A Prospective Comparison of Fertilizability of in vitro Matured Human Oocytes Obtained from Stimulated Cycle: Conventional Versus ICSI

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과배란유도 주기에서 얻어진 체외성숙 난자의 수정능: 고식적 체외수정시술과 세포질내정자주입법의 비교

지병철[1,2] · 안소정[1] · 문정희[1] · 황은주[2] · 서창석[1,2,3*] · 김석현[2,3] · 문신용[2,3]

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목 적: 고식적 체외수정시술과 세포질내정자주입법을 사용하여 체외성숙 난자를 수정시키고 수정률과 배발달율을 비교하고자 하였다.

연구방법: 2007년 1월부터 2008년 8월까지 난소과자극과 체외수정시술을 받은 59명의 여성에서 135개의 배아소포 단계의 미성숙난자만을 얻어 75 mIU/mL rFSH, 0.5 IU/mL rhCG, 10 ng/mL rEGF가 포함된 체외성숙용 배양액에서 최대 48시간까지 배양하였다. 체외성숙된 난자는 고식적 체외수정방법 (n=41) 또는 세포질내정자주입법 (n=94)을 이용하여 수정시켰으며 이후 배발달율을 관찰하였다.

결 과: 고식적 체외수정군과 세포질내정자주입군에서 체외성숙률은 각각 51.2%와 59.6%였고, 수정률은 각각 71.4%와 80.4%로 두 군간에 통계적으로 유의한 차이는 없었다. 배발달율도 두 군에서 비슷하게 관찰되었다.

결 론: 난소과자극을 통해 얻은 미성숙난자는 고식적 체외수정법을 사용하더라도 세포질내정자주입법과 동등한 수정률과 배발달율을 보임을 확인하였다. **[Korean. J. Reprod. Med. 2009; 36(4): 249-254.]**

중심단어: 미성숙난자, 체외수정, 세포질내정자주입

Human immature oocyte has provided a useful germ cell source for infertility treatment not only in unstimulated but also stimulated in vitro fertilization (IVF) cycles.[1,2] It has been successfully applied to natural or modified natural IVF treatment, and provided new female germ cell source for oocyte donation, fertility preservation and even for embryonic stem cell via somatic cell nuclear transfer.[3~7]

Until now, much progress has been made to enhance maturational potency and developmental capacity of immature human oocyte.[8] In most laboratories, immature oocytes were allowed to be mature and then fertilized by intracytoplasmic sperm injection (ICSI) method.[2,9]

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This practice was mainly based on the observations that fertilization by ICSI almost doubled fertilization rate of immature oocytes obtained from unstimulated IVF cycles.[10,11]

However, there were no current reports with regards to the efficacy of ICSI as a fertilization method of immature human oocyte obtained from stimulated IVF cycle. In this report, we collected human germinal vesicle (GV) stage oocytes from stimulated IVF cycles and prospectively compared the fertilization and cleavage rates achieved by conventional IVF or ICSI.

MATERIALS AND METHODS

A total of 59 consecutive women were included in whom at least one GV stage oocyte was obtained after ovarian hyperstimulation and IVF from Jan 2007 to Oct 2008. The mean age of women was 34.2±4.0 years with a range of 26~45 years. The infertility factors were identified as the following: tubal (n=19), unexplained (n=14), ovulatory (n=10), male (n=9), uterine (n=4), and endometriosis (n=3). A smoking habit was not noted in any of the study subjects.

Ovarian hyperstimulation was performed using hMG (Pergonal; Serono, Geneva, Switzerland) or recombinant FSH (rFSH; Gonal-F; Serono) beginning on day 3 of menstrual cycle. The pituitary was suppressed by multiple-dose protocol of GnRH antagonist (Cetrotide; Serono). Follicular development was monitored with periodic vaginal ultrasounds and serum levels of estradiol. When dominant follicles averaged 19 mm in diameter, ovulation was triggered by 250 μg recombinant hCG (rhCG; Ovidrel; Serono). An ultrasound-guided retrieval of oocytes was performed 35~36 hours later.

The collected cumulus-oocyte complexes (COCs) were assessed according to the presence or absence of a GV or the first polar body (PB) by stereomicroscope (×200). Immature oocytes were defined by the absence of the first PB and then classified as GV stage or metaphase I (MI) stage depending on visible GV. Isolated GV stage oocytes were then cultured in IVM medium up to 48 hrs; commercial medium (Cook-BL, Brisbane, Australia) supplemented with rFSH 75 mIU/mL (Serono), rhCG 0.5 IU/mL (Serono) and rEGF 10 ng/ml (Invitrogen, Carlsbad, CA). All oocytes were cultured in 1 mL of each IVM medium for up to 48 hrs in an atmosphere of 5% CO_2 and 95% air with high humidity.

After IVM, they were stripped with 85 IU/mL hyaluronidase (Cook) and mechanical pipetting until completely denuded of their cumulus cells. Maturation was considered when they have the first PB. The matured oocytes were then fertilized by conventional method (41 GV oocytes) or ICSI (94 GV oocytes). The insemination method was randomly allocated with a ratio of 2:3 using random number generator (except obligatory ICSI cycles). The spermatozoa were obtained on the day of oocyte retrieval and kept at room temperature for up to 48 hrs. Normal fertilization was confirmed when two distinct pronuclei are present 16~18 hrs later. Embryo culture was performed as described previously.[2]

All data in the present study were analyzed using SPSS (Windows version 12.0). The Student's t test was used to compare parametrical variables between two groups and Chi-square test was used for the comparison of two proportions. A P-value of <0.05 (two-tailed) was considered statistically significant.

RESULTS

There was no significant difference in the mean age of women, duration of infertility, women's body mass index, the primary cause of infertility and previous IVF trials between conventional IVF group and ICSI group (Table 1). Tubal factor was a main cause in both groups but male factor was relatively predominant in ICSI group. ICSI as a fertilization method of mature oocytes

Table 1. Comparison of fertilization and cleavage rate of immature human oocytes obtained from stimulated cycles inseminated by two method

	Conventional IVF	ICSI	
Number of patients	21	38	
Age of female (years)	33.0±4.1*	34.8±3.9*	NS
Duration of infertility (years)	2.8±1.7*	2.7±1.3*	NS
Women's body mass index (kg/m^2)	21.7±2.8*	21.6±2.8*	NS
Infertility diagnosis			NS
tubal factor	8 (38.1%)	11 (28.9%)	
unexplained	5 (23.8%)	9 (23.7%)	
male factor	0 (0%)	9 (23.7%)	
ovulatory factor	4 (19.0%)	6 (15.8%)	
uterine factor	2 (9.5%)	2 (5.3%)	
endometriosis	2 (9.5%)	1 (2.6%)	
Number of previous IVF trials	0.8±1.0*	0.5±0.9*	NS
Stimulation regimens			NS
hMG	2	0	
recombinant FSH	19	38	
Number of cycles destined to ICSI	1	7	
	Total		
Number of GV stage oocytes	41	94	
Number of in vitro matured	21 (51.2%)	56 (59.6%)	NS
Number of fertilized	15 (71.4%)	45 (80.4%)	NS
Number of cleaved	14 (93.3%)	42 (93.3%)	NS
	Excluding ICSI cycle		
Number of GV stage oocytes	39	84	
Number of in vitro matured	19 (48.7%)	50 (59.5%)	NS
Number of fertilized	13 (68.4%)	39 (78.0%)	NS
Number of cleaved	13 (100%)	38 (97.4%)	NS

*Mean ± SD
IVF: in vitro fertilization, ICSI: intracytoplasmic sperm injection, NS: not significant, GV: germinal vesicle

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was used in eight cycles due to poor sperm quality or previous low fertilization rate. The numbers of GV oocytes in conventional IVF group and ICSI group were 41 and 94 respectively, and the proportion of GV

oocytes amongst total retrieved oocyte in stimulated IVF cycle was not different significantly between two groups (20.0% and 27.6% respectively, p=0.0632).

When GV oocytes were matured in vitro within 48 hours, there was no significant difference in the maturation rate between the two groups. The fertilization rate was higher in the ICSI group but no statistical significance was noted; 71.4% vs. 80.4%. The cleavage rate was also similar between two groups. Exclusion of obligatory ICSI cycles (one cycle in conventional group and seven cycles in ICSI group) did not change the results on the outcome. Unfortunately, there were no transferable embryos in all subjects; most embryos derived from immature oocytes exhibited poor quality with severe fragmentation or cleavage arrest.

DISCUSSION

Intracytoplasmic sperm injection (ICSI) has been regarded as necessary for fertilization of in vitro matured oocytes even in conditions where sperm parameters are not impaired. Theoretically, compared with conventional IVF, the success rates with ICSI might be higher because entry of the spermatozoon into the oocyte is artificially achieved and does not rely on a chance. However, prospective reports of insemination of human in vitro-matured oocytes are scarce.

In the study by Barnes et al., 43% of mature oocytes from normal ovaries and 26% of in vitro matured oocytes from PCOS women were fertilized after insemination.[12] The reason for poor fertilization rates after standard insemination is still unclear but hardening of the zona pellucida of the oocyte caused by prolonged culture period could be responsible for the decreased fertilization, and theoretically, ICSI is used to overcome the problem of zona hardening.[13,14]

In a comparative study, Hwang et al. demonstrated that in vitro matured oocytes obtained from women undergoing cesarean section had higher fertilization rates by ICSI, but similar embryonic developmental quality than conventional IVF.[10] In a more recent work, a higher fertilization rate was obtained with ICSI and higher pregnancy rate and implantation rate was achieved in conventional IVF.[11]

In the present study, we demonstrated that conventional insemination has comparable fertilization and cleavage potential compared with ICSI as the insemination method of immature human oocytes obtained from stimulated cycle. This finding was somewhat inconsistent with previous reports. The important difference between the two studies was ovarian stimulation or not. It is likely that the characteristics of immature human oocytes obtained from stimulated cycle are different with those from unstimulated cycle. A recent report indicated that the immature oocytes from stimulated cycles had larger diameter than those from unstimulated cycles and they grew during in vitro culture in contrast to oocytes from unstimulated cycles.[15] Although oocytes from stimulated cycles remained at the GV stage despite stimulation, they might acquire growing potential during stimulation with coincidental increment of size.

In the present study, we tried to culture the embryos further but almost all the embryos degenerated beyond the eight-cell stage. For providing more useful information, further study about the developmental competence of in vitro matured oocytes would be needed.

For application of our results on the area of in vitro-matured oocyte, further larger studies should be performed. And also, further fundamental researches are needed with regards to the characteristics of in vitro matured oocytes including behavior of zona pellucida to achieve better developmental competence of immature oocytes.

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= **Abstract** =

Objective: The aim of this study was to compare the fertilization and cleavage rates of human in vitro matured oocytes after fertilized by conventional in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI).

Methods: A total of 135 GV stage oocytes were obtained from 59 women who received ovarian stimulation and IVF during Jan 2007 to Oct 2008. Ovarian hyperstimulation was performed using hMG or recombinant FSH with GnRH antagonist and then ovulation triggered by recombinant hCG. The immature oocytes obtained from stimulation cycles were cultured in IVM medium up to 48 hrs; commercial medium supplemented with rFSH 75 mIU/mL, rhCG 0.5 IU/mL and rEGF 10 ng/mL. The in vitro matured oocytes were fertilized by conventional IVF (41 GV oocytes) or ICSI method (94 GV oocytes).

Results: Maturation rate were 51.2% and 59.6% in conventional IVF group and ICSI group, respectively. There was no significant difference in fertilization rates between two groups; 71.4% and 80.4%, respectively. The cleavage rate was also similar in two groups.

Conclusion: The presented data suggest that conventional IVF has comparable fertilization and cleavage potential compared with ICSI as the insemination method of immature human oocytes obtained from stimulated cycle.

Key Words: Immature oocyte, in vitro fertilization, Intracytoplasmic sperm injection