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Correlation between *in vitro* maturation and expression of LH receptor in cumulus cells of the oocytes collected from PCOS patients in HCG-primed IVM cycles

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BACKGROUND: The aim of this study was to investigate whether in vitro maturation (IVM) and blastocyst development of oocytes collected following HCG-primed IVM cycles of PCOS patients are correlated with their cumulus cell (CC) patterns and further to investigate mRNA expression of the receptors for FSH, LH and epidermal growth factor (EGF) in the CCs with each pattern. METHODS: Patients who underwent IVM were primed with 10 000 IU of HCG 36 h before oocyte aspiration. The isolated cumulus–oocyte complexes were divided into three groups according to the CC patterns: oocytes with dispersed CCs (group A), oocytes with compacted CCs (group B) and oocytes with sparse CCs (group C). Oocyte maturation and blastocyst development were compared among three groups. The expression of the mRNA for FSH, LH and EGF receptors in group A and B was analysed by semi-quantitative RT–PCR. RESULTS: The maturation rate of group A was significantly higher than those of group B and C. The rate of blastocysts in group A was significantly higher than those of group B and C. mRNA expression of the LH reeptor in group A was more abundant than that of group B. CONCLUSIONS: These results suggest that the presence of dispersed CCs at oocyte collection may be positively correlated with the rates of oocyte maturation and blastocysts in HCG-primed IVM cycles. In addition, the expression of LH receptor in CCs may be correlated with the CC pattern of oocytes at collection.

Key words: cumulus cell pattern/HCG/human/immature oocytes/IVM

Introduction

Current protocols of ovarian stimulation using IVF could be replaced with the retrieval of immature human oocytes followed by in vitro maturation (IVM). The major benefits of IVM treatment include avoidance of the risk of ovarian hyperstimulation syndrome (OHSS), reduced cost and less complicated treatment. The IVM of immature human oocytes has been successfully applied for many years in superovulated cycles (Nagy *et al.*, 1996), natural cycles (Russell *et al.*, 1997) and polycystic ovarian syndrome (PCOS) patients (Trounson *et al.*, 1994).

A typical polycystic ovary (PCO) has several antral follicles up to ~10 mm in diameter around the periphery of the ovary. Although these follicles are not actively growing, they contain immature oocytes, which may be capable of further development (Polson *et al.*, 1988; Clayton *et al.*, 1992; Trounson *et al.*, 1994; Barnes *et al.*, 1996). However, the rates of maturation, fertilization and cleavage of immature oocytes obtained from PCOS patients are lower than those obtained from non-PCOS patients (Barnes *et al.*, 1996; Trounson *et al.*, 1998), which is thought to be related to a low pregnancy rate.

To compensate for these problems, several attempts have been made to improve the viability of IVM oocytes by gonadotrophin stimulation prior to oocyte collection (Wynn *et al.*, 1998; Jaroudi *et al.*, 1999). Recently, Chian *et al.* (2000) reported that higher rates of oocyte maturation and pregnancies were achieved in patients with PCOS by HCG priming. It was reported previously that oocytes with various cumulus cell (CC) patterns were collected at the time of oocyte retrieval in HCG-primed IVM cycles (Son *et al.*, 2001). However, no studies on the maturation of oocytes obtained from HCG-primed IVM cycles of PCOS women based on CC patterns have been reported.

The maturation of oocytes depends on the communication between follicular cells and on the presence of gonadotrophin receptors. CCs respond to gonadotrophin and secrete various substances that play a role in nuclear and cytoplasmic maturation (Chian *et al.*, 1999). FSH is important for the development of pre-ovulatory follicles *in vivo* (Macklon and Fauser,

2000) and for induction of LH receptors (LH-Rs) (Gougeon, 1996). Furthermore, in vitro studies have shown that meiotic resumption in cumulus–oocyte complexes (COCs) was induced by epidermal growth factor (EGF) (Gomez *et al.*, 1993; Kobayashi *et al.*, 1994; Lorenzo *et al.*, 1994). It was also reported that EGF alone or associated with gonadotrophin induces cumulus expansion and promotes maturation of bovine and mouse oocytes during in vitro culture (Lonergan *et al.*, 1996; De La Fuente *et al.*, 1999). Based on these studies, it is hypothesized that FSH receptor (FSH-R), LH-R and EGF-receptor (EGF-R) in CCs account for the high maturation rate of oocytes obtained from PCOS patients in IVM cycles with HCG priming prior to oocyte collection. Therefore, the aims of this study were to compare IVM and blastocyst development of the immature oocytes obtained from HCG-primed IVM cycles of PCOS women with respect to CC patterns and to investigate mRNA expression of FSH-R, LH-R and EGF-R in each CC pattern.

Materials and methods

Patients

A total of 35 women underwent 40 IVM cycles. The average age of the patients was 33.3 ± 2.7 years (mean SEM) with a range of 28–37 years. Patients with PCOS presented with anovulation ($n = 6$), PCOs visible on ultrasound ($n = 29$), elevated serum testosterone concentrations, >10 IU/ml LH in serum on menstrual cycle day 2 and a minimum 3 year history. The patients had a high risk of OHSS. To initiate the IVM treatment cycle in the anovulatory patients, the patient received i.m. injections of progesterone (Progest; Samil Pharmacology, Seoul, Korea) for 10 days. A withdrawal bleed occurred within 3 days after the last dose. Ovarian follicle development was monitored by transvaginal ultrasonography (Aloka, Tokyo, Japan) beginning on cycle days 3 and 5 (onset of withdrawal bleed = day 0). The patient was given 10 000 IU of HCG (IVF-C, LG Chemical, Korea) on cycle days 7 and 13 based on the patient's cycle length and endometrium thickness. The procedures were reviewed and approved by the Institutional Review Board of the Maria Infertility Hospital.

Retrieval of immature oocytes

After 36 h, oocytes were aspirated with a 19-gauge aspiration needle (Cook, Eight Mile Plains, Queensland, Australia) under the guidance of transvaginal ultrasound. A portable aspiration pump was used with a pressure of between 80 and 100 mmHg. The aspirates were collected in tubes containing pre-warmed heparinized Ham's F-10 medium that contained bicarbonate and HEPES supplemented with 0.3% human serum albumin. Follicular aspirates were placed into a 70 μm pore size filter (Becton Dickinson, NJ) which had been prerinsed with the medium. The filtrate was washed several times with medium by vigorous pipetting using a 10 ml serological pipette (Becton Dickinson) to remove erythrocytes and small cellular debris. The retained cells were resuspended in the medium. The COCs were then isolated under a stereomicroscope and washed twice in the same medium. All COC handling procedures were conducted in a mini-chamber under a 5% CO_2 atmosphere at 37°C.

IVM and embryo culture

The isolated COCs were divided into three groups according to the CC patterns: oocytes with dispersed CCs (group A), with compacted CCs (group B) and with sparse CCs (group C). Dispersed CCs were enclosed by an expanded CC and one or two layers of corona cells around germinal vesicle (GV) oocytes. Compacted CCs possessed 4–5 layers of corona cells and CCs completely covering the GV oocytes. Sparse CCs had very few coronal cells covering the zona pellucida of GV oocytes (Figure 1). The atretic and denuded COCs were discarded. The mature eggs collected at the time of egg collection were not included in this study. The COCs of each group at the GV stage were cultured separately in 1 ml of IVM medium in a 4-well culture dish. The IVM medium consisted of YS medium (Yoon *et al.*, 2001) supplemented with 30% heat-inactivated human follicular fluid, 1 IU/ml FSH, 10 IU/ml HCG and 10 ng/ml recombinant human (rh)EGF (Daewoong Pharmaceutical Co., Korea) (Son *et al.*, 2002). The human follicular fluid was prepared using the method described by Chi *et al.* (1998). The COC oocytes were cultured in IVM medium at 37°C in 5% CO_2, 5% O_2 and 90% N_2. After 24 h culture, the oocytes were denuded of CCs with 0.03% hyaluronidase (Sigma Chemical Co., St Louis, MO) and mechanical pipetting. Oocyte nuclear maturation was assessed from the presence of the first polar body under the dissecting microscope. Following examination, immature oocytes remaining at GV or metaphase I (MI) stage were cultured in the same medium and the meiotic status

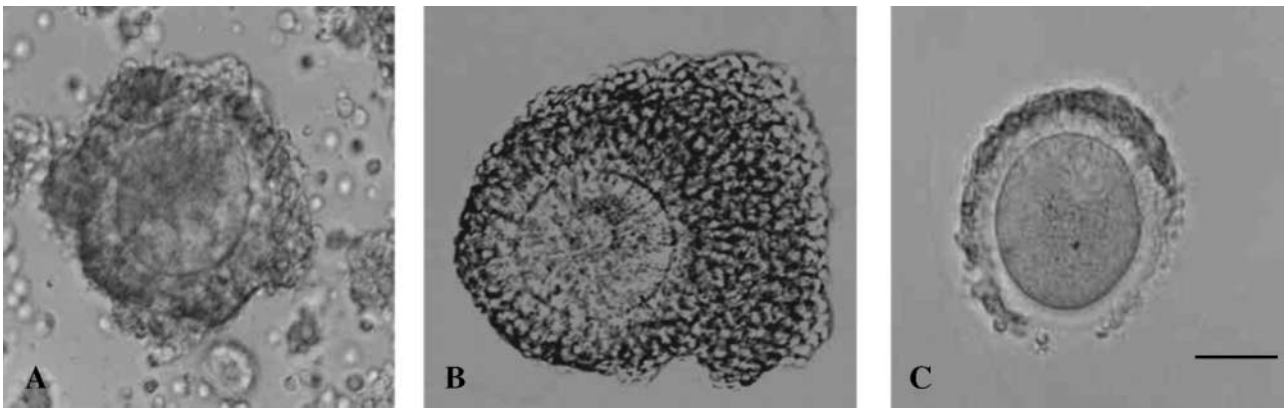

Figure 1. Human immature oocytes retrieved at the time of oocyte collection. (**A**) A GV-stage oocyte with a dispersed CC pattern just after oocyte retrieval (group A). (**B**) A GV-stage oocyte with a compacted CC pattern just after oocyte retrieval (group B). (**C**) A GV-stage oocyte with a sparse CC pattern (group C) (original magnification ×200), bar = 50 μm.

Table I. Oligonucleotide primers used for RT–PCR

Gene	Primer type	Forward primer (5′–3′)	Reverse primer (5′–3′)	Annealing temperature (cycles)	Product size (bp)
FSH receptor[a]	Outer	ATGATGTTTTCCACGGAGCC	ACCATATCAGGACTCTGAGG	50°C (30)	335
	Nested	AAAAGCTTGTCGCCCTCATG	ACCATATCAGGACTCTGAGG		
LH receptor[b]	Outer	GCATCTGTAACACAGGCATC	CATCTGGTTCAGGAGCACAT	55°C (35)	342
	Nested	GCAGAAGATGCACAATGGAG	CTCTCAGCAAGCATGGAAGA		
EGF receptor[c]	Outer	GGACGACGTGGTGGATGCCG	GGCGCCTGTGGGGTCTGAGC	61°C (30)	163
	Nested	CCTCATCCCACAGCAGGGCTTC	GCTGTATCGCTGCAAGAAGCTGTC		
GAPDH[d]		CGGAGTCAACGGATTTGGTCGTAT	AGCCTTCTCCATGGTGGTGAAGAC	60°C (30)	306

[a]Oktay *et al.* (1997).
[b]Mandai *et al.* (1997).
[c]Chia *et al.* (1995).
[d]Wong *et al.* (1994).

re-examined at 48 h and finally at 72 h of culture. ICSI was used to fertilize the mature oocytes recovered from each group. Fertilization was assessed 17–19 h after insemination for the appearance of two distinct pronuclei (2PN) and two polar bodies. The zygotes were co-cultured with CCs in 10 μl YS medium supplemented with 10% human follicular fluid (Yoon *et al.*, 2001). The CCs for co-culture were prepared using the method reported by Yoon *et al.* (2001). Embryos were cultured on day 4 or day 6 after oocyte retrieval. Blastocyst culture was performed in each group which had ≥3 good-quality embryos on day 2 after insemination. Of 40 IVM cycles, six cycles were transferred at blastocyst stage on day 6. In the cycles, blastocyst development was examined among embryos derived from three groups.

Isolation of cumulus cells

CCs were collected from sibling GV-stage eggs at the time of egg collection in 10 patients undergoing IVM cycles (group A, $n = 10$; group B, $n = 10$). CCs were isolated by two needles and then washed three times with phosphate-buffered saline (PBS). After removing the blood cells, each type of CC was stored at −70°C for RNA extraction.

RNA isolation and reverse transcription

Total RNA from CCs of each group was isolated using an RNeasy RNA isolation kit (Quiagen, USA). Prior to each reverse transcription reaction, 1 μg of total RNA was dissolved in 10 μl of water and treated with RNase-free DNase (Gibco-BRL, USA) to remove contaminating genomic DNA for 15 min at room temperature. After the incubation, DNase was heat inactivated at 65°C for 15 min. Total RNA (1 μg) was reverse transcribed at 40°C for 1 h in a 20 μl reaction mixture using 100 ng of random hexanucleotide primers and 6 IU of AMV reverse transcriptase (Gibco-BRL) in the presence of cDNA synthesis buffer (Gibco-BRL), 125 mmol/l dNTPs mixtures (Gibco-BRL), 5 mmol/l dithiotheritol (Gibco-BRL) and 40 U of RNase inhibitor (RNasin; Gibco-BRL). The reaction was initiated by adding 1 μl (200 U) of Superscript II reverse transcriptase to each tube, mixed and incubated at 25°C for 10 min. The tubes were then transferred to 40°C and incubated for 60 min. The resultant cDNA mixtures were heated at 95°C for 5 min before storage at −20°C. Negative controls were performed by omission of reverse transcriptase.

Semi-quantitative PCR

Semi-quantitative PCR to compare the expression of FSH-R, LH-R and EGF-R mRNA was performed using a GeneAmp PCR System 2400 (Perkin Elmer, USA). Equal loading was monitored by glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression (Wong *et al.*, 1994). The PCR amplification was carried out by adding 25 μl of PCR mixture containing 20 mmol/l Tris–HCl (pH 8.4), 1.5 mmol/l $MgCl_2$, 50 mmol/l KCl, 0.2 mmol/l dNTP and 2.5 U of Taq polymerase. The PCR conditions were 95°C denaturation, annealing specific for primers (as Table I), 72°C extension, each step 1 min with a final extension of 5 min. For nested PCR, 1 μl of primary product was added to 49 μl of freshly prepared mix as above. Annealing temperatures and cycle numbers were optimized for each phase (Table I). PCR products were analysed by electrophoresis on a 3% agarose gel and stained with ethidum bromide. The density of PCR products was measured by Gel Doc 2000 (Bio-Rad Laboratories, USA). RT–PCR experiments for the FSH-R, LH-R and EGF-R genes and for the control GAPDH gene were repeated 10 times.

Statistical analysis

The rate of IVM, fertilization, cleavage and blastocyst development among each group was compared with the χ^2 test using the Statistical Analysis System (SAS Institute, USA) software package. Semi-quantitative RT–PCR experiments between two groups were expressed as means ± SEM ($n = 10$), and their statistical comparison was done by analysis of variance (ANOVA) followed by the Duncan's multiple range test.

Results

IVM and embryo development

A total of 35 women (age 33.3 ± 2.7 years) underwent 40 IVM cycles for this study. A total of 787 oocytes (mean = 18.3 ± 11.8) were retrieved. The percentage of oocytes at either the MI or MII stage at the time of oocyte retrieval was 8.1% (64 out of 787) and these eggs were not included in this study. There was no difference in the number of GV oocytes recovered from group A (5.3 ± 3.2), B (7.9 ± 4.3) and C (5.0 ± 2.4). The atretic COCs were 6.9% (50 out of 723, discarded), while the proportions of COCs in groups A, B and C were 29.1% (211 out of 723), 43.4% (314 out of 723) and 20.5% (148 out of 723), respectively (Table II). Figure 2 shows the time course of maturation to MII stage in oocytes according to their CC patterns. The maturation rates until 24 h of culture in group A, B and C were 72.0 ± 19.6% (152 out of 211), 30.6 ± 16.8% (96 out of 314) and 48.0 ± 31.5% (71 out of 148), respectively. The maturation rates until 48 h of culture in group A, B and C were 83.4 ± 12.8% (176 out of 211), 58.3 ± 11.9% (183 out of

Table II. Comparison of maturation, fertilization and cleavage rates in oocytes derived from three groups

Parameter	Group A	Group B	Group C
No. of oocytes cultured	211	314	148
No. of MII oocytes (%)	183 (86.7)*	193 (61.5)	101 (68.2)
No. of 2PN oocytes (%)	156 (85.2)	148 (76.7)	85 (84.1)
No. of cleaving 2PN embryos (%)	145 (93.0)	129 (87.2)	76 (89.4)

*Significant difference among three groups: $P < 0.001$.
Group A = oocytes with dispersed CCs; group B = oocytes with compacted CCs; group C = oocytes with sparse CCs.

314) and 58.1 ± 21.9% (86 out of 148), respectively. Therefore, the oocyte maturation was faster in group A than in group B and C ($P < 0.001$). In addition, the maturation rate (86.7 ± 9.6%, 183 out of 211) until 72 h of culture in group A was significantly higher than those in group B (61.5 ± 24%, 193 out of 314) and C (68.2 ± 22.9%, 101 out of 148) ($P < 0.001$). Table II shows the results obtained in the three groups defined according to CC patterns. There were no significant differences in the rates of 2PN and cleavage among these three groups. However, blastocyst development in group A (40%, 10 out of 25) was significantly higher than those of group B (23.3%, seven out of 30) and group C (23.1%, three out of 13) ($P < 0.05$) (Table III).

Semi-quantitative RT–PCR analyses of FSH-R, LH-R and EGF-R mRNA expression

RT–PCR experiments were performed 10 times in two groups of CC patterns and the same results were always obtained. GAPDH as a housekeeping gene was detected in two groups of CC patterns, yielding a 306 bp product

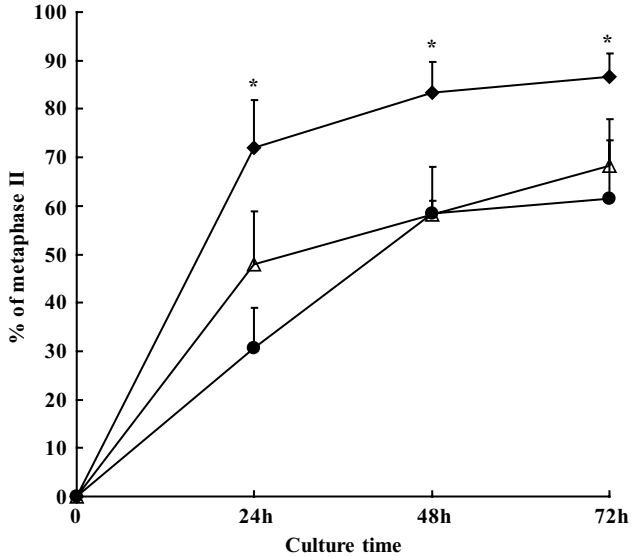

Figure 2. The time course of the completion of human oocyte maturation to MII (extrusion of first polar body) of oocytes cultured based on the CC pattern at the time of oocyte retrieval. Group A = oocytes with dispersed CCs (filled triangles); group B = oocytes with compacted CCs (filled circles); group C = oocytes with sparse CCs (open squares). *Significant difference among three groups at the time point ($P < 0.001$).

Table III. Comparison of blastocyst formation rate of 2PN derived from oocytes of the three groups

Parameter	Group A	Group B	Group C
No. of 2PN oocytes	25	30	13
No. of blastocysts (%)	10 (40)*	7 (23.3)	3 (23.1)

*Significant difference among three groups: $P < 0.001$. Group A = oocytes with dispersed CCs; group B = oocytes with compacted CCs; group C = oocytes with sparse CCs.

(Figure 3A). The mRNA expression of FSH-R, LH-R and EGF-R in each CC pattern was examined using semi-quantitative RT–PCR and compared with that of GAPDH as a housekeeping gene (Figure 3A). Gel electrophoresis of each group revealed bands of 335, 342 and 163 bp, corresponding to the regions of the human FSH-R, LH-R and EGF-R genes amplified (Figure 3A). Semi-quantitative RT–PCR confirmed these qualitative observations, showing that expression of FSH-R and EGF-R in group A was comparable with that in group B. However, compared with group B, significantly abundant expression of LH-R mRNA was detected in group A (Figure 3A and B).

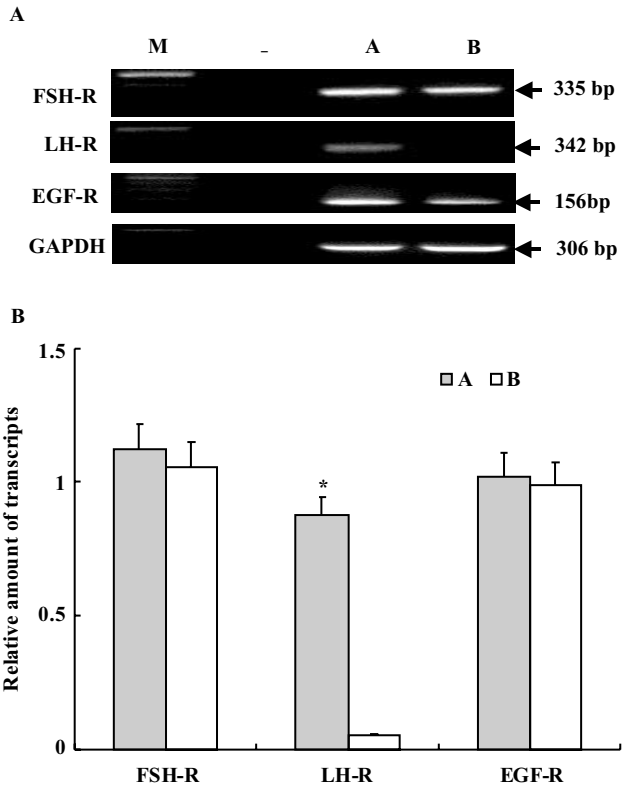

Figure 3. The semi-quantitative RT–PCR for internal control of GAPDH and the gene expression of FSH receptor (FSH-R), LH receptor (LH-R) and EGF receptor (EGF-R) in group A and B. (**A**) The gene expression of FSH-R (335 bp), LH-R (342 bp) and EGF-R (163 bp) mRNA in the two groups. Expression is indicated by the ratio of applicant density of the target genes over that of GAPDH (306 bp). (**B**) Relative amount of FSH-R, LH-R and EGF-R transcripts in group A (A: closed bar) and group B (B: open bar). Bars are means ± SEM of 10 experiments. M = molecular weight marker; – = negative control; A = group A (dispersed CCs), B = group B (compacted CCs). *$P < 0.001$ compared with group A and B (LH-R expression).

Discussion

The results from this study indicate that IVM of oocytes collected from HCG- primed IVM cycles of PCO(S) patients is correlated with their CC patterns. Immature oocytes with various CC patterns showed differences in oocyte function *in vitro*, which included the proportion maturing and the rate of blastocyst development. In addition, it is demonstrated that the expression of the LH-R is related to the CC pattern, which is associated with oocyte maturation.

Recent studies of the treatment of PCOS patients with HCG before oocyte recovery showed an increased rate of oocyte maturation (Chian *et al.*, 2000). Similar clinical results were obtained with a similar approach in the IVM programme for patients with PCOS (Son *et al.*, 2002). The current results showed that the COC patterns at the time of oocyte retrieval in HCG-primed IVM cycles of PCO(S) patients were different in oocytes with dispersed, compacted and sparse CC patterns. A similar observation has been reported by Son *et al.* (2001), which determined the COC pattern in HCG-primed IVM cycles for patients with a high risk of OHSS. Moreover, the maturation speed and rate of oocytes with dispersed CCs after culture in this study were significantly higher than those with compacted and sparse CCs. About 72% of oocytes with dispersed CCs reached the MII stage by 24 h of culture, which was similar to maturation and morphology of GV-stage oocytes obtained from superovulated IVF cycles. Cha and Chian (1998) reported the different time course of germinal vesicle breakdown (GVBD) for GV-stage oocytes recovered from superovulated IVF patients and from unstimulated IVF patients, which was contributed to by different patterns of CCs in GV-stage oocytes. In the study, up to 12 h of culture, 80% of GV oocytes recovered from superovulated IVF patients underwent GVBD, whereas all the oocytes recovered from untreated patients still had intact GVs (Cha and Chian, 1998). It was also found that the oocytes with dispersed CC patterns only appeared in HCG-primed IVM cycles compared with oocytes in non-primed and low dose HMG-primed IVM cycles (Son *et al.*, 2001). Therefore, it is considered that differences in the maturation speed and rates of oocytes with different CC patterns might be due to the difference in priming of follicles by HCG before oocyte recovery and the activity of the CCs with the follicles.

Son *et al.* (2002) reported that MII-stage oocytes could be collected at the time of oocyte retrieval in HCG-primed IVM cycles of women with PCOS. We also found 8% of mature eggs at the day of egg retrieval in this study, and these were from oocytes with dispersed CCs. There was no difference in embryo quality morphologically between embryos derived from immature oocytes either with or without MII oocytes at the time of oocyte retrieval. Recently, Chian *et al.* (2004) reported that maturational and developmental competence of immature oocytes was not detrimentally affected by the presence of a dominant follicle of 19 mm at the time of egg retrieval. Russell (1998) reported a marked decrease in the rates of maturation, fertilization and transfer among cycles in which immature oocytes were retrieved when a dominant follicle of $\geq$14 mm was present at the time of retrieval. In our study, we only measured follicle diameter just before HCG was given to the patients. The size of the leading follicle was ~10 mm. Therefore, the leading follicle size at the time of egg retrieval was probably 11–13 mm in diameter after HCG priming. Therefore, we think that the developmental competence of immature oocytes may not be detrimentally affected by the presence of a $<$14 mm leading follicles at the time of egg retrieval. However, further studies to clarify the correlation of leading follicular size and developmental capacity of sibling immature oocytes in an IVM programme are necessary.

During follicular growth up to the pre-ovulatory stage, numerous genes are activated and inactivated in the developing oocyte and surrounding mural granulosa cells and CCs (Salustri *et al.*, 1989). The induction of LH-R by FSH is mediated by the FSH-induced increase in intracellular cyclic AMP (Erickson *et al.*, 1979, Erickson *et al.*, 1982). The LH-induced events induce GVBD. LH also induces CC expansion, which involves the secretion of a hyaluronic acid-rich proteoglycan matrix from CCs (Salustri *et al.*, 1989). However, in this study, LH-R mRNA was found to be highly expressed on the dispersed CCs in spite of no FSH induction. In addition, the expression of FSH-R and EGF-R mRNA was not different between dispersed CCs and compacted CCs collected from priming HCG in IVM cycles of PCO(S) patients. This was probably due to the difference in the stage of follicle development at the time of oocyte retrieval after HCG administration.

It was reported that FSH-R mRNA in the immature rat ovary could already be localized in granulosa cells of small follicles (Nakamura *et al.*, 1991; Lapolt *et al.*, 1992). Treatment with pregnant mare's serum gonadotrophin (PMSG) to stimulate follicle growth resulted in a marked increase of FSH-R mRNA expression and FSH-binding sites, whereas subsequent administration of HCG to induce ovulation and luteinization significantly decreased FSH-R expression (Nakamura *et al.*, 1991; Lapolt *et al.*, 1992). EGF-R was detected by immunostaining and RT–PCR on CCs of all stages of porcine follicle development (Singh *et al.*, 1995). Immunoreactivities for EGF-R were expressed simultaneously in the human oocytes of primordial, primary, preantral and antral follicles (Qu *et al.*, 2000). In human ovary, LH-R mRNA increased from pre-ovulatory follicles to the corpus luteum of the midluteal phase (Minegishi *et al.*, 1997). Therefore, based on these reports, it is thought that some follicles at the time of HCG priming in PCOS patients might already have LH-R, resulting in the oocytes retrieved having a dispersed CC pattern. Similarly, compact CCs around oocytes following the administration of large doses of HCG (1000 IU) could be due to the presence of insufficient LH-R in order to induce the CC response *in vivo*. Meanwhile, the oocytes with sparse CCs might be mixed with oocytes that removed CCs mostly by high pressure on ovum aspiration and that were entering the degenerative process. A well-defined further study is needed to ascertain this possibility.

Barnes *et al.* (1996) reported that those oocytes first reaching MII were shown to be the most competent to develop

into blastocysts. Our results showed that the rate of blastocysts derived from oocytes with dispersed CCs was significantly higher than those of oocytes with compacted and sparse CCs, even though the number of embryos examined was small. Therefore, the results from this study suggest that the presence of dispersed CCs at the time of oocyte retrieval may be positively correlated with oocyte maturation and blastocyst development in HCG-primed IVM cycles. In addition, the results also indicate that the expression of the LH-R in CCs may be correlated with the CC pattern at the time of oocyte retrieval.

In conclusion, the CC pattern at the time of aspiration plays a predictive role in the maturation of oocytes recovered in HCG-stimulated IVM cycles, and may be a relevant parameter in the development of technology with higher rates of successful oocyte maturation and developmental competence of embryos in vitro.

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