

불임 여성의 난소로부터 회수된 미성숙 난자의 체외 성숙과 배양에 관한 연구

마리아 병원¹, 충남대학교 동물자원학부, 형질전환 복제돼지 연구센터²

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Study on *In Vitro* Maturation and Culture of Immature Oocytes Collected from Ovaries of Infertile Women

Seok Yoon Lee¹, Won Young Son¹, San Hyun Yoon¹, Won Don Lee¹,
Chang Sik Park², Jin Ho Lim¹

¹Maria Hospital, Seoul, ²Division of Animal Science & Resources, Research Center for
Transgenic Cloned Pigs, Chungnam National University, Daejeon, Korea

Objective: This study was performed to examine the maturation and the development to the blastocyst stage of immature oocytes collected from patients with high risk of ovarian hyperstimulation syndrome (OHSS).

Materials and Methods: Cumulus-oocyte complexes (COCs) were collected following only HCG-priming for non stimulated IVF-ET cycles of the patients. At the time of oocyte collection, COCs were classified into three groups in accordance with their appearance (Group I: oocytes with dispersed cumulus cells; Group II: oocytes with compacted cumulus cells; Group III: oocytes with sparse cumulus cells). The *in vitro* maturation and blastocyst development rates of the COCs were compared among these groups. From August 2001 to June 2002, 48 IVM/IVF-ET cycles from 42 patients (mean age: 32.4±3.8 years) were performed. To prevent the occurrence of OHSS, the patients were primed with 10,000 IU HCG alone 36 h before oocyte collection without gonadotropin stimulation. Oocytes were aspirated on cycle days from 7 to 13. The normal COCs were classified into three groups according to their appearance. The aspirated immature oocytes were cultured in YS maturation medium containing 30% (v/v) human follicular fluid (HFF), 1 IU/ml FSH, 10 IU/ml HCG and 10 ng/ml rhEGF. Fertilization was induced by intracytoplasmic sperm injection (ICSI). All zygotes were co-cultured with cumulus cells in 10 µl YS medium containing 10% HFF until day 7 after oocyte collection. Blastocyst transfer was performed on day 5 after ICSI.

Results: The mean number of oocytes cultured in the IVM/IVF cycles was 24.7±10.6. Of 1185 COCs, those assigned to Group I, II and III were 470 (39.7%), 414 (35.0%) and 301 (25.4%), respectively. The maturation rate (94.5%, 444/470, p<0.05) in Group I was significantly higher than those of Group II (62.8%, 260/414) and Group III (73.1%, 220/301). Especially, 30.9% of COCs in Group I (145/470) was matured on the day of oocyte aspiration. There were no differences in the rates

of fertilization and cleavage among the three groups. The development rate to the blastocyst stage in Group I (54.6%, 206/377, $p < 0.05$) was also significantly higher than those in Group II (33.0%, 68/206) and Group III (30.1%, 52/173). Twenty-four clinical pregnancies (50.0%) was obtained and 22 pregnancies (45.8%) are ongoing. Implantation rate in the present study was 24.6%.

Conclusion: These results suggest that there is a positive correlation between the appearance of COCs and the developmental competence of the immature oocytes in non stimulated IVM/IVF cycles.

Key Words: Immature oocyte, Blastocyst, HCG-priming

(*In vitro* Fertilization, IVF) (cytoplasm)
 1 (quality)
 (controlled , 가
 ovarian hyperstimulation, COH) 9,12 (go-
 (exogenous gonadotrophin) nadotrophin)
 가 (atresia) IVM
 COH , Chian
 (human chorionic gonadotrophin, HCG)
 가 (polycystic ovarian syndrome, PCOS)
 , COH (ovarian
 hyperstimulation syndrome, OHSS) 13
 . OHSS 가 HCG
 , COH , Son
 OHSS HCG
 (mild OHSS; 1 2), OHSS (cumulus cell, CC)
 (moderate OHSS; 3 4), OHSS 14 가
 (severe OHSS; 5 6)
 OHSS COH
 IVF (im- IVM HCG
 mature oocyte) (*In-vitro* maturation, IVM)
 COH IVM , IVM (blastocyst stage embryo)
 OHSS 가
 1.
 , 4-11 OHSS가 가 2001 8 2002 6
 IVM OHSS
 , 가
 (IVM/IVF-ET)
 42 (32.4±3.8 years)

48
 2.
 7 13
 36 HCG 10,000 IU (IVF-C, LG chemical, Korea)
 19 gauge (Cook, Eight Mile Plains, Queensland, Australia)
 80~100 mmHg
 40 IU/ml (Choongwae Pharmacology, Hwasung, Korea) 0.3% (bovine serum albumin, BSA) HEPES (H-6147, Sigma) bicarbonate가 Ham's F-10 (N-6635, Sigma) 70 µm mesh (Falcon 1060, Life Technologies) mesh
 10 ml pipette (Becton Dickinson & company, NJ, USA)

3.
 (cumulus-oocyte complexes, COCs)
 1 dispersed cumulus cell (CC), 2 compacted CC, 3 sparse CC
 가
 YS¹⁵
 YS 30% HFF, 1 IU/ml FSH, 10 IU/ml HCG 10 ng/ml rhEGF (Recombinant human epidermal growth factor, Daewoong pharmaceutical Co., Korea) 가¹⁶ HFF Chi¹⁷ HFF

가 18 mm ,
 가 40 ,
 HFF 3,000 rpm 30
 56 30
 HFF 0.22 µm filter (Millex-GV; Millipore, Bedford, MA) filtering 15 ml tube 4 ml -70
 5% CO₂, 5% O₂ 90% N₂
 37 IVM
 1 , 0.03% hyaluronidase (Sigma, St Louis, MO, USA) pasteur pipette (Becton Dickinson & company, NJ, USA)
 1 (first polar body) 가
 (germinal vesicle, GV)
 (metaphase I, MI)

2
 3 가
 4.

가
 가
 50 ml specimen cup (Green cross, Korea)
 30
 , 10% HFF 가 Ham's F-10 3 ml
 가 3,000 rpm 5
 1 ml percoll
 3,000 rpm 20
 2 swim up

5.
 (Intra-cytoplasmic sperm injection, ICSI)
 ICSI가 10% HFF가
 가 YS 5% CO₂, 5% O₂ 90% N₂ 37

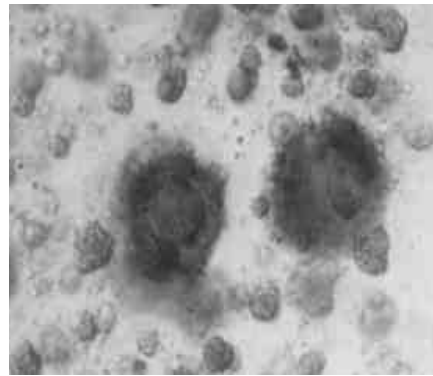
16~20
 , 2
 2 (pronucleus, PN)
 (zygote) 20%
 HFF가 가 YS (monolayer)
 (co-culture) 2
 가 7
 가
 Dokras
 19
 6.
 (endometrium)
 36 HCG 10,000 IU
 . Estradiol (E₂) valerate (Progynova; Schering, Berlin, Germany) 6 mg Progest 100 mg

(fetal heartbeat)

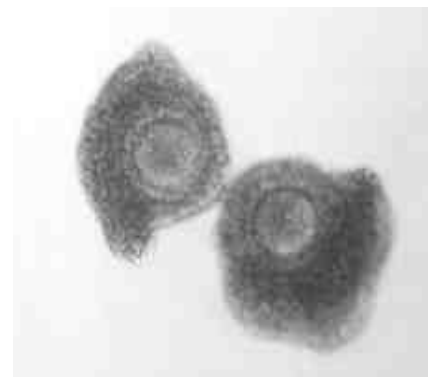
7.
 statistical
 analysis system (SAS) package (SAS Institute, Cary, NC, USA) χ^2 test

가 ;
 (polycystic ovary syndrome, PCOS)
 (27), (unexplained) (6), (anovulatory) (2), (tubal factor) (4)
 (uterus factor) (3)
 (Table 1).

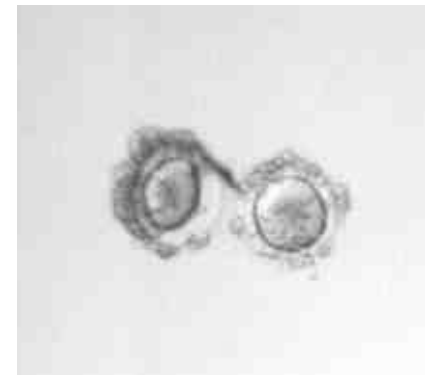
progesterone (Progest, Samil Pharmacology, Seoul, Korea)
 3 가
 1,185 (24.7±10.6)



A



B



C

Figure 1. The morphology of human immature oocytes at the time of oocyte collection. (A) GV-stage oocytes with dispersed CC appearance (group I). (B) GV-stage oocytes with compacted CC appearance (group II). (C) GV-stage oocytes with sparse CC appearance (group III). (Original magnification 200X).

cumulus cell
 dispersed (1), compacted (2), sparse (3

(Figure 1). (260/414), 3 73.1% (220/301), 1 가

(Table 2). 1, 2, 3 470 (39.7%), 414 Table 3 (35.0%), 301 (25.4%)

1 94.5% (444/470), 2 62.8%

1 30.9% 145

2, 3 90.2% (424/470), 34.5% (143/414), 61.5% (185/301)

2, 3 94.3% (443/470), 61.4% (254/414), 71.8% (216/301)

1 2, 3

3 94.5% (444/470) 2, 3 62.8% (260/414), 73.1% (220/301) (p<0.05).

Table 1. The distribution of patients according to their infertility factors

Parameter	No. of patients	Percentages
PCOS	27	64.3
Unexplained	6	14.3
Anovulatory	2	4.8
Tubal factor	4	9.5
Uterus factor	3	7.1
Total	42	100

PCOS = polycystic ovary syndrome

Table 2. The maturation, fertilization, and cleavage rates of the oocytes classified according to the appearance of their cumulus cells

Parameter	Group I	Group II	Group III
No. oocytes collected (mean ± SD)		1308 (27.3 ± 11.9)	
No. oocytes cultured (mean ± SD)		1185 (24.7 ± 10.6)	
No. of oocytes cultured	470	414	301
No. of MII oocytes (%)	444 (94.5)*	260 (62.8)	220 (73.1)
No. of 2PN oocytes (%)	377 (85.0)	206 (79.2)	173 (78.6)
No. of oocytes cleaved (%)	358 (95.0)	165 (80.1)	149 (86.1)

*p<0.05, when compared group I with group II or III
Group I: oocytes with dispersed CC; Group II: oocytes with compacted CC; Group III: oocytes with sparse CC

Table 3. Effect of culture period on oocyte maturation rates of the oocytes

Parameter	No. (%) of MII oocytes			
	Day 0	Day 1	Day 2	Day 3
Group I	145 (30.9)*	424 (90.2)*	443 (94.3)*	444 (94.5)*
Group II	0 (0)	143 (34.5)	254 (61.4)	260 (62.8)
Group III	0 (0)	185 (61.5)	216 (71.8)	220 (73.1)

*p<0.05, when compared group I with group II or III
Group I: oocytes with dispersed CC; Group II: oocytes with compacted CC; Group III: oocytes with sparse CC

HCG 가 Barnes MII가

Cha Chian IVF 9 dispersed

GV (germinal vesicle break-down, GVBD) 22 1

80% IVF GV 2 3 IVM

GVBD가 HCG dispersed

(IE) 가 , IVM HCG

IVF , IVF GV , IVM dispersed

HCG 가 IVM

(145/470)가 MII 1 30.9%

2 3 가 1 2 3 1 2

HCG (folliculogenesis) hormone receptor, LHR가 (luteinizing

LH 가 , di-

HCG (10,000 IU) 2 LH 가 3 가

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