

인간포배기 배아의 효과적인 유리화 동결법의 개발을 위한 연구

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Study on the Development of Efficient Vitrification of Human Blastocysts

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Objective: The purpose of this study was to evaluate the survival rate of vitrified blastocyst according to the freezing vessels, equilibration time in cryoprotectant and artificial dehydration method.

Methods: Human blastocysts were vitrified after loading onto the plastic straw, open-pulled straw (OPS), electron microscopy grid (EM grid) for 1.5 min or 3 min. They also were directly plunged into LN₂ within 30sec. For artificial shrinkage of blastocysts, 36 gauge fine needle was pushed at the cellular junction of the trophectoderm into the blastocoele cavity until it shrank without damage of inner cell mass.

Results: The survival rate of vitrified blastocysts on plastic straw, OPS, EM grid as freezing vessels were 26.7, 13.0 and 60.5%, respectively. The survival rate of EM grid was significantly higher than that of plastic straw and OPS (p<0.05). For 1.5 min equilibrium, the survival rates of early blastocyst (EB), middle blastocyst (MB) and late blastocyst (LB) were 64.4, 81.0, and 20.0% respectively. For 3 min equilibrium, the survival rates of EB, MB, and LB were 69.9, 50.0 and 57.5% respectively. The survival rates of EB and MB were significantly higher than that of LB in 1.5 min equilibrium group (p<0.05), however, the significance was not observed in 3 min equilibrium groups. In cytoplasmic shrinkage before vitrification, the survival rates of EB, MB and LB were 92.9, 100 and 75.9% respectively. The survival rate of MB was significantly higher than that of LB (p<0.05). The survival rates of vitrified blastocysts by artificial dehydration and slow-frozen blastocysts were not significantly different as 88.9 and 66.7%, respectively.

Conclusion: This study showed that the vitrification of human blastocysts using EM grid and artificial dehydration is an effective method. Therefore, these methods would be an useful techniques for blastocyst cryopreservation.

Key Words: Cryopreservation, Artificial dehydration, Human blastocysts

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5 lene glycol, ficoll sucrose³ ethy-
(EFS)
4-8

가

가 가 가

2

6 plastic straw,
EM grid, cryoloop

가 가

가 가

electron microscopy grid (EM grid)
9,10 Park
가 가⁷

(gel)

가

glycerol, ethylene glycol, dimethyl sulphoxide, pro-
pylene glycol 1.
sucrose, treha-
lose IVF-ET
83

ficoll, polyvinylpyrrolidone (PVP), polyethylene
glycol (PEG)

가

2. GnRH-agonist (Buserelin, Suprefact, FSH/hMG, 17~18 hCG (pro-fasi, Serono), 36~38, 10% YS 1x, 10⁵ cells/ml, 37, 5% CO₂, 14~18, 37, 5% CO₂, 24, 20% hFF 가 YS, 5, 2 가, (1, 3, 5~6), EB (), MB (), LB ()¹¹

3) (1) Plastic straw
0.25 ml plastic straw EFS40 EG-
20 1.5 straw 1
5 straw 10

(2) Open pulled straw (OPS)
OPS EG20 1.5
EFS40
1 μl
0.5 M Sucrose 가
OPS
OPS

(3) EM grid
400 mesh copper EM grid (IGC 400; Pelco International) EG20 1.5~3
EFS40 EM grid
30

3. 4)
36 gauge needle (Hamilton, 90033)
ethylene glycol (EG: Sigma, E-9129) 가 (PBS) 20% (v/v)
(EG20), 40% (v/v) EG, 18% (w/v) EM grid
Ficoll (MW 70,000; Sigma, F-2878), 0.3 M Sucrose EG20
가 (EFS40) 3 EFS40
0.5 M 0.25 M Sucrose (30)
가
2) 4. -
EG20 1.5~3, EFS40 hFF 가 YS 3~4 20%
30 ~1 1~5 CO₂ 18~20 37, 5%
0.5 M, 0.25 M Sucrose
5, Sucrose 가 5

Table 1. Comparison of recovery rates and survival rates of vitrified-thawed blastocysts according to the freezing vessel

Freezing vessels*	No.(%) of embryos		
	Examined	Recovered	Survived
Plastic straw	15	15 (100)	4 (26.7) ^a
OPS	23	23 (100)	3 (13.0) ^a
EM grid	83	81 (97.6)	49 (60.5) ^b

^{a,b}: Different superscripts within column indicated significant differences (p<0.05)

*: OPS: open-pulled straw, EM grid: electron microscopy grid

Table 2. Effect of equilibration time before vitrification on survival rates of vitrified-thawed blastocysts using EM grid

Equilibration time (min)	Developmental stage*	No.(%) of embryos		
		Examined	Recovered	Survived
1.5	EB	46	45 (97.8)	29 (64.4) ^a
	MB	22	21 (95.5)	17 (81.0) ^a
	LB	15	15 (100)	3 (20.0) ^b
	Total	83	81 (97.6)	49 (60.5)
3.0	EB	23	23 (100)	16 (69.6) ^a
	MB	15	14 (93.3)	7 (50.0) ^a
	LB	40	40 (100)	23 (57.5) ^a
	Total	78	77 (98.7)	46 (59.8)

^{a,b}: Different superscripts within column indicated significant differences (p<0.05)

*: EB: early blastocyst, MB: middle blastocyst, LB: late blastocyst

5. SAS (statistical analysis system) chi-square (χ^2) test

2. EG20

Table 1

plastic straw 26.7% (4/15), OPS 13.0% (3/23), EM grid 60.5% (49/81)

plastic straw, OPS 26.7% (4/15), OPS 13.0% (3/23), EM grid 60.5% (49/81)

Table 2

EM grid

EB 1.5 64.4% (29/45) 3 69.6% (16/23)

MB 1.5 81.0% (17/21) 3 50.0% (7/14)

LB 3 57.5% (23/40) 1.5

MB 1.5

LB 3 가

(p<0.05).

Table 3. Effect of artificial blastocoele shrinkage on survival rate of vitrified-thawed blastocysts using EM grid

Artificial shrinkage*	Developmental stage**	No.(%) of embryos		
		Examined	Recovered	Survived
+	EB	14	14 (100)	13 (92.9) ^{a,b}
	MB	17	17 (100)	17 (100) ^a
	LB	54	54 (100)	41 (75.9) ^b
	Total	85	85 (100)	71 (83.5)
-	EB	12	12 (100)	8 (66.7) ^{a,c}
	MB	21	20 (95.2)	12 (60.0) ^{a,c}
	LB	39	38 (97.4)	16 (42.1) ^d
	Total	72	70 (97.2)	36 (51.4)

^{a,b,c,d}: Different superscripts within column indicated significant differences (p<0.05).

*: +: blastocoele artificial puncture, -: not puncture

** : EB: early blastocyst, MB: middle blastocyst, LB: late blastocyst

Table 4. Effect of freezing methods on survival rate after freezing-thawing human blastocysts

Freezing methods	No.(%) of embryos		
	Examined	Recovered	Survived
Slow freezing	15	15 (100)	10 (66.7) ^a
Vitrification	18	18 (100)	16 (88.9) ^a

^a: Values within columns having same superscripts are not significantly different

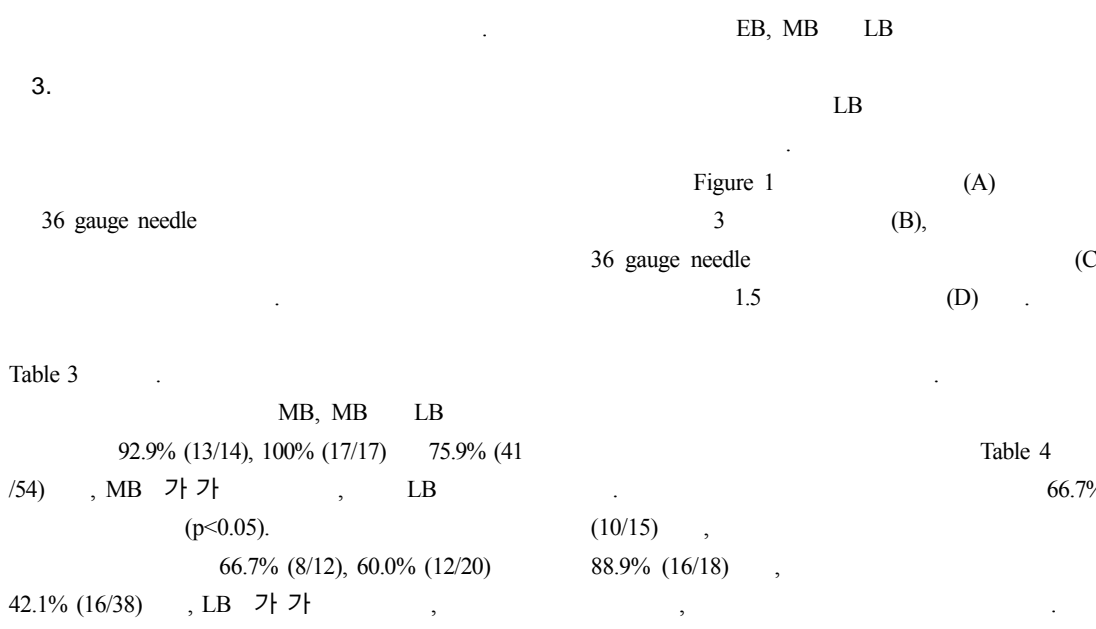


Table 3

92.9% (13/14), 100% (17/17), 75.9% (41/54), MB 가 가 , LB (p<0.05). 66.7% (8/12), 60.0% (12/20), 42.1% (16/38), LB 가 가 ,

Table 4

66.7%



Figure 1. A series course of vitrification of human blastocysts using EM grid ($\times 100$). Human expanded blastocyst (A) were equilibrated in EG20 for 3 min (B). Shrank embryos (C) after physical puncture using 36G needle (*; C) were equilibrated in EG20 (D) for 1.5 min.

¹⁴ Vajta

. Vajta

OPS 90%

¹⁵ Chen

plastic straw , OPS

plastic straw , EFS ,¹⁶ OPS

, Kong OPS glass mi-

cropipette

¹⁷

74~89%

^{6,12}

plastic straw

¹³ , plastic straw

straw

plastic straw

, straw

OPS , OPS Niemann 가

EM grid Martino

, straw EM grid

⁷

^{9,10} Park plastic

EM grid

EM grid

EM grid 가 .

가 , 가 , 가 ,

가 , 가

Tachikawa 2 가 2

, 3 , 12 , 18 , 19-21

가

22

23

LB 가 2 , 5,7 EB 가

가 , EB

, 5,7

가

EG20 EFS40 2 EB , MB

LB 1.5 가

, 6

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