

IVF - ET Program blastocyst

Blastocyst

The Studies on the Development of Human Blastocyst Embryos in IVF - ET Program

. The development of human blastocyst embryos by co-culture with Cumulus cells

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=Abstract=

This study was carried out to investigate the development rates of human embryos co-cultured with cumulus cells to each blastocyst stage. Human zygotes were co-cultured on cumulus cell monolayer in YS medium supplemented with 20% hFF. On day 2, if patient had four or more "good" embryos (regular blastomeres without fragmentation), embryos were further cultured for 72hrs. Blastocysts on day 5 were classified into early blastocyst(ErB), early expanding blastocyst(EEB), middle expanding Blastocyst(MEB), and expanded blastocyst(EdB) on the basis of their morphological aspects of trophoctoderm cells and blastocoele. Subsequently, maximum 3 of best blastocysts were transferred in 486 cycles.

The results in this study were as follows : Patients who had four or more "good" embryos on day 2 were 498 persons, but patients who were not transferred

even an embryo due to failure in development to the blastocyst stage on day 5 were 12 persons (2.4%). The development rate of embryos to the blastocyst stage was 58.2% (2,885/4,957) on day 5, and the rates that developed to the ErB, EEB, MEB, and EdB stage were 15.0% (743/4,957), 14.9% (739/4,957), 14.4% (714/4,957), and 13.9% (689/4,957), respectively. Total 1366 blastocysts were transferred in 486 cycles (mean number=2.81). The implantation rate and the ongoing implantation rate obtained by observing the number of G-sac and FHB were 29.9% (409/1,366) and 22.5% (308/1,366), respectively. The clinical pregnancy rate was 51.2% (249/486), and the ongoing pregnancy rate was 39.1% (190/486). Among women who were ongoing pregnancy, women with singleton were 50% (95/190), women with twin were 37.9% (72/190), and women with triplet were 12.1% (23/190). Although triplet pregnancy rate in this study was high such as 12.1%, because many blastocysts with high viability were produced in our co-culture system using cumulus cells on day 5, we really believe that a multiple pregnancy except twin should not occur by selecting good embryos for maximum two blastocyst transfer.

These results demonstrate that autologous cumulus cells may be used for the production of blastocysts with high developmental competence, and the use of autologous cumulus cells to be collected easily, and to be treated conveniently at OPU must be an effective means for obtaining high implantation and pregnancy rate.

GIFT가 IVF-ET

,

program

60 70%

가

program

가

가

,

가 , 가 (Quinn, 1994).
가 (Pickering et al., 1995),
25 30% ,
. Gandolfi (1987, 1989) , 가
Wiemer (1989) ,
(1989), Bonso (1989)
(ampullary cells) 69% ,
. Menezo (1992) Olivennes
(1994) VERO 41%, 60%
. Hela
(Ben-Chetrit et al., 1996).
, VERO Hela
, ,
, 가 (Quinn & Margalit, 1996 ; Freeman et al., 1995).
. YS
10% 가 2PN 4
, 가 3

1.

1997 4 9
498 .

2.

10 0.6 ml GnRH-agonist(Buserelin, Serono)
 2 3 hCG 2 3 ampule FSH/hMG
 hMG(Serono) 0.3 ml Buserelin . 17 18 mm
 가 2 10,000 IU hCG 38
 . 37 , 5% CO₂
 (Manipulation chamber,)
 10% (hFF) 가 YS
 37 , 5% CO₂ (Forma) .

3.

10% 가 Ham's F-10 3 ml 300 × g 5
 1 ml percoll
 300 × g 20 . percoll pellet
 pasteur pipette 2 swim up
 , 1 2 × 10⁵ 1 ml 가

4.

YS (, 1994) . NaCl(110 mM), KCl(5 mM), CaCl₂ · 2H₂O(1 mM), MgSO₄ 7H₂O(0.8 mM), NaHCO₃(20 mM) KHCO₃(5 mM)
 Taurine(0.2 mM), Glutamine(1 mM) EDTA(0.1 mM) Sodium lactate(3 mM),
 Sodium pyruvate(0.4 mM), 10 ml antibiotic, antimycotic solution 가 .
 MEM non-essential amino acids vitamin, RPMI 1640 1/2 amino acid가
 .
 10% hFF가 가 YS , 20% hFF 가 YS
 . 37 , 5% CO₂

5.

30 gauge
 (thecae cell) , 2
 1 . 0.0003% hyaluronidase가 가 10%
 hFF YS ,
 (single cell suspension) 1×10^6 cells/ml
 10 μ l . 3 4 (monolayer)
 hyaluronidase
 2 ,
 . 18 37 , 5%
 CO₂ .

6.

18
 30 gauge , , 2
 20% hFF가 가 YS
 . 2 4
 498 , 4,957
 . 1 1

7.

4 5 Dokras (1993)
 (Vacuolated morulae) ,
 . Early
 Blastocyst(ErB), Early Expanding Blastocyst(EEB), Middle Expanding
 Blastocyst(MEB), Expanded Blastocyst(EdB) . 5 1
 가 , ICM (Inner Cell
 Mass) trophectoderm cells

가 3 가 . 5
가 24 가 가

8.

(fetal heart beat) 4 5 (gestational sac) .

97 4 9
 , 2 가 4
 498
 Table 1 2 2
 4,957 20% hFF가 YS
 5 6 2,885
 58.2% ErB 743 (15.0%),
 EEB 739 (14.9%), MEB 714 (14.4%), EdB 689 (13.9%)

Table 1. The development rates of human embryos to each blastocyst stage on day 4 after co-culture with cumulus cells.

Co-culture with cumulus cells	
No. of cycle	498
No. of ET cycle	486
No. of 2PN	4,957
No. of blastocyst embryos (%)	2,885 (58.2)
EB (%)	743 (15.0)
EEB (%)	739 (14.9)
MEB (%)	714 (14.4)
EdB (%)	689 (13.9)

YS

3

10 μl

가 가 ,
()

가 가

58.2%

YS+gp

glucose

phosphate

36.2%

50.9%

7.3%

(1996),

Freeman (1995) 68%

Plachot (1993) 40%,

Quinn (1996) 50%

가 가

가

(Positive conditioning) (Bonso et al., 1989). Gandolfi (1989)

(Growth Factor)

가 . Chia (1995)

가

ICM cell trophectoderm cell

가

(conditioned medium)

가

80

가

pronase

(Kobayashi et al., 1992).

가

가 positive conditioning

가

(negative

conditioning)

Bavister(1992)

가

가

YS

가

가

가

, pH CO₂
가 가

58.2%

,

3

51.2%

virus

YS

, 10%

가 가

가

가

가

가
(51.2%)
,
가
(39.1%)
.

1.	2	4			498	5
	1				12 (2.4%)	
2.	498	4,957	2PN	4		
2,885		(58.2%)				
			ErB, EEB, MEB	EdB	743 (15.0%),	739 (14.9%),
714 (14.4%)	689 (13.9%)					
3.	486	1,366				2.81
	가					
4.			409	308		
		29.9%	22.5%			
5.		486	429	(51.2%)		, 190
	(39.1%)					
6.		190				95 (50.0%),
72 (37.9%)	23 (12.1%)					

virus

. 20% 가 YS 4 5

IVF-ET program 가 가

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가 가
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