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Comparison of Vitrification and Slow Freezing-thawing Method on 1-cell Zygotes

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Objective: This study was conducted to examine the effect of vitrification on the survival and in vitro development of mice 1-cell zygotes.

Method: Effects of exposure to vitrification solution and vitrification, with different concentrations of the cryoprotectant solution, were examined. The 1-cell zygotes were also subjected to a slow freezing-thawing method to compare with vitrification method. Solution composed of ethylene glycol (6.0 M, 5.0 M, 4.0 M) and sucrose (1.0 M) were used as cryoprotectant. The experiments employed the method loading the embryos on electron microscope grids.

Results:

I. The effects of exposure in vitrification solution

1-cell zygotes were non-toxic at all concentrations of the vitrification solution showing the survival rate between 88.1% and 97.5%. Development into 2-cell was more successful in the higher concentrations of the vitrification solution. Therefore, higher concentrations of the vitrification solution do not seem to cause any problems in vitrification procedure.

II. The effects of vitrification method

1-cell zygotes showed the survival rate between 78.8% and 92.4%. The lowest and the highest survival rate was observed in the 6.0 M and 4.0 M vitrification solution, respectively. 2-cell development rates varied from 77.6% to 91.3%. Blastocyst development rate was shown highest in 5.0 M and the lowest in 4.0 M solution. Therefore, the highest 2-cell and blastocyst development rate was observed in 5.0 M solution.

III. Comparison of vitrification and slow freezing-thawing method on 1-cell zygotes

This experiment showed that 1-cell zygotes had the highest survival and development rates in 5.0 M vitrification solution. Vitrified group of 1-cell zygotes, in the 5.0 M vitrification solution, were compared with the group processed in slow freezing-thawing method. The development rate into 2-cell and blastocyst as well as the survival rate were higher in the vitrified group than in the slowly frozen group.

Conclusion: 1. The results demonstrate that the best cryoprotectant is a 5.0 M vitrification solution for 1-cell zygotes. 2. Vitrification method significantly increases the survival rate of the 1-cell zygote

and its development into 2-cell and blastocyst. Equilibration and exposure time during the vitrification was remarkably short in this experiment. Total time, from the exposure to vitrification solution to storage in the liquid nitrogen, was taken only 90 seconds. In contrast, the slow freezing-thawing method have taken more than four hours. Taken together, we presume that the overall time used for the procedure contributes to the results as an important parameter. 3. The loading of 1-cell zygotes on the EM grid is technically more simple and takes less time than the straw or cryo vial method.

Key Words: Cryoprotectant, Vitrification, Ethylene glycol, Electron microscope grid

1972 Whittingham

DMSO (dimethyl sulfoxide)

가 .⁷²¹ 1 BDF₁ (C₅₇BL/DBA)
(cryoprotective 6~8
agents : CPAs) , 10~12 DBA , 10 /14
DMSO, PROH, glyce- light cycle . preg-
rol , ethylene glycol nant mare's serum gonadotropin (PMSG; G-4877, Sigma,
USA) 7.5 IU 48 human
glucose sucrose , .^{1,6,10,17-20,22,23} chorionic gonadotropin (HCG; C-8554, Sigma, USA) 7.5
IU . HCG
1:1
. .
5,7,12,15 , .^{11,12}
(species) 7 2. 1 -
. HCG 18~22
1985 Rall Fahy¹⁶가 (vitrification) 1 -
67 IU/ml hyaluronidase (Si-
gma, H-3506)
가 .^{1,2,17,22,23} D-PBS 50 mg/ml bovine
serum albumin (BSA; A-9647, Sigma, USA) 가
가 Dulbecco's phosphate buffered saline (D-PBS; 21300-017,
GibcoBL, USA) 37 ,
(ice crystallization) , 5.0% CO₂, 100% (Forma Sci-
automa- entific, 1058) 10% 가 mo-
tic freezer가 10% modified HTF (Basal XI) 1
. .
1 - 가 .
가 .
. 3.
. 10% 가 mo-

dified HTF (Basal XI) 5 . 37 , 5.0% CO₂, 100%
 , 10% 가
 4. modified HTF (Basal XI) 1 .
 ethylene glycol (Sigma, E-9129) 6.0 M, 5.0 M, 4.0 M
 , ethylene glycol 3 mg/ml BSA가 가 D-
 1.0 M sucrose (Sigma, S-1888) 가 . Ethylene PBS 1.5 M PROH 가 ,
 glycol sucrose 10% fetal bovine serum 1.5 M PROH 3 mg/ml BSA가 가 D-
 (FBS) 가 D-PBS (GibcoBRL, 11500-022) 1.0 M, 0.5 M .
 , 0.2 μm filter (Gelman Science, 4192) 1 - 0.3 ml 1.5 M
 sucrose 0.5 M PROH cryo vial (Nunc, 375299)
 , 10% FBS가 가 D-PBS 0.25 cryo vial 30 ,
 M 0.125 M . cryo vial -6 -1 /min
 1 10~ 5
 15 mouth pipet pipetting 10% FBS가 (seeding) . 5
 가 D-PBS -0.5 /min -80
 6.0 M, 5.0 M, 4.0 M 20 -196
 (25) . Pipetting 4 .
 electron microscope grids (EM cryo vial -100
 grids; Gilder 400 M, Cu, v/100, Ted Pella, Inc, Redding, 5 8.0 /min
 CA) . EM grid (20) 5
 0.5 M, 0.25 M, 0.125 M .
 10% FBS가 D-PBS 1.0 M PROH 가 5 , 0.5 M
 10% FBS가 D-PBS 5 , 3 mg/ml BSA가 가
 D-PBS 5 D-PBS 5
 1 -
 5.
 EM grid
 -196 .
 1 30
 24 4 가
 EM grid
 10 . 37 wammmer plate
 0.5 M, 0.25 M, 0.125 M, 8.
 10% FBS가 가 D-PBS 1 CCD-color TV
 , 10% FBS가 가 D-PBS camera system (Polaroid video recorder)

Table 1. In vitro development of 1-cell zygotes exposed to vitrification solutions (VS)

Conc. of VS	Total No.	Survival rate (%) ^a	2-cell embryos (%) ^b	Blastocyst / Survival (%) ^c
Control	87	100 ± 0.0	93.9 ± 4.9	90.4 ± 3.7
6.0 M	86	88.1 ± 9.5	92.9 ± 16.0	55.7 ± 23.4**
5.0 M	76	93.5 ± 6.1	88.7 ± 15.9	56.6 ± 22.3**
4.0 M	88	97.5 ± 3.5	59.4 ± 14.6*	33.8 ± 12.5**

* Development rate into 2-cell embryos; p<0.05, ** Development rate into blastocyst; p<0.05

^a Mean ± SD of survival rate (%); No. of survived 1-cell zygotes/No. of total zygotes

^b Mean ± SD of 2-cell development rate (%); No. of 2-cell embryos/No. of survived 1-cell zygotes

^c Mean ± SD of development rate into blastocyst (%); No. of blastocyst embryos/No. of survived 2-cell zygotes

Table 2. In vitro development of 1-cell zygotes stored at -196 by vitrification

Conc. of VS	Total No.	Survival rate (%)	2-cell embryos (%)	Blastocyst / Survival (%)
6.0 M	108	78.8 ± 22.6	77.6 ± 23.2	55.3 ± 17.6
5.0 M	85	87.0 ± 15.3	91.3 ± 11.7	62.0 ± 14.7
4.0 M	99	92.4 ± 11.2	78.3 ± 20.3	33.8 ± 8.9*

* Development rate into blastocyst; p<0.05

Table 3. In vitro development of 1-cell zygotes. They are exposed to or vitrified in vitrification solution or processed in slow freezing-thawing method

Group	Total No.	Survival rate (%)	2-cell embryo (%)	Blastocyst / Survival (%)
Vitrified (5.0 M)	85	87.0 ± 15.3	91.3 ± 11.7	62.0 ± 14.7
Slow freezing	64	62.7 ± 4.2	49.5 ± 4.2	19.0 ± 5.1

Vitrified and slow freezing group show significantly different results (p<0.001).

200 . 93.9±4.9%

9. 90.4 ± 3.7% (Table 1).

1 -

, 2-

88.1~97.5%

SPSS pc version 7.5 one way AN- 6.0 M

OVA , p<0.05 , 2- 4.0 M 59.4±

14.6% 6.0 M 5.0 M

(p<0.05).

4.0 M 33.8±

12.5% 5.0 M 6.0 M 56.6±22.3%

1. 1 - 55.7 ± 23.4%

(p<0.05) (Table 1, Fig-

1 - 2 - ure 1).

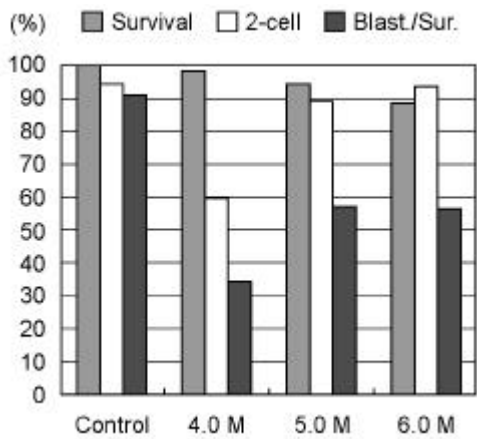


Figure 1. In vitro development of 1-cell zygotes exposed to vitrification solution.

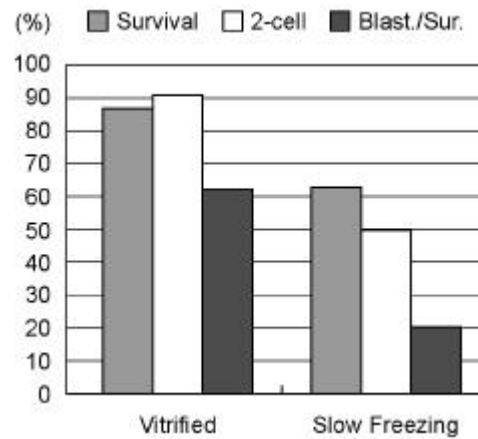


Figure 3. In vitro development of 1-cell zygotes. They are exposed to or vitrified in 5.0 M vitrification solution or processed in slow freezing-thawing method.

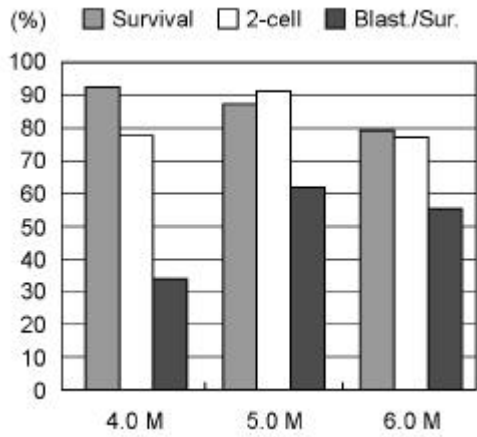


Figure 2. In vitro development of 1-cell zygotes stored at -196 by vitrification.

1 - EM grid
 hyaluronidase 가
 . Figure 5 24 2 -
 , Figure 6 4
 .
 3. 1 -
 -
 가 5.0
 M -
 - 62.7 ± 4.2% 2 -
 49.5 ± 4.2%
 19.0 ± 5.1% . , 2 -
 -
 (p<0.001)

(Table 3, Figure 3).

2. - 1 -
 - 6.0 M 78.8 ±
 22.6%, 5.0 M 87.0 ± 15.30%, 4.0 M 92.4 ±
 11.2% , 2 -
 6.0 M 77.6 ± 23.2%, 5.0 M
 91.3 ± 11.7%, 4.0 M 78.3 ± 20.3% 5.0 M
 가 . 4.0 M 가 , ,
 33.8 ± 8.9% 5.0 M 62.0 ± 14.7% 6.0 M 가 ,
 55.3 ± 17.6%
 (p<0.05) (Table 2, Figure 2). Figure 4 - 4.20

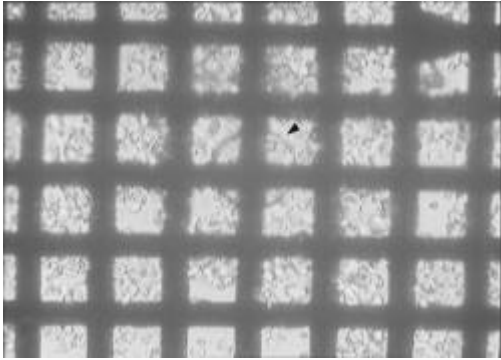


Figure 4. 1-cell zygotes (arrowhead) on EM grid after vitrification and thawing (× 200).

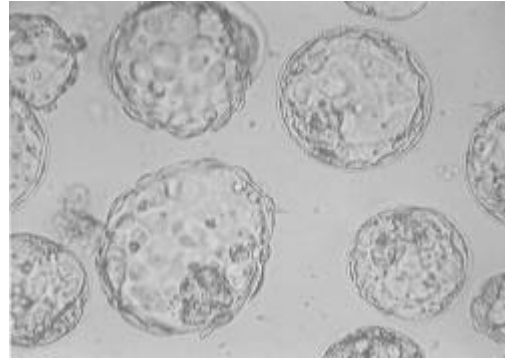


Figure 6. Blastocyst after vitrification and thawing (× 200).



Figure 5. 2-cell stage embryos after vitrification and thawing (× 200).

17 (1.5 M 2 M)
 3.5 M 7.5 M
 DMSO, 1,2-PROH, glycerol 가
 ethylene glycol
 3 10
 -80
 , -100
 Ali Shelton¹²
 VS14 (5.5 M ethylene glycol 1.0 M sucrose가)
 6.0 M 4.0 M ethylene glycol
 EM grid Martino¹³
 (EM grid) . Ali Shelton
 Swiss outbred 2- 81%
 - 71%
 BDF₁
 5.0 M ethylene glycol 1.0 M sucrose가
 93.5±6.1%
 - 87.0±15.3%
 Ali Shelton

가 가
 가
 가
 가
 (supercooled)
 가
 가

1990 Kasai¹⁰ 40% ethylene glycol, straw 6.0 M 4.0 M
30% ficoll, 20% sucrose (EFS40) 98% 5.0 M
51% EFS-
40^{22-24, 13} 가 2 -
1996 49.5±4.2%
Martino¹³ 5.5 M 4.0 M ethylene glycol 1.0 M 0.5 M sucrose가 77.6~91.3%
35 20
, sample container ethylene glycol
straw EM grid -
straw가 34%, EM grid가 51~72% 가
EM grid -
15% 가 , EM grid
1- EM grid straw cryo vial
ethylene glycol 1.0 M sucrose가 가
1-
straw Kasai (1990)
1996 Kim²³ EFS40 (ethylene glycol 40%가)
. Kim EFS40 1-
1
2- 85.5%,
53.2% , 1-
78.8~92.4% , 2- 77.6~91.3%
EFS40
5.0 M EFS40
1- 5.0 M
, 2-
(p<0.001). ethylene glycol 가
,⁹ ethylene glycol (organogenesis)
.⁸ 가 가

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