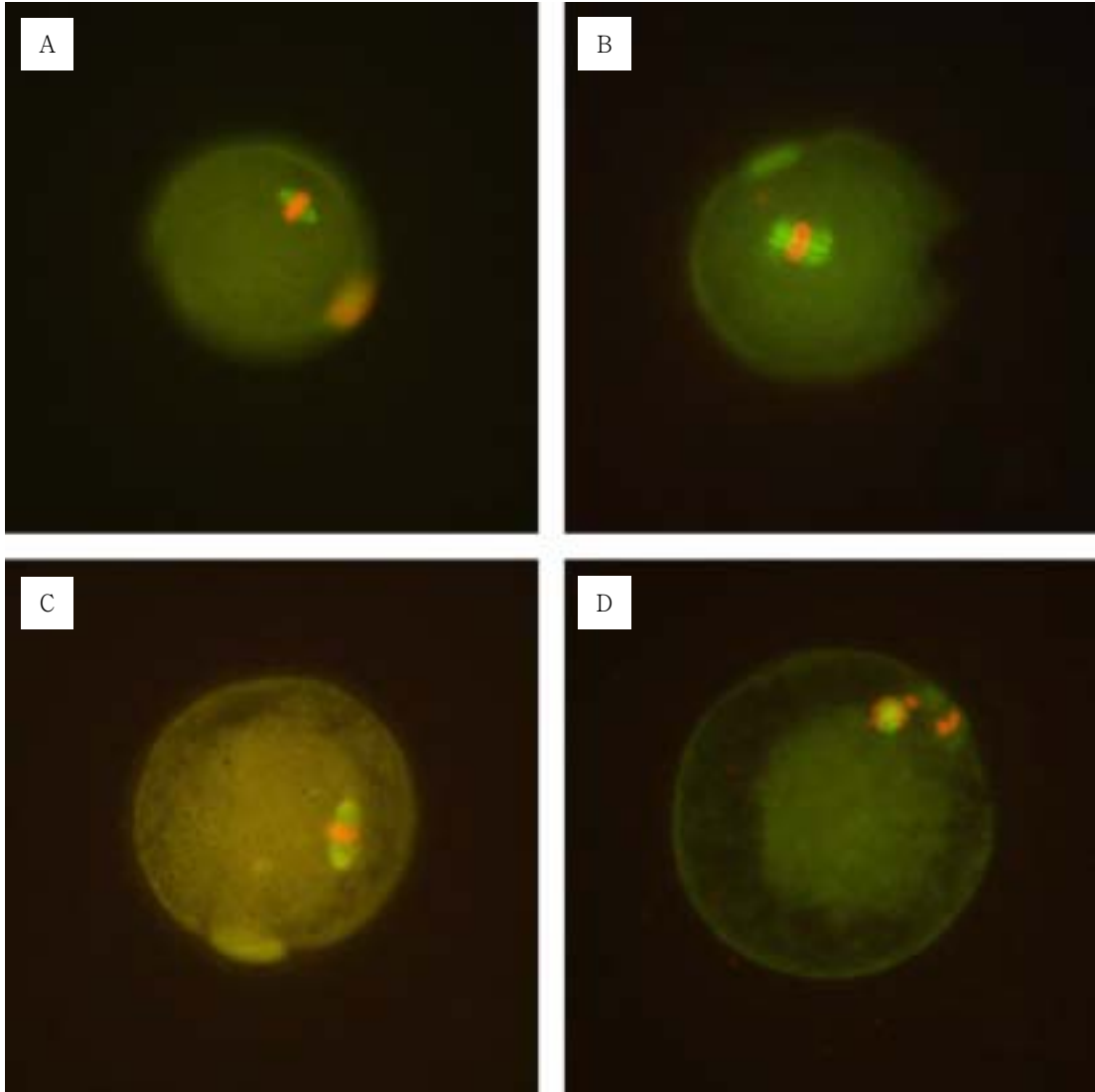
a vs. b, p<0.01; c vs. d, p<0.05.

**Table 2.** Spindle and chromosome configurations of mouse ovulated oocytes after vitrification

	Cryo loop	EM grid
No. of oocytes	75	33
Spindle morphology		
Normal	52 (69.3%)	20 (60.6%)
Slightly abnormal	5 (6.7%)	1 (3.0%)
Abnormal	18 (24.0%)	12 (36.4%)
Chromosome morphology		
Normal	49 (65.3%)	19 (57.6%)
Slightly abnormal	3 (4.0%)	2 (6.1%)
Abnormal	23 (30.7%)	12 (36.4%)

Two groups were not significantly different.

57.6%) (Table 2). PROH 24.4% (24.4% vs. 62.7%,  $p < 0.01$ ).  
 가 cryo loop EM grid (67.7% vs. 53.5%,  $p < 0.05$ ). Chen<sup>10</sup> CPS, OPS, EM grid 79%, 63%, 39% EM grid 53.5% cryo loop OPS, Lane Gardner<sup>23</sup> cryo loop DMSO ethylen-glycol 99.3% tubule microfilament, micro- Park<sup>24</sup> EM grid 73.8% spindle 11-12, 13,20-22 Gook<sup>4</sup> PROH spindle 69.3% 60.6% 4% , Aigner<sup>18</sup> cryo loop Park<sup>24</sup> DMSO 69% EM grid



**Figure 1.** Mouse ovulated oocytes after vitrification were stained with fluorescein isothiocyanate (FITC) to visualize spindle (green) and propidium iodide (PI) to visualize chromosomes (red). A, normal spindle and chromosomes; B, slightly abnormal spindle; C, slightly abnormal chromosomes; D, abnormal spindle and chromosomes.

spindle 65.5% 60.6% , CPS, OPS EM grid spindle  
<sup>25</sup> EM grid ethylenglycol, ficoll sucrose 89~95% 3  
microtubule 가 49.1% , .<sup>10</sup>  
. Chen <sup>9</sup> 가 .  
OPS 가  
spindle 78% 87%

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