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Ethylene Glycol (EG)  
1,2-Propanediol (PROH)<sup>\*</sup>

Effect of Ethylene Glycol (EG) and 1,2-Propanediol (PROH) on the  
Survival and the Development of Mouse and Human Embryos  
after Slow Freezing/Rapid Thawing Protocol

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**Objective:** The aim of this study were to compare the effects of EG and PROH on cryopreservation of mouse and human embryos, and to find the optimal protocol for embryo freezing.

**Methods:** Human embryos derived from fertilized eggs showing 3 pronuclei (PN) and mouse embryos were divided into two groups respectively: dehydrated with 1.5 M EG + 0.2 M sucrose or 1.5 M PROH + 0.2 M sucrose using the slow freezing method. Moreover mouse embryos were controlled the exposure time of cryoprotectant during dehydration or rehydration steps.

**Results:** The survival rates of human embryos were 79.2% (84/106) in EG group and 77.9% (88/113) in PROH group. In mouse embryos, the survival and development rates up to blastocyst were 70.6% (245/347), 44.1% (123/279) in EG group and 62.1% (198/319), 45.1% (123/279) in PROH group, respectively. However, in EG group, partially damaged embryos after thawing were decreased compared to PROH group. In combination group, when the exposure time during dehydration and rehydration were reduced, the survival and embryonic developments were increased slightly, but not significant.

**Conclusion:** Cryopreservation of mouse and human embryos at cleavage stage by using EG or PROH exhibited no statistical difference in the survival rate and/or developmental rate to blastocyst. However, the use of EG for cryopreservation of embryos might reduce the exposure time of the cryoprotectant because of a high permeation of EG and result in lessen its toxic effects.

**Key Words:** Ethylene glycol, Slow freezing, Human embryos, Mouse embryos

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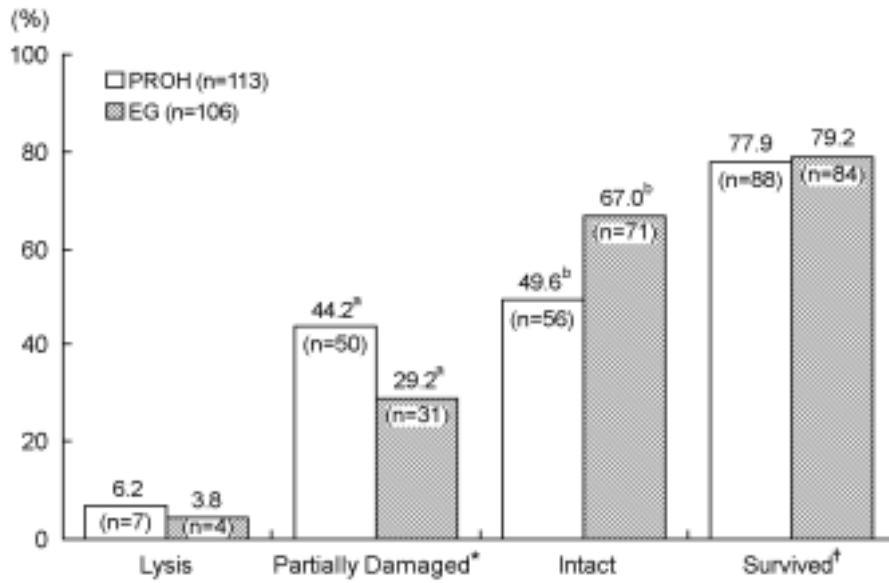
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가 .  
PROH EG 가  
가 가  
7-9 EG , ,  
가  
(OHSS)  
가 , 10-13 EG  
1983 Trounson Mohr . 11 가  
4-8 1.5 M dime-  
thylsulphoxide (DMSO) -80 가  
PROH  
(1984) 1.45 M DMSO . 1 Zeilmaker Glycerol  
-40 . 2 14,15 EG 가  
Lassalle (1985) 4 가 7,9  
1.5 M 1,2-propanediol (PROH) + 0.1 M  
sucrose -30  
3  
1983 DMSO PROH  
가  
ethylene glycol (EG)  
1.  
1)  
3~4 ICR pregnant mare's  
serum gonadotropin (PMSG, Intervet international B.V)  
5 IU , 48 human chorionic  
gonadotropin (hCG, Intervet) 5 IU  
. 42~44 h  
2  
10% synthetic serum substitute (SSS, Irvine Scien-  
tific Co.)가 가 preimplantation I (P-I, Irvine)  
4 가 EG 6-8  
. 2 day2 .  
day6  
10% SSS가 가 blastocyst (Irvine)

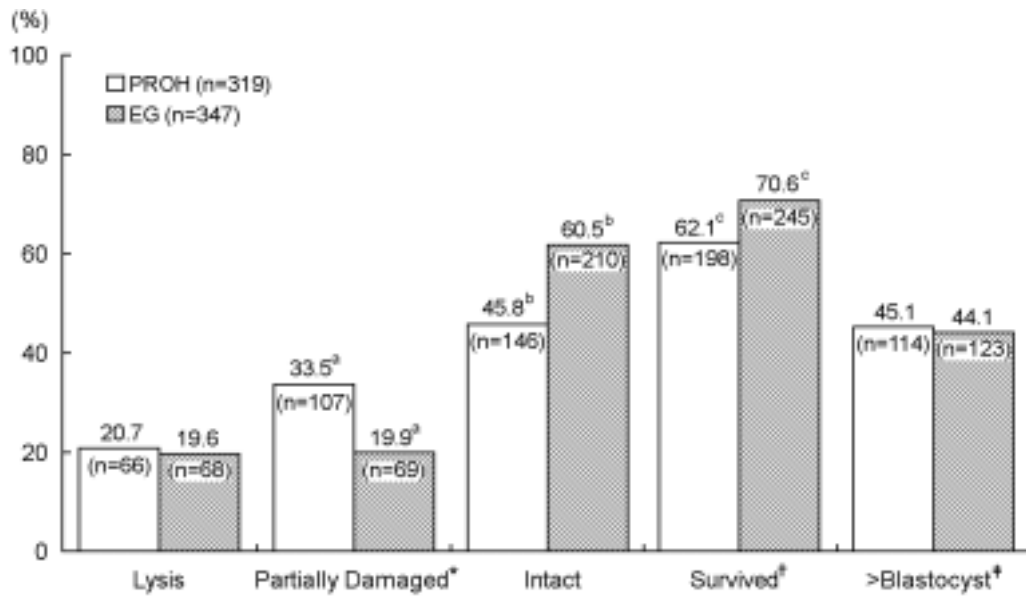
2) 3PN  
 GnRH agonist follicle stimulating hormone (FSH)/  
 human menopausal gonadotrophin (hMG)  
 18 mm 가 2  
 10,000 IU hCG (propasi, Se-  
 rono) hCG  
 36~38 h  
 4~6  
 (intracyto-  
 plasmic sperm injection, ICSI)  
 10% SSS가 가 P-I  
 14~18 h (pronuclei, PN)  
 , 3  
 4-8

2.  
 1) PROH EG  
 20% fetal bovine  
 serum (FBS, GIBCO BRL)가 가 Dulbecco's  
 phosphate-buffered saline (DPBS, GIBCO)  
 , 1.5 M ethylene glycol (EG,  
 Sigma Chemical Co.) 1,2-propanediol (PROH, Si-  
 gma), 0.2 M sucrose (Sigma)  
 20% FBS - DPBS, 0.5 M EG, 1.0 M EG, 1.5  
 M EG, 1.5 M EG + 0.2 M sucrose  
 5, 5, 5, 10, 5  
 PROH  
 0.25 ml plastic-straw loading 24  
 (Kryo 10 serise III, Planer)  
 . 24 -7 2 /  
 min , 5  
 (seeding) . -39 0.3/  
 min  
 straw  
 40 37 water bath 40 49.6% , EG , 3.8%, 29.2%,  
 . 4 (equilibration) 67.0% . lysis가  
 , 1 M EG + 0.2 M sucrose, 0.5 PROH (p<0.05),  
 M EG + 0.2 M sucrose, 0.2 M sucrose, 20% lysis가 EG

FBS-DPBS 5, 5, 5, 5  
 , PROH  
 2) PROH EG  
 1  
 3) EG  
 EG  
 1, 2  
 ( ) EGF1 (5-5-5-10-  
 5), EGF2 (3-3-3-6-1), EGF3 (1-1-1-2-1)  
 EGF1 ( ) EGT1  
 (5-5-5-5), EGT2 (3-3-3-3), EGT3 (1-1-1-1)  
 3.  
 Chi-square test  
 p 0.05  
 가  
 3PN  
 EG (n=106) PROH (n=113)  
 (Figure 1).  
 . PROH  
 , lysis 6.2%, lysis가  
 44.2%, lysis가  
 , EG , 3.8%, 29.2%,  
 lysis가  
 PROH (p<0.05),  
 lysis가 EG



**Figure 1.** Survival rate of human embryos frozen-thawed with 1.5 M PROH or 1.5 M EG. The human embryos were derived from 3PN. The values with same superscripts are significantly different ( $p < 0.05$ ).  
 \*The Partially damaged embryos include the embryos having more than 1 intact blastomere.  
 †The survived embryos include the embryos showing more than half intact blastomeres.



**Figure 2.** Survival and developmental rates of mouse embryos frozen-thawed with 1.5 M PROH or 1.5 M EG. The values with same superscripts are significantly different ( $p < 0.05$ ).  
 \*The Partially damaged embryos include the embryos having more than 1 intact blastomere.  
 †The survived embryos include the embryos showing more than half intact blastomeres.  
 ‡>Blastocyst include the embryos developed to blastocyst, hatching, and hatched blastocyst.

**Table 1.** Effects of exposure time during freezing and thawing on development of mouse embryos frozen with 1.5 M EG + 0.5 M sucrose

|               | Group                | No. of embryos frozen-thawed | No. of embryos survived (%) | No. of embryos developed to blastocyst (%) |
|---------------|----------------------|------------------------------|-----------------------------|--|
| Freezing step | Control <sup>‡</sup> | 138                          | 135 (97.8)                  | 114 (82.6) <sup>a</sup>                    |
|               | EGF1 <sup>*</sup>    | 123                          | 121 (98.4)                  | 94 (76.4) <sup>ab</sup>                    |
|               | EGF2 <sup>*</sup>    | 96                           | 92 (95.8)                   | 69 (71.9) <sup>b</sup>                     |
|               | EGF3 <sup>*</sup>    | 101                          | 94 (93.1)                   | 62 (61.4) <sup>c</sup>                     |
| Thawing step  | Control <sup>‡</sup> | 98                           | 95 (96.9)                   | 75 (76.5) <sup>a</sup>                     |
|               | EGT1 <sup>†</sup>    | 79                           | 76 (96.2)                   | 41 (51.9) <sup>b</sup>                     |
|               | EGT2 <sup>†</sup>    | 74                           | 68 (91.9)                   | 48 (64.9) <sup>ab</sup>                    |
|               | EGT3 <sup>†</sup>    | 76                           | 75 (98.7)                   | 48 (63.2) <sup>ab</sup>                    |

<sup>\*</sup>The exposure time during freezing step of EGF1 is 5-5-5-10-5, EGF2 is 3-3-3-6-3, and EGF3 is 1-1-1-2-1 (minute)

The exposure time during thawing step is 5-5-5-5 (minute)

<sup>†</sup>The exposure time during freezing step is 5-5-5-10-5 (minute)

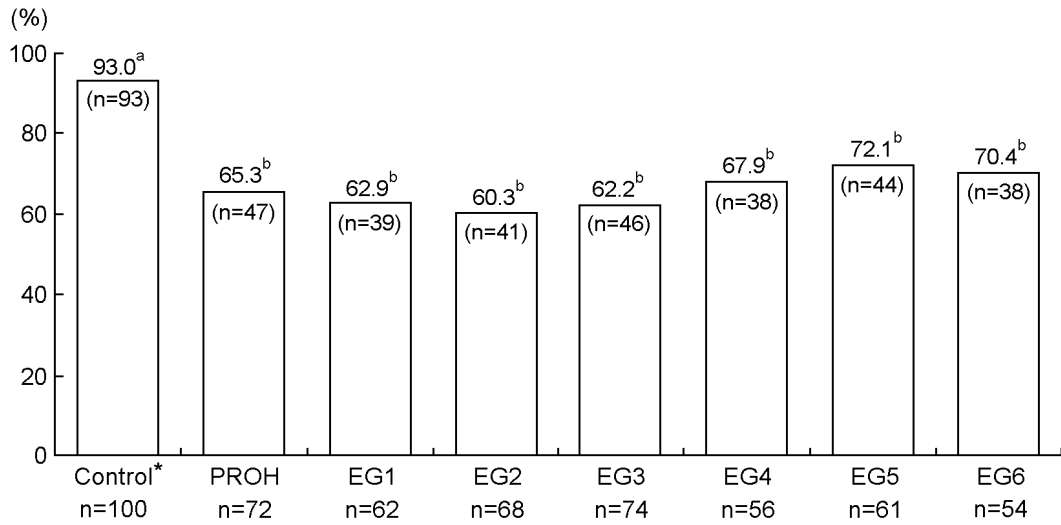
The exposure time during thawing step of EGT1 is 5-5-5-5, EGT2 is 3-3-3-3, and EGT3 is 1-1-1-1 (minute)

<sup>‡</sup>A control group was cultured for 5 days without being exposed to the cryoprotectant solution or frozen

The values with different superscripts in the column are significantly different (p<0.05)

(p<0.01). 50% lysis가 lysis PROH 20.6% (52/253), lysis가 EG 28.0% (78/279) EG (p<0.05). PROH가 77.9%, EG가 79.2% EG

3  
EG (n=347) PROH (n=319) (Table 1). EGF1 EGF2  
ICR 76.4%, 71.9% EGF3  
(Figure 2). , control EGF2, 3 , EGF1, 2  
. PROH EGF3 .  
EG lysis 20.7% 19.6% EGT2 EGT3  
, lysis가 33.5% 가 64.9%, 63.2% EGT1  
19.9% PROH가 (p<0.001), lysis가 EGT1  
45.8% 60.5% EG가 가 control 가 .  
(p<0.001), 3PN  
50%  
lysis가 lysis가 EGF1 (5-5-5-10-5) EGF2 (3-3-3-6-3)  
PROH가 62.1%, EG , EGT1 (5-5-5-5) EGT-  
가 70.6% (p<0.05). 2 (3-3-3-3) EGT3 (1-1-1-1)가  
3 , EGT1  
가 3  
PROH 45.1% (114/253), EG PROH 가  
44.1% (123/279) . (Figure 3). Day5  
PROH 24.1% (61/253), EG  
16.5% (46/279) PROH (p<0.05), PROH EGT1



| Group | Freezing 5 step (minute) | Thawing 4 step (minute) |
|-------|--------------------------|-------------------------|
| PROH  | 5-5-5-10-5               | 5-5-5-5                 |
| EG1   | 5-5-5-10-5               | 5-5-5-5                 |
| EG2   | 5-5-5-10-5               | 3-3-3-3                 |
| EG3   | 5-5-5-10-5               | 1-1-1-1                 |
| EG4   | 3-3-3-6-3                | 5-5-5-5                 |
| EG5   | 3-3-3-6-3                | 3-3-3-3                 |
| EG6   | 3-3-3-6-3                | 1-1-1-1                 |

**Figure 3.** Combination of exposure time during freezing and thawing step. Developmental rates to blastocyst of mouse embryos frozen-thawed with 1.5 M PROH or 1.5 M EG were investigated. Blastocyst include the embryos developed to blastocyst, hatching, and hatched blastocyst. The values with different superscripts are significantly different ( $p < 0.05$ ). \*A control group was cultured for 5 days without being exposed to the cryoprotectant solution or frozen

65.3% 62.9% , EG5 EG-  
 6가 72.1%, 70.4% 가  
 PROH EG1  
 가 .<sup>11</sup> EG PROH  
 3PN  
 lysis  
 EG PROH 1 2  
 lysis가  
 PROH가 EG  
 (Figure 1, Figure 2). lysis가  
 EG가 PROH  
 PROH EG가

PROH osmotic swelling<sup>17</sup> lysis가

EG lysis가

EG가

Figure 3 EG 5-5-5-5 (EG5) 3-3-3-6-3 1-1-1-1 (EG6) 5-5-5-10-5, (EG1) 3-

EG가

lysis가 50% 가 가 , PROH EG (diffu- sion) (equilibration) 가

PROH day5 lysis가

EG PROH EG2 EG3 가

PROH EG 1-2-1 (EGF3) 1-1-

가 (Table 1)

DMSO PROH가 1980 가

EG<sup>19</sup> 가<sup>17</sup>

EG 3PN

PROH lysis

가 EG

PROH 가 3

가 PROH 2 3, 5, 7 2, 4, 6, 8 가<sup>3</sup>

, 1.5 M

6-8  
 , 2-5  
 ,  
 EG PROH  
 .  
 가  
 .  
 EG PROH  
 .  
 2PN  
 IVF  
 3PN  
 .  
 EG가 PROH  
 .  
 EG PROH  
 lysis가  
 가 , lysis가  
 .  
 가  
 가  
 .  
 ,  
 PROH  
 .

1. Trounson A, Mohr L. Human pregnancy following cryopreservation, thawing and transfer of and eight-cell embryo. *Nature* 1983; 305: 707-9.
2. Zeilmaker GH, Alberta AT, van Gent I. Two pregnancies following transfer of intact frozen-thawed embryos. *Fertil Steril* 1984; 42: 293-26.
3. Lassalle B, Testart J, Renard JP. Human embryo features that influence the success of cryopreservation with the use of 1,2-propanediol. *Fertil Steril* 1985; 44: 645-51.

4. Kasai M, Nishimori M, Zhu SE, Sakurai T, Machida T. Survival of mouse morulae vitrified in an ethylene glycol-based solution after exposure to the solution at various temperatures. *Biol Reprod* 1992b; 47: 1134-9.
5. Kasai M, Komi JH, Takakamo A, Tsudera H, Sakurai T, Machida T. A simple method for mouse embryo cryopreservation in a low toxicity vitrification solution, without appreciable loss of viability. *J Reprod Fertil* 1990; 89: 91-7.
6. Cocero MJ, Moreno S, Aguilar B. Ultrastructural characteristics of fresh and frozen-thawed bovine embryos using two cryoprotectants. *Biol Reprod* 2002; 66: 1244-58.
7. Shaw JM, Ward C, Trounson AO. Evaluation of propanediol, ethylene glycol, sucrose and anti-freeze proteins on the survival of slow-cooled mouse pronuclear and 4-cell embryos. *Hum Reprod* 1995; 10: 396-402.
8. Chi HJ, Koo JJ, Kim MY, Joo JY, Chang SS, Chung KS. Cryopreservation of human embryos using ethylene glycol in controlled slow freezing. *Hum Reprod* 2002; 17: 2146-51.
9. Emiliani S, Bergh MV, Vannin A, Biramane J, Englert Y. Comparison of ethylene glycol, 1,2-propanediol and glycerol for cryopreservation of slow-cooled mouse zygotes, 4-cell embryos and blastocysts. *Hum Reprod* 2000; 15: 905-10.
10. Cho HJ, Son WY, Yoon SH, Lee SW, Lim JH. An improved protocol for dilution of cryoprotectant from vitrified human blastocysts. *Hum Reprod* 2002; 9: 2419-22.
11. Newton H, Fisher J, Arnold JRP, Pegg DE, Faddy MJ, Gosden RG. Permeation of human ovarian tissue with cryoprotective agents in preparation for cryopreservation. *Hum Reprod* 1998; 13: 376-80.
12. Park SP, Kim EY, Kim DI, Park NH, Won YS, Yoon SH, et al. Simple, efficient and successful vitrification of bovine blastocyst using electron microscope grids. *Hum Reprod* 1999; 14:



- 2838-43.
13. Yoon TK, Chung HM, Lim JM, Han SY, Ko JJ, Cha KY. Pregnancy and delivery of healthy infants developed from vitrified oocytes in a stimulated in vitro fertiliation-embryo transfer program. *Fertil Steril* 2000; 74: 180-1.
  14. Ali J, Shelton N. Design of vitrification solution for the cryopreservation of embryos. *J Reprod Fertil* 1993; 99: 471-7.
  15. Summerfeld V, Niemann H. Cryopreservation of bovine in vitro produced embryos using ethylene glycol in controlled freezing or vitrification. *Cryobiology* 1999; 38: 95-105.
  16. Guerif F, Bidault R, Cadoret V, Couet M, Lanasac J, Royere D. Parameter guiding selection of best embryos for transfer after cryopreservation: a reappraisal. *Hum Reprod* 2002; 17: 1321-6.
  17. Mukaida T, Wada S, Takahashi K, Pedro PB, An TZ, Kasai M. Vitrification of human embryos based on the assessment of suitable conditions for 8-cell mouse embryos. *Hum Reprod* 1998; 13: 2874-9.
  18. Burns WN, Gaudet TW, Martin MB, Ramiro Leal Y, Schoen H, Eddy CA, et al. Survival of cryopreservation and thawing with all blastomeres intact identifies multicell embryos with superior frozen embryo transfer outcome. *Fertil Steril* 1999; 72: 527-32.
  19. Paynter SJ, O'Neil L, Fuller BJ, Shaw RW. Membrane permeability of human oocytes in the presence of the cryoprotectant propane-1,2-diol. *Fertil Steril* 2001; 75: 532-8.
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