

=ABSTRACT=

Viability of Frozen-Thawed Embryos According to Developmental Stage in Mouse Embryo Cryopreservation

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Object : To compare the viability after thawing of cryopreserved embryos according to developmental stage and decide the cryopreservation strategy

Methods : Total 538 mouse embryos (One, 2, 4, 8-cell, blastocyst, each 151, 136, 101, 98, 52, respectively) were cryopreserved and thawed using 1,2-propanediol and glycerol as cryoprotectant with slow cooling and rapid thawing technique, and compared the recovery, cleavage, blastocyst development rate, and calculated the recovery and reexpansion rate in blastocyst cryopreservation.

Results : Highest recovery, cleavage and blastocyst development rate were obtained from one cell, 8-cell stage freezing, 90.1%, 89.5% and 76.7%, respectively. In recovery rate, there was no significant difference among developmental stage, but in cleavage rate, there was significant difference between 2 and 8-cell group ($p < 0.05$). In blastocyst development rate, there was significant difference between 2 and 8 cell, 4 and 8-cell group ($p < 0.05$). Recovery and reexpansion rate of frozen-thawed blastocyst was 73.1%, 52.6%, respectively.

Conclusion : Eight-cell embryo may be the best developmental stage for cryopreservation and blastocyst freezing also may be the promising technique.

Key Words : Mouse Embryos Cryopreservation, Viability, Developmental stage

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20-40%

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* 1999

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BCF₁(C₅₇BL/6XCBA)

2-4 5 IU PMSG(pregnant mare serum gonadotropin) 48 5 IU hCG(IVF-C,)

1:1

hCG 18-20 TCM 199 30 gauze (Oocyte-Cumulus Complex) 0.1% Hyaluronidase

3-5 3-5

20% TCM 199 2 , 4

1-2 , 8 24, 48, 72 가 (anucleated fragmentation)가

2. , 2, 4, 8 (1) 20% Propanediol 가 (0.2 μm filter) D-PBS 1.2-1.5 M PROH 5 ml Sucrose 0.342 gm 가 0.2 M Sucrose

PBS + 20% hFCS 5

0.5 M, 1.0 M, 1.5 M PROH, 1.5 M PROH +

0.2 M Sucrose 5 Plastic Straw 3-4 가 (Kryo 10 Series, Planner) . -7 2 5 Forceps 5 0.3 -39 5

(2) 4 (TCM 199 with 20% hFCS) Straw 40 30 1.5 M PROH 5 0.5 M PROH, 0.2 M Sucrose in PBS + 20% hFCS, PBS + 20% hFCS 5

3. (1) PBS 20% Glycerol 가, 1,2,4,6,8% Glycerol 10 8% Glycerol 가 37 8% Glycerol Straw 37 18 5 18 5 -7 1 -7 5 0.3 -37 5

(2) Straw 10 30 30 Straw 8, 6, 5, 4, 3, 2, 1% Glycerol 5, 10, 12, 12, 14, 14, 16 PBS 2-3

4. - 2 4

Table 1. Recovery, cleavage and blastocyst development rates after freezing and thawing according to cleavage stage.

Cleavage stage	No. of frozen embryos (Experiment times)	No. of recovered embryos (Recovery rate)	No. of cleaved embryos (Cleavage rate)	No. of blastocyst development (Blastocyst develop. rate)
2 PN	151(13)	136(90.1%)	109(80.1%)	81(59.6%)
2 cell	136(12)	117(86.0%)	80(68.4%)	65(55.6%)
4 cell	101(11)	88(87.1%)	71(80.7%)	46(52.3%)
8 cell	98(12)	86(87.8%)	77(89.5%)	66(76.7%)

*Recovery rate : No. of normal microscopic feature after the removal of freezing solution/No. of frozen embryos × 100
 **Cleavage rate : No. of cleaved embryos/No. of recovered embryos × 100
 ***Blastocyst development rate : No. of blastocyst development/No. of recovered embryos × 100

Table 2. Blastocyst viability after freezing and thawing

Experiment orders	No. of frozen embryos	No. of recovered embryos (Recovery rate)	No. of blastocyst reexpansion (reexpansion rate)
1	10	7(70%)	4(57.1%)
2	10	8(80%)	5(62.5%)
3	10	7(70%)	3(42.9%)
4	12	9(75%)	4(44.4%)
5	10	7(70%)	4(57.1%)
Total	52	38(73.1%)	20(52.6%)

* Recovery rate : No. of normal microscopic feature after the removal of freezing solution/No. of frozen embryos × 100
 ** Reexpansion rate : No. of reexpanded blastocyst/No. of recovered blastocyst × 100

50% 가
 24
 24 2

가

5.

Chi square, t-test
 P<0.05

538
 , 2 , 4 , 8 ,
 151, 136, 101, 98, 52 -
 90.1%, 86.0%, 87.1%, 87.8%

가

80.1%, 68.4%, 80.7%, 89.5% 8

가 가 (P<0.05),
 4 8 가 2
 4 가 (P<0.05),
 가 (Table 1).
 5 52
 73.1%,
 52.6% (Table 2),

8 가 가
 8 가 가
 73.1% 52.6%

Trounson Mohr DMSO(Dimethylsulfoxide)

8

1972 Whittingham 1983
 1984 Zeilmaker

1985 Cohen 1,13,15,16 DMSO, PROH(1,2-Propanediol), (>1 /), (<0.5 /), (<10 /)

1999 Fugger 2-4 2-6

Demoulin 7,20,21 2, 4 가 2 , Lassalle 4, 8

2,22 가 4, 8

1,10,17 가 8 Trounson Gly- cerol DMSO 4-8

Freemann 4-12 8 가 Quinn 2, 4, 8 8 가 90%

4,10,23,25 가 Mandelbaum PROH Sucrose 2-3 가 Trounson 2-8 가 Kondo 1-3 ()

11 1988 Cohen 106 - 8) 70% 15% 35%

Kalström

Sucrose Horne PROH (2-4)

18 Gordts 94%, 37.5% 79% 2-6 12,15,24,26,27 가 Fehilly

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: 538 (, 2- , 4- , 8- , , 151, 136, 101, 98, 52)

1.2-Propanediol, Glycerol

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가 (90.1%), 8 가

89.5%, 76.7%

8 2 4 2

73.1%, 52.6%

: 8 가

가

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