A 38 h interval between hCG priming and oocyte retrieval increases *in vivo* and *in vitro* oocyte maturation rate in programmed IVM cycles[†]

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BACKGROUND: Our aim was to evaluate whether extending the interval between human chorionic gonadotrophin (hCG) priming and immature oocyte retrieval increases the oocyte maturation rate following *in vitro* maturation (IVM). **METHODS:** This study was performed retrospectively. IVM was performed on 113 polycystic ovary syndrome patients ($n = 120$ cycles). Oocyte collection was performed either 35 h (Group 1; $n = 76$) or 38 h (Group 2; $n = 44$) after 10 000 IU of hCG priming. Following oocyte retrieval, oocyte maturity was assessed and the remaining immature oocytes were cultured in IVM medium up to Day 2. **RESULTS:** The number of *in vivo* matured oocytes collected was significantly higher in Group 2 (13.6%, 114/840 versus 7.3%, 96/1312 in Group 1) ($P < 0.01$); the oocyte maturation rate after Day 1 was significantly higher ($P < 0.01$) in Group 2 (46.3 versus 36.0% in Group 1); and clinical pregnancy (40.9 versus 25%) and implantation rates (15.6 versus 9.6%) were better in Group 2 than those in Group 1. **CONCLUSIONS:** The results suggest that extending the period of hCG priming time from 35 to 38 h for immature oocyte retrieval promotes oocyte maturation *in vivo* and increases the IVM rate of immature oocytes. Therefore, oocyte retrieval after 38 h of hCG priming may improve subsequent pregnancy outcome in cycles programmed for IVM treatment.

Keywords: hCG priming; *in vitro* maturation (IVM); oocyte collection time; polycystic ovarian syndrome (PCOS); pregnancy

Introduction

There is an increasing interest in *in vitro* maturation (IVM) as well as natural-cycle in *in vitro* fertilization (IVF) and minimal stimulation regimes in the past few years. Although numerous studies on IVM of immature human oocytes have been performed, compared with the controlled ovarian hyperstimulation (COH) cycles, the efficiency of current IVM techniques is still suboptimal in terms of the number of mature oocytes obtained, embryo developmental competence and implantation rates. Therefore, several attempts have been made to improve the viability of IVM oocytes by gonadotrophin stimulation prior to oocyte collection (Wynn *et al.*, 1998; Chian *et al.*,

2000). Chian *et al.* (1999, 2000) reported good pregnancy rates by hCG priming (10 000 IU) before immature oocyte retrieval from women with polycystic ovary syndrome (PCOS) and this has been confirmed by some other groups (Lin *et al.*, 2003; Son *et al.*, 2007).

There are several embryological studies evaluating the advantages of using hCG priming in IVM cycles (Yang *et al.*, 2005; Son *et al.*, 2006, 2007). First, it has been reported that immature oocytes with dispersed cumulus cells (CCs) only appeared at the time of oocyte retrieval in the hCG-primed IVM cycles and that immature oocytes with dispersed CC showed better IVM and higher embryo developmental potential than those with compacted or sparse CC (Yang *et al.*, 2005; Son *et al.*, 2006). Secondly, a few *in vivo* matured oocytes with dispersed CC at the time of collection can be

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obtained even in IVM cycles (Son *et al.*, 2002, 2005, 2006), and these have produced better quality embryos than those derived from *in vitro* matured oocytes, resulting in better clinical pregnancy rates (Son *et al.*, 2008). Based on the above embryological observations, it is very important to find ways to increase the number of oocytes with dispersed CC at the time of retrieval in order to improve *in vivo*/IVM rates, embryo developmental competence and clinical outcome.

It has been reported that a prolonged interval between hCG administration and oocyte retrieval in conventional COH cycles increased the production of oocytes with fully expanded CC, follicular maturation and embryo developmental competence (Gudmundsson *et al.*, 1990; Bokal *et al.*, 2005; Raziel *et al.*, 2006). In IVM cycles, however, the effects on the embryological aspects and clinical outcome of oocytes obtained following a prolonged interval of 38 h between hCG priming and oocyte retrieval in PCOS patients have not been evaluated. Therefore, this study was conducted to evaluate whether extending the interval between hCG priming (from 35 to 38 h) and the time of oocyte retrieval increased the number of *in vivo* matured oocytes and improved the *in vitro* oocyte maturation rate as well as the clinical outcome in PCOS patients undergoing IVM treatment cycles.

Materials and Methods

The initial IVM study was approved by the Research Institute of the Hospital. The IVM treatment was explained to the patients, and informed consent was obtained.

Patients

A total of 113 PCOS patients (120 cycles) were included in this retrospective study. Between January 2006 and February 2007, immature oocyte collection was performed after 35 h hCG priming (Group 1; $n = 76$), and between February 2007 and November 2007, oocyte retrieval was performed after 38 h hCG priming (Group 2; $n = 44$). There were no differences in the characteristics of the IVM cycles between the two groups in terms of age, duration of infertility, largest follicle diameter at retrieval, endometrial thickness at retrieval or distribution of various follicle diameters on hCG priming day (Table I). A 10 000 IU dose of hCG was administered in both groups. Women with amenorrhea received vaginally 300 mg of progesterone (Prometrium; Schering, Pointe-Claire, Québec, Canada) once daily for 10 days to induce withdrawal bleeding. A baseline ultrasound scan was obtained for all women between Days 2 and 4 of menstrual bleeding to ensure that no ovarian cysts were present and to assess the antral follicle count

(AFC) (Tan *et al.*, 2002). Transvaginal ultrasound scans were repeated on Day 8 of the cycle or on the day of hCG administration.

Oocyte collection

Oocyte retrieval was performed between Days 10 and 16 of the menstrual cycle based on the length of the patient's cycle. A 10 000 IU dose of hCG was administered when endometrial thickness reached at least 6 mm. The number and measurement of follicles diameters was calculated on the day of hCG administration. The largest follicle and endometrial thickness were measured by ultrasound scan just before oocyte collection. The size of the largest follicle was calculated as a mean of two measurements in a two-dimensional plane. Transvaginal ultrasound-guided collection of oocytes was performed using a 19-gauge aspiration needle (K-OPS-7035-RWH-ET, Cook, Australia) with a reduced aspiration pressure of 7.5 kPa. The aspirates were collected in tubes with prewarmed heparinized saline. Collection started with the largest follicle and oocyte maturity was assessed. Subsequent collection of oocytes without follicle size measurement was performed according to accessibility of the follicles after which the maturity level of the oocyte was identified. To avoid the possibility of missing oocytes with a small amount of CC, the remaining follicular aspirates were filtered using 70 μ m mesh in hole size (Falcon, Becton–Dickinson & Company, NJ, USA), washed three times with oocyte wash medium (Cooper Surgical, CT, USA) that contained HEPES buffer supplemented with recombinant human serum albumin, and the oocytes isolated under a stereomicroscope.

In vitro maturation

The oocytes collected were assessed for nuclear maturity under the dissecting microscope with high magnification ($\times 80$) using the sliding method (Chian *et al.*, 2000; Son *et al.*, 2006). If no germinal vesicle (GV) was observed in the oocyte cytoplasm, the cumulus masses were removed with hyaluronidase and mechanical pipetting, and reassessment of maturity was performed. Oocytes that were mature on the collection day (Day 0: 0–6 h) were inseminated on the same day, whereas the immature oocytes (GV- or GV breakdown (GVBD)-stage) were cultured in IVM medium (Cooper Surgical, CT, USA) supplemented with 75 mIU/ml FSH and LH. Following culture on Day 1 (24–30 h), the oocytes were denuded of CCs with hyaluronidase and mechanical pipetting. After examination, immature oocytes remaining at GV- or GVBD-stage were further cultured in the same medium and the meiotic status was re-examined on Day 2 (48–52 h culture).

IVF, embryo development and transfer

Matured oocytes were inseminated by intracytoplasmic sperm injection (ICSI) using the partner's spermatozoa. ICSI was performed at least 1 h after observing first polar body (PB) extrusion as suggested by Hyun *et al.* (2007). Fertilization was assessed 17–19 h after

Table I. Characterization of hCG-primed IVM cycles in Groups 1 and 2.

Variables	Group 1 (hCG + 35 h)	Group 2 (hCG + 38 h)	P value
No. of cycles (age, mean $\pm$ SD)	76 (32.6 $\pm$ 3.4)	44 (33.1 $\pm$ 3.9)	NS
Duration of infertility (years $\pm$ SD)	3.8 $\pm$ 0.4	3.7 $\pm$ 0.3	NS
Endometrial thickness at collection (mm, mean $\pm$ SD)	8.6 $\pm$ 1.8	8.7 $\pm$ 1.6	NS
Diameter of largest follicle at collection (mm, mean $\pm$ SD)	11.6 $\pm$ 4.0	11.6 $\pm$ 3.0	NS
No. of follicles on the day of hCG priming			
≤ 6 mm (mean $\pm$ SD)	25.1 $\pm$ 11.2	23.7 $\pm$ 9.6	NS
7–9 mm (mean $\pm$ SD)	9.5 $\pm$ 4.5	10.4 $\pm$ 6.3	NS
≥ 10 mm (mean $\pm$ SD)	2.1 $\pm$ 1.8	1.9 $\pm$ 1.7	NS

Group 1: IVM cycles where oocytes were collected 35 h after hCG; Group 2: IVM cycles where oocytes were collected 38 h after hCG.

insemination for the appearance of two distinct pronuclei and two polar bodies. The zygotes were cultured in Embryo Maintenance Medium (Cooper Surgical, CT, USA). Embryonic development was assessed on Day 2 (41–43 h) and on Day 3 (65–67 h) after insemination according to the regularity of blastomeres, the percentage and pattern of anucleate fragments, and all dysmorphic characteristics of the embryos. For this study, we defined embryos as good quality if they had at least three-cells on Day 2 and six-cells on Day 3, contained <20% anucleate fragments and exhibited no apparent morphological abnormalities. Embryos showing blastomere multi-nucleation, poor cell adhesion, uneven cell division and cytoplasmic abnormalities were defined as low quality. The best quality embryos were transferred on Day 2 or 3 after ICSI.

Endometrium preparation

For endometrial preparation, patients received estradiol (E_2) valerate (Estrace; Roberts Pharmaceutical, Mississauga, Canada), starting on the day of oocyte retrieval. If the endometrial thickness was 6–8 mm, an 8–10 mg dose was given, and if it was ≥ 8 mm, a 6 mg dose was administered, all in divided doses. Luteal support was provided by administering 50 mg of progesterone daily intra-muscularly starting on the day of ICSI and continued, along with E_2 valerate, until 12 weeks of gestation, if the pregnancy test was positive.

Statistical analysis

Statistical analyses were performed using the χ^2 , Fisher's exact or *t*-test as appropriate. All *P*-values quoted are two-sided, and values <0.05 indicate statistical significance. Analyses were performed using the SPSS statistical package (SPSS, Inc., Chicago).

Results

Fig. 1 shows the cumulus oocytes complex (COC) retrieved from experimental groups as reported previously (Son *et al.*, 2006). Oocytes classified as having dispersed, compacted or sparse CC appearances were collected. Fig. 2 shows the percentage of oocytes classified according to the appearance of their cumulus at the time of oocyte retrieval in the two groups. This figure shows that a higher number of oocytes with dispersed CCs appeared in Group 2 (64.3%) compared with that in Group 1 (37.5%) ($P < 0.01$).

Table II summarizes the embryological aspects and clinical outcome in the hCG-primed IVM cycles of Groups 1 and 2. On the day of oocyte retrieval, the number of *in vivo* matured oocytes collected was significantly higher ($P < 0.01$) in Group 2 (13.6%, 114/840) than in Group 1 (7.3%, 96/1312). All the *in vivo* matured oocytes collected in both groups showed dispersed CCs. Fig. 3 shows the mean percentage of

oocytes at different stages of maturity on Days 0, 1 and 2 for both groups. In both groups, all the oocytes that were still at the GVBD-stage following 6 h culture on the day of retrieval became mature by Day 1, together with a high proportion of GV-stage oocytes. In contrast, in both groups, most of the oocytes that mature by Day 2 were derived from oocytes that were at the GVBD-stage on Day 1 (Fig. 3). The IVM rate after Day 1 culture was significantly higher ($P < 0.01$) in Group 2 (46.3%) than in Group 1 (36.0%) (Fig. 4). The IVM rate up to Day 2 was also significantly higher ($P < 0.05$) in Group 2 (68.2%) compared with that in Group 1 (62.3%) (Fig. 4).

Table III details the rates of maturation, fertilization, cleavage and embryo development according to maturation time in the two groups. The good quality embryo rate, defined as the number of good quality embryos divided by the number of normal fertilized oocytes, was significantly higher in the embryos derived from the *in vivo* matured oocytes (Group 1 = 64.9%, Group 2 = 62.4%) than from oocytes matured *in vitro* on Day 1 (Group 1 = 46.2%, Group 2 = 42.9%) in both groups ($P < 0.01$) (Table III). In both groups, the rates of fertilization, cleavage and good quality embryos in oocytes matured *in vitro* on Day 2 were significantly lower than those in oocytes matured *in vitro* on Day 1, as well as *in vivo* matured oocytes in the both groups ($P < 0.01$). However, there was no significant difference in the fertilization and embryo developmental competence in relation to the hCG priming time.

Although fertilization, cleavage and good quality embryo rates were comparable between the two groups, the mean number of all embryos available for transfer was significantly higher in Group 2 compared with that in Group 1 (9.4 ± 5.9 versus 7.4 ± 5.7 embryos) ($P < 0.01$) (Table II). Of the 280 embryos transferred in Group 1, 53 (18.9%), 178 (63.6%) and 49 (17.5%) were derived from oocytes matured *in vivo*, and those *in vitro* matured on Day 1 and Day 2, respectively. In Group 2, 34.4 (55/160), 63.1 (101/160) and 2.5% (4/160) of embryos transferred were derived from oocytes matured *in vivo*, and those *in vitro* matured on Days 1 and 2, respectively. Therefore, more embryos produced from *in vivo* matured oocytes were transferred in Group 2 than in Group 1. The clinical pregnancy rate in Group 2 (40.9%) was higher than that in Group 1 (25%), but this was not statistically significant ($P = 0.1$). The implantation rate in Group 2 (15.6%) was higher than that of Group 1 (9.6%), although the difference did not reach statistical significance ($P = 0.07$).

Table IV details a comparison of clinical pregnancies following transfer of the embryos derived from oocytes with

Figure 1: COC morphology of oocytes obtained in hCG-primed IVM cycles.

(a) A GV stage oocyte with dispersed CCs (b) a GV stage oocyte with compacted CCs (c) a GV stage oocyte with sparse CCs. Original magnification ($\times 400$). GV, germinal vesicle.

Figure 2: Incidence of oocytes with CC appearances of either dispersed (■), compacted (▨), or sparse (□). Group 1: IVM cycles where oocytes were collected 35 h after hCG. Group 2: IVM cycles where oocytes were collected 38 h after hCG. (a versus b) $P < 0.01$.

different maturation times in hCG-primed IVM cycles. In both groups, we observed higher clinical pregnancy rates in cycles where embryos had been derived from *in vivo* matured oocytes than in those where embryos had been derived from *in vitro* matured oocytes.

Discussion

This study demonstrates that in programmed IVM cycles an extension of the interval between hCG administration and oocyte collection from 35 to 38 h results in a higher number of *in vivo* and *in vitro* matured oocytes.

Although there have been conflicting reports on the clinical outcome of FSH- or hCG priming before IVM (Mikkelsen *et al.*, 1999; Söderström-Anttila *et al.*, 2005), the advantages of hCG-primed IVM cycles compared with that of non- or FSH-primed IVM cycles have been reported (Son *et al.*, 2006, 2008). Technically, the CC expansion (dispersal) of the oocytes caused by a high dosage of hCG (10 000 IU) facilitates

Figure 3: Mean percentage of oocytes (mean % $\pm$ SD) at Metaphase II- (□), GVBD- (▨), or GV- (■)-stage on collection day (Day 0), on Day 1 and on Day 2 culture in Group 1 (hCG + 35 h) and Group 2 (hCG + 38 h).

Day 0: 0–6 h after culture, 1: 24–30 h after culture, Day 2: 48–52 h after culture. GVBD=germinal vesicle breakdown, GV=germinal vesicle. (a versus b) $P < 0.01$, (c versus d) $P < 0.01$.

detachment and expulsion of the COC mass from the follicle during the aspiration and thus makes oocyte collection easier in hCG-primed IVM cycles than in non- or FSH-primed IVM cycles (Son *et al.*, 2006). Thus, oocytes with a dispersed CC appearance can be obtained at the time of oocytes retrieval only in hCG-primed IVM cycles (Yang *et al.*, 2005; Son *et al.*, 2006). Those oocytes having dispersed CC are easy to identify under a stereomicroscope without any filtration method, similar to the standard IVF technique (Son *et al.*, 2008). Regarding the embryological aspects, those immature and *in vivo* matured oocytes with dispersed CC collected in hCG-primed IVM cycles have shown good embryo developmental competence (Son *et al.*, 2002, 2006, 2008; Yang

Table II. Maturation, fertilization, development and pregnancy rates in Groups 1 and 2.

Variables	Group 1 (hCG + 35 h)	Group 2 (hCG + 38 h)	P value
No. of cycles	76	44	
No. of cycles with <i>in vivo</i> matured oocytes (%)	38 (50)	36 (81.8)	$P < 0.01$
No. of oocytes collected (mean $\pm$ SD)	1312 (17.3 $\pm$ 9.0)	840 (19.1 $\pm$ 9.5)	NS
No. of oocytes matured on collection day (%)	96 (7.3)	114 (13.6)	$P < 0.01$
Mean $\pm$ SD (range)	1.3 $\pm$ 2.1 (0–12)	2.6 $\pm$ 2.8 (0–12)	$P < 0.01$
No. of oocytes cultured <i>in vitro</i>	1216	726	
No. of oocytes matured <i>in vitro</i> on Day 1(%)	438 (36.0)	336 (46.3)	$P < 0.01$
No. of oocytes matured <i>in vitro</i> on Day 2(%)	320 (26.3)	159 (21.9)	NS
No. of MII oocytes matured <i>in vitro</i> (%)	758 (62.3)	495 (68.2)	$P < 0.05$
No. of total MII oocytes (%)	854 (65.1)	609 (72.5)	$P < 0.01$
No. of normal fertilized oocytes (%)	623 (73.0)	447 (73.4)	NS
No. of embryos cleaved (%)	560 (89.9)	413 (92.4)	NS
Mean no. of embryos available for ET	7.4 $\pm$ 5.7	9.4 $\pm$ 5.9	$P < 0.01$
No. of transferred embryos (mean $\pm$ SD)	280 (3.7 $\pm$ 1.0)	160 (3.6 $\pm$ 0.9)	NS
No. of clinical pregnancies (%)	19 (25.0)	18 (40.9)	$P = 0.1$
No. of implantations (%)	27 (9.6)	25 (15.6)	$P = 0.07$

Group 1: IVM cycles where oocytes were collected 35 h after 10 000IU hCG; Group 2: IVM cycles where oocytes were collected 38 h after 10 000 IU hCG. NS, non significant.

Figure 4: Time course of oocyte maturation during culture *in vitro* of human immature oocytes in cycles where oocytes were collected either 35 h after hCG (Group 1, □) or 38 h after hCG (Group 2, ■). Day 0: 0–6 h after culture, Day 1: 24–30 h after culture, Day 2: 48–52 h after culture. * $P < 0.01$ or ** $P < 0.05$. Significant difference between two groups at the time point.

et al., 2005). Therefore, the dispersed CC pattern at the time of aspiration in hCG-primed IVF cycles can be predictive of higher maturation rates and of embryonic developmental competence. In this study, 38 h of hCG priming produced a significantly higher number of oocytes with dispersed CC than did 35 h of priming.

Administration of hCG is normally used to substitute the LH surge and triggers the final oocyte maturation before retrieval. In COH cycles, several reports have been published on the effect of extending the time between oocyte collection and hCG injection on oocyte maturation and embryonic developmental competence. Although Bjercke *et al.* (2000) could not find any difference between 34 and 38 h of oocyte exposure to hCG, other studies suggested that prolonging the interval to oocyte retrieval increased the rate of *in vivo* oocyte maturation and the number of high quality cleaving embryos in COH cycles (Gudmundsson *et al.*, 1990; De Vits *et al.*, 1994; Raziel *et al.*, 2006). Recently, a similar clinical approach was adopted in another IVF clinic and the positive effects were

Table IV. Details in comparison of clinical pregnancies following transfer of the embryos derived from oocytes matured according to maturation day in hCG-primed IVF cycles.

ET of embryos derived from oocytes matured on	Group 1 No. of CP/no. of ET cycles (%)	Group 2 No. of CP/no. of ET cycles (%)
Day 0	3/6 (50.0)	3/5 (60.0)
Day 0+Day 1	6/18 (33.3)	11/25 (44.0)
Day 0+Day 1+Day 2	2/6 (33.3)	0/2 (0.0)
Day 1	6/22 (27.3)	4/11 (36.4)
Day 1+Day 2	1/19 (5.3)	0/1 (0.0)
Day 2	1/5 (20.0)	—
Total clinical pregnancy rate (%)	19/76 (25.0)	18/44 (40.9)

CP, clinical pregnancies; ET, embryo transfer. Group 1: IVF cycles where oocytes were collected 35 h after hCG; Group 2: IVF cycles where oocytes were collected 38 h after hCG.

confirmed after performing COH in patients who had a high percentage of immature oocytes in previous cycles (Raziel *et al.*, 2006). The investigators assumed that the longest interval would yield oocytes in which all cellular and nuclear maturation processes would have been completed and therefore the mature oocytes collected would be more likely to develop into 2PN zygotes and normally cleaving embryos.

For hCG-primed IVF programs, we considered two methods to increase the number of oocytes with dispersed CC: a time-dependent and dose-dependent response on hCG priming. The effect of a dose-effective response 36 h after hCG priming has already been studied on embryological aspects in IVF cycles (Gulekli *et al.*, 2004). In that study, the authors compared the IVF, fertilization and embryo development rates between oocytes collected after 10 000 and 20 000 IU hCG priming in patients who underwent IVF cycles. Although the fertilization rate was significantly lower in the group that had been stimulated with 20 000 IU hCG compared with that in the group stimulated with 10 000 IU, the maturation and cleavage rates were similar between the two groups. Based on the finding of that study, it seems that there is no dose-dependent effect of the hCG on *in vivo* or/and *in vitro* oocyte maturation in IVF cycles. However, there was no mention about the difference in CC appearance and *in vivo* matured oocytes in the report.

Therefore, this study was conducted to evaluate a time-dependent effect on hCG-primed IVF cycles. In comparison with COH cycles, there is no risk of ovulation by delaying

Table III. Details in embryology of hCG-primed IVF cycles in Groups 1 and 2.

Groups	Group 1 (hCG + 35 h)			Group 2 (hCG + 38 h)		
	Day 0	Day 1	Day 2	Day 0	Day 1	Day 2
No. of MII oocytes	96	438	320	114	336	159
No. of fertilized oocytes (%)	74 (77.1) ^a	344 (78.5) ^a	205 (64.1) ^b	85 (74.6) ^a	268 (79.8) ^a	94 (59.1) ^b
No. of cleaved embryos (%)	72 (97.3) ^a	332 (96.5) ^a	156 (76.1) ^b	84 (98.8) ^a	256 (95.5) ^a	73 (77.7) ^b
No. of good quality embryos (%*)	48 (64.9) ^a	159 (46.2) ^b	40 (19.5) ^c	53 (62.4) ^a	115 (42.9) ^b	15 (16.0) ^c

Group 1: IVF cycles where oocytes were collected 35 h after hCG; Group 2: IVF cycles where oocytes were collected 38 h after hCG.

*The number of good quality embryos divided by number of fertilized oocytes.

^a versus ^b $P < 0.05$.

^{a,b,c} Different letters superscripts denote significant differences ($P < 0.01$) among values within row in each hCG timing group.

oocyte collection after hCG stimulation in patients undergoing IVM cycles since the largest follicle size at the time of hCG administration is 8–12 mm. In the present study, a positive correlation was observed between delayed collection time after hCG priming and the number of oocytes with dispersed CC generated in hCG-primed IVM cycles (Fig. 2). This finding is in agreement with Gudmundsson *et al.* (1990), who described a significantly higher number of oocytes with fully expanded CC after delayed oocyte retrieval (39 h) in COH cycles. Therefore, we believe that prolonging the interval between hCG administration and oocyte collection in IVM cycles is a valuable treatment to collect more oocytes stimulated in an *in vivo* environment for PCOS patients.

In the present study, we assessed whether the presence of a high number of oocytes with dispersed CC at the time of collection in hCG-primed IVM cycles was positively correlated to *in vivo* and *in vitro* oocyte maturation. The rate of *in vivo* matured oocytes in Group 2 (13.6%, mean = 2.6) was significantly higher compared with that in Group 1 (7.3%, mean = 1.3) (Table II). Therefore, there was a higher chance of retrieving *in vivo* matured oocytes at the time of retrieval in Group 2 (hCG + 38 h) compared with that in Group 1 (hCG + 35 h), even when the largest follicle size was similar between both groups (Table I). Actually, the smallest follicle size from which an *in vivo* matured oocyte was collected was 8 mm in Group 1, whereas in Group 2 it was as small as 6 mm. This is in agreement with Bokal *et al.* (2005), who found a significantly higher number of MII oocytes in HCG + 38 h group than in hCG + 34 h in COH cycles. When we analyzed the embryological aspects of both groups in detail, we confirmed that oocytes matured *in vivo* possessed a significantly higher developmental potential than those oocytes matured *in vitro* (Table III) for both groups, resulting in higher clinical pregnancy rates in cycles that had transferred embryos derived from *in vivo* matured oocytes (Table IV).

Although there was no difference in the mean percentage of GVBD-stage oocytes on collection day between the two groups (Fig. 3), the IVM rate after Day 1 of culture in Group 2 was significantly higher (46.3%) than that of Group 1 (36%) (Fig. 4), suggesting that the follicles in Group 2 can produce GV-stage oocytes that possess the potential to mature faster *in vitro* by extending hCG stimulation for three more hours. As reported previously (Barnes *et al.*, 1996; Son *et al.*, 2005), those oocytes that first reach MII stage in IVM cycles are usually the most competent to develop into blastocysts. The *in vitro* matured oocytes after Day 1 produced better quality embryos than those that had matured late (Day 2) in both Groups 1 and 2 (Table III). Therefore, this study demonstrated that an increased number of oocytes with dispersed CC following prolonged hCG stimulation was positively correlated with the number of *in vivo* and *in vitro* matured oocytes. However, there was no significant difference in fertilization and embryonic developmental competence between oocytes matured on the same day in Groups 1 and 2 (Table III).

As shown in Table IV, in both groups, the clinical pregnancy rate in cycles where embryos were derived from *in vivo* or/and Day 1 *in vitro* matured oocytes was higher than that where embryos were derived from late matured oocytes (Day 2).

Most of the cycles in Group 2 had transferred embryos derived from oocytes matured *in vivo* and/or *in vitro* on Day 1 compared with cycles in Group 1 (Table IV). Therefore, the trend of better pregnancy outcome in the hCG + 38 h IVM cycles is attributed to the increase in the quantity of embryos available to select for transfer, since the percentages of oocytes matured *in vivo* and those matured faster *in vitro* were improved with prolonged hCG exposure. In fact, the mean number of all embryos available in Group 2 was significantly higher than in Group 1 (9.4 and 7.4 embryos, respectively) (Table II).

The present study indicates that a larger cohort of oocytes matured *in vivo* and *in vitro* can be recruited by permitting hCG action for three more hours. In COH cycles, Bokal *et al.* (2005) suggested that the longer exposure to hCG increased follicular vascularization and thus oocyte quality due to a high concentration of vascular endothelial growth factor (VEGF). Therefore, it is tempting to speculate that in IVM cycles the hCG action for three more hours would allow follicles to produce more VEGF, increasing oocyte maturation. However, in IVM cycles, it is hard to confirm the effect of VEGF since it is difficult to get follicular fluid from each follicle due to its small size. Another possibility could be that even though there is no direct evidence on the appearance of LH-receptors (LH-R) in folliculogenesis, it is possible that some small follicles with insufficient LH-R might have responded following exposure for a longer time to hCG (35 versus 38 h), resulting in an increased number of oocytes with dispersed CC and a reduced number of oocytes with compact or sparse CC. Interestingly, LH-R expression has been confirmed in dispersed CCs of oocytes generated from hCG-primed IVM cycles (Yang *et al.*, 2005). Willis *et al.* (1998) have reported that unstimulated granulosa cells retrieved from anovulatory women with polycystic ovaries responded to LH in small follicles of 4 mm. In spite of the exposure to large doses of hCG (10 000 IU) for three more hours, the presence of compact CCs around oocytes could be due to LH-R which is still insufficient to induce the CC response *in vivo*. Meanwhile, the oocytes with sparse CCs might be mixed with oocytes with CCs removed mostly by pressure on ovum aspiration and that were entering the degenerative process. A well-defined study is needed to ascertain these possibilities.

In conclusion, our results suggest that the extension of the interval between hCG priming and oocyte retrieval to 38 h in programmed IVM cycles promotes *in vivo* and *in vitro* oocyte maturation. The prolonged exposure to hCG seems to be a promising alternative protocol to be used in IVM cycles.

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