

Effects of Different Infusion Frequency of Liquid Nitrogen on Human Embryo Development and Pregnancy Rates after Freezing and Thawing

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Objective: To investigate the efficacy of high infusion frequency of liquid nitrogen on pregnancy in human embryo after freezing and thawing.

Materials and Methods: 150 infertile patients underwent 162 consecutive thawing-ET cycles. In the high infusion frequency group (Group A), 47 patients (50 cycles) underwent cryopreservation with high infusion frequency of liquid nitrogen. In the low infusion frequency group (Group B), 103 patients (112 cycles) underwent cryopreservation with low infusion frequency of liquid nitrogen. We analyzed the clinical characteristics, fertilization rates, development of embryo, good quality embryo ratio, implantation rates, and pregnancy rates between these two groups.

Results: There was no difference between the groups with regard to clinical characteristics (mean age, infertility duration, infertility factors, hormone profile), mean number of oocyte retrieval, fertilization rates, and mean embryo number of transfers. The survival rates in group A was 64.9% (228 of 350 embryos), and among the 228 embryos 190 embryos (83.3%) which progressed to the two- to eight-cell stage. After thawing, the embryo numbers were 65 (34.2%), 29 (15.3%), 35 (18.4%), and 37 (19.5%) of grades 1, 2, 3, and above 4, respectively. The survival rates in group B was 63.8% (482 of 755 embryos), and among the 482 embryos 465 embryos (96.5%) which progressed to the two- to eight-cell stage. After thawing, the embryo numbers were 106 (22.8%), 94 (20.2%), 89 (19.1%), and 112 (24.1%) of grades 1, 2, 3, and above 4, respectively. There was no difference in embryo quality change after the freezing-thawing procedure between the groups. Implantation rates (31.1% vs. 34.3%) were not significant. However hCG positive rates in group A (40%) were higher than group B, but not statistically significant. Clinical pregnancy rate (26% vs. 25.9%), on going pregnancy rates (>20 weeks) were not significant (26% vs. 25%).

Conclusion: We compared embryo quality change, survival rates, and pregnancy rates between high

infusion frequency group and low infusion frequency group and the results were similar between the two groups. Therefore, high infusion frequency of liquid nitrogen for cryopreservation is a worthy method to preserve in human embryos.

Key Words: Human embryos, Cryopreservation, High infusion frequency of liquid nitrogen

가 .
 가가 , 가
 , , 가
 , H₂O₂ DNA 가
 .
 가 , ,
 가
 1.
 -196 1997 1 2000 12
 150 gonadotropin (FSH, Metrodin; Serono, Rome, Italy; hMG, Pergonal; Serono, Rome, Italy) GnRH analogue (GnRH-a, leuprolide acetate depot, 1.875 mg SC single dosage; Takeda Chemical Industries, Ltd., Japan; Leuprolide acetate, 1 mg/d; Abbott laboratories, Chicago, IL; Supermon, 0.5 mg three times per day; Hoechst, Frankfurt, Germany) long protocol
 2.
 (apoptosis) A
 가 B
 가 47 103 17
 6 ~18 mm 가 2
 가 10,000 IU hCG

hCG 34~36

3.

37 , 5% CO₂ incubator

B3 media (BMikorea, Suwon, Korea) , 4~

8 percoll gradient 300 G 15

2

ICSI (intracytoplasmic sperm injection)
(insemination) . 24 2 (2
pronucleus, 2 PN)

가

(high-frequency infusion
(HFI; 120 infusion/min): CryoMagic, Booil Industry,
Seoul, Korea; low-frequency infusion (LFI; 50 infusion/
min): Kryo-10, Planar, UK)

program

1,2-propranediol (PROH; Sigma,
St. Louis, MO, USA) 20% fetal bovine serum (FBS;
Gibco BRL, N.Y., USA) 가

2

Sol. A (1.5 M PROH)

15 Sol. B (1.5 M
PROH + 0.2 M sucrose) 15

Sol. B

0.25 ml straw 1 10

-2 /min -7 , -7

가 forcep straw

10

. -7 -35 -0.3 /min

straw

-196

(500 /min) , straw

30

10

4 Sol. 1
(1.0 M PROH + 0.2 M sucrose), Sol. 2 (0.5 M PROH +

0.2 M sucrose), Sol. 3 (0.2 M sucrose), Sol. 4 (PBS +
20% FBS) 30 µl

oil

5 0.4% bovine serum albumin (BSA,
Sigma, MO, USA) mHTF

4.

37 , 5% CO₂ incubator 48
(cleavage of oocyte) (fragmenta-
tion) TDT (SET T.D.
T.: Laboratoire CCD, France) .⁸

5.

Serhal Craft
.⁹ , 3 estradiol valerate
(Progynova, Schering AG, Germany) 6 mg/day

10

가 7 mm

progesterone in oil (Progest,
Korea) 50~100 mg/day

4

6.

progesterone in oil

100 mg , micronized proges-
terone (Utrogestan, Besins, France)

600 mg 2

10~12

β-hCG ,

12

7.

SPSS for Windows release 7.5
Independent Sample Test Chi-Square Test
p<0.05

Table 1. Comparison of clinical characteristics between HFI and LFI groups

	HFI Group	LFI Group
No. of cycles/cases	50/47	112/103
Age	31.32 ±3.08	31.23 ±4.05
Gravida	1.24 ±1.09	1.62 ±1.37
Parity	0.36 ±0.85	0.28 ±0.62
Infertility duration (m)	39.4 ±27.60	43.88 ±38.89
LH (mIU/mL)	7.88 ±6.21	6.05 ±3.47
FSH (mIU/mL)	10.47 ±20.71	7.07 ±4.86
E2 (pg/mL)	34.32 ±30.26	54.58 ±78.45
P4 (pg/mL)	0.96 ±2.01	1.98 ±4.54

Values are means ±SD. p>0.05

HFI: High frequency infusion, LFI: Low frequency infusion

Table 2. The survival rate and embryo development between groups

	HFI Group (cycles=50)	LFI Group (cycles=112)
Mean no. of oocytes retrieval	19.97 ±6.21	18.99 ±9.24
Mean no. of 2 PN	14.56 ±7.41	14.93 ±7.72
Fertilization rates (%)	77.0	83.2
Survival rates (%) of thawed embryos	64.9	63.8
Mean no. of embryo per transfer	4.54 ±2.10	4.30 ±1.84
Embryo development (2~8 cells)	190/228	465/482
Grade I	65 (34.2%)	106 (22.8%)
Grade II	29 (15.3%)	94 (20.2%)
Grade III	35 (18.4%)	89 (19.1%)
Grade IV	37 (19.5%)	112 (24.1%)

p>0.05

Table 3. Clinical results between HFI group and LFI group

	HFI Group (cycles=50)	LFI Group (cycles=112)
Implantation rates (%)	31.1%	34.3%
No. (%) of positive hCG	20 (40%)	39 (34.8%)
No. (%) of clinical pregnancy	13 (26%)	29 (25.9%)
No. (%) of ongoing pregnancy (>20 weeks)	13 (26%)	28 (25%)

p>0.05

hormone assay, , hor

가 (Table 1, 2).

A

64.9% B 63.8%

. A

228 , 2 8

190 . grade grade 1

65 (34.2%), grade 2 29 (15.3%), grade 3

35 (18.4%), grade 4 37 (19.5%)

B

482 , 2 8

465 . grade grade

1 106 (22.8%), grade 2 94 (20.2%), grade 3

89 (19.1%), garde 4 112 (24.1%)

(Table 2).

A

B

31.1%

34.3% , hCG 40%

34.8%

.

26% 25.9%,

26% 25% 가

(Table 3).

15,16

(solution effects) (ice formation), (osmotic cell shrink) (ice crystals) (cooling phase) (ice formation and dehydration phase) -196 (rapid thawing phase)

가 가 가 가 (migratory recrystallisation), 가 (dissolved gases), (cold shock) 14,17-19

freezing), (vitrification) (ultrarapid freezing) (slow shock) 14,17-19

가 Trounson, programmable controlled rate cell freezer (cryoprotective agent)

Gordts 70% 20% 11.4% 9.0% 15.5% 11.4% 3,4,20 (migratory recrystallization)

11.4% 9.0% 15.5% 11.4% (granules) (Frederik and Busing, 1980) runaway ATP

Rall Fahy 17,21-26

13, Florescent recovery after photobleaching (FRAP)

9% 88% 0% 53% 14

가 -0.3~0.4 /min 가 1 -80 -30 50 가

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