

## Cumulus Free Simple Media

### **In vitro Fertilization and Embryo Development in Simple Media of the Frozen-Thawed Cumulus-free Mouse Oocytes Cryopreserved by Vitrification**

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**Objective:** To observe the capability of fertilization and embryo development including blastocyst formation of the oocytes in simple media after thawing of the cryopreserved cumulus-free mouse oocytes by vitrification method.

**Methods:** Oocytes were collected from 5 to 6 weeks old ICR female mice, and were denuded from the cumulus cells by 0.1% hyaluronidase. Recovered mature oocytes in study group were cryopreserved by vitrification method using EM grid for 5 ~7 days. In brief, oocytes were exposed in dPBS containing 1.5 M EG and 5.5 M EG+1 M sucrose for 2.5 minutes and 20 seconds each, and then executed vitrification by plunging in LN2 after loading on EM grid. Thawing treated by exposure of 1, 0.5, 0.25 and 0.125 M sucrose solution for 2.5 minutes each in order and used for experiments. Spermatozoa aspirated from the epididymis of 12 weeks old ICR male mice were used for insemination after capacitation. T6 media containing 0.4% BSA were used for fertilization and development.

**Results:** Survival and fertilization rates after thawing were 76.9% and 79.6% respectively. Fertilization rate was lower ( $p < 0.005$ ) than that of control group (92.9%). There was no difference in embryo developmental rates from 2-cell to morula, however, the blastocyst formation rate and mean cell numbers of blastocysts in study group (63.3%,  $58.9 \pm 9.2$ ) were lower compared with those of control group (76.1%,  $63.5 \pm 8.9$ ).

**Conclusion:** Vitrification is an effective method for mouse mature oocyte cryopreservation with high survival and fertilization rate after thawing. And in simple media, fertilization rates and embryo development of frozen-thawed mouse oocytes are satisfactory.

**Key Words:** Frozen-Thawed by vitrification, Fertilization, Embryo development

1978

가 (assi-

sted reproductive technology)

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5가 126-1,



1 cc , 6.

0.1% hyaluronidase drop ,

3 polar body

1 × 10<sup>6</sup>/ml 가

4. . 5~6 (2PN)

1)

1.5 M ethylene glycol (EG, Sigma)

가 Dulbecco's buffered saline (dPBS, Gibuco)

2.5 5.5 M EG 1 M sucrose가

가 dPBS 20 electron mi-

croscope grid (EM grid, Gilder Co., West Chester, PA)

bisbenzimidazole solution (Hoechst 33258, 100 μM, Sigma)

loading dPBS 3

-196 7 (Olympus, Japan)

UV filter (×400)

2)

1, 0.5, 0.25, 0.125 M sucrose가 가

dPBS EM grid 1 M

sucrose organ dish 2.5~3

0.5, 0.25, 0.125 M 2.5

3

8. Student's t-test

5.

12

(epidid-

ymis) , paraffin

oil 1 cc

drop 가

1.5~2 (Figure 1),

2PN (Figure 2).

1. - 가

147

125 가 85.0%

113 가 76.9%

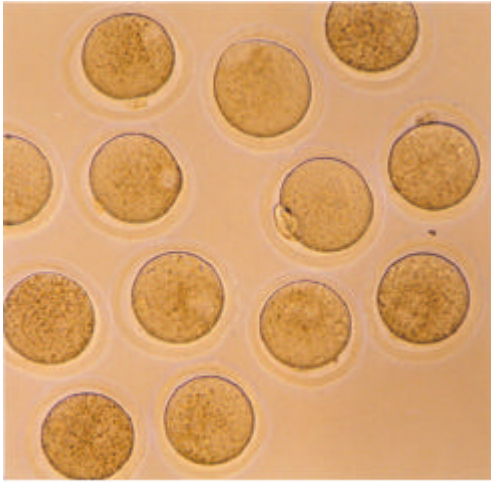
(Table 1).

1 가

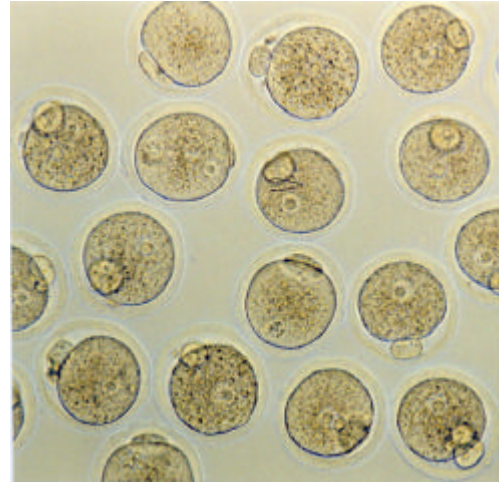
**Table 1.** Comparison of fertilization rates between control and cryopreservation group

	No. of examined oocytes	No. of recovered oocytes (%)	No. of survived oocytes (%)	No. of fertilized oocyte (%)
Control	198	-	-	184 (92.9)
Frozen	147	125 (85.0)	113 (76.9)	90 (79.6)*

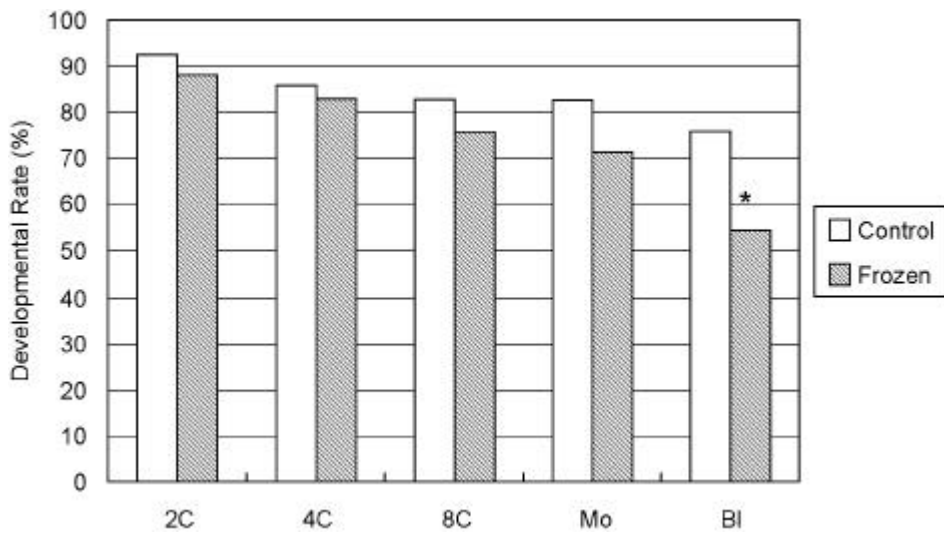
\*: p<0.005



**Figure 1.** Photograph of oocytes after thawing of cryopreservation group (×100).



**Figure 2.** Photograph of fertilized oocytes after in-vitro fertilization of cryopreservation group (×100).



**Figure 3.** Comparison of embryo developmental rates between control and cryopreservation group. \*:  $p < 0.05$

198 , - 가 T6 drop  
 , 184 2 cell, 4  
 92.9% 79.6% cell, 8 cell morula가 92.4, 85.9, 82.6  
 가 (p<0.005) 82.6% , 91.1, 84.4,  
 78.9 76.7%

(Table 1).  
 2.

(Figure 3).

100 μM EDTA가

(Figure 4a, b),



가 , cumulus free  
 - , 1,18  
 - Park 20  
 - 35~70% 가  
 , 19,20 Lane 1 F1 mouse oocyte  
 nylon loop (73.4%) 69.7%  
 laser zona drilling  
 (5 μm) (79.6%) (chromosome abnormality)  
 가 가  
 가 가  
 가 가  
 가 cytoskeletal system  
 - cytoskeletal system  
 . 20-22  
 가  
 in situ  
 . 21,22 Chen 23  
 ICR mouse -  
 58% , Park 20  
 cumulus enclosed  
 22.2%, cumulus free 7.1%  
 , 63.3%  
 .  
 cumulus enclosed 24 -  
 , cytoskeletal system  
 ,  
 (complex media) ,

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