Article

Effect of gonadotrophin priming on in-vitro maturation of oocytes collected from women at risk of OHSS

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Abstract

This study examined the effects of human menopausal gonadotrophin (HMG) or human chorionic gonadotrophin (HCG) priming on cumulus-oocyte complex (COC) morphology, oocyte maturation and embryo development in patients undergoing in-vitro maturation (IVM) cycles. The patients were primed with nothing (group 1), low-dose HMG (group 2) or 10,000 IU HCG (group 3) before oocyte retrieval. COC with dispersed cumulus cell appearance was only observed in group 3. In addition, 11% of metaphase II stage oocytes at the time of retrieval were collected from group 3. Oocyte maturation in vitro in group 3 was faster than that in groups 1 and 2. The blastocyst development rate of residual embryos after embryo transfer in group 3 was significantly higher than that of groups 1 and 2 (P < 0.05). These results suggest that HCG priming may stimulate the COC, promote oocyte maturation, and improve developmental competence in IVM cycles.

Keywords: HCG, HMG, human, immature oocyte, in-vitro maturation

Introduction

Immature oocyte retrieval combined with in-vitro maturation (IVM) is an attractive alternative to conventional IVF, with several advantages: the avoidance of the risk of ovarian hyperstimulation syndrome (OHSS), reduced cost, and less complicated treatment. Although it is possible to mature and fertilize human oocytes obtained from unstimulated cycles, the pregnancy rate after IVM has in general been low (Barnes et al., 1996; Trounson et al., 1996; Trounson et al., 1996; Trounson et al., 1998). To overcome these problems, some studies have focused on improved culture media; other studies have tried to optimize the quality of the oocyte by stimulation with oestradiol (Russell et al., 1997) or gonadotrophins (Wynn et al., 1998).

Several authors have reported IVM of oocytes retrieved from ovaries exposed to gonadotrophin stimulation prior to oocyte collection. Wynn et al. (1998) have indicated that mild ovarian stimulation with FSH prior to oocyte collection in human IVM studies improved both the number of oocytes retrieved and the maturation rate. However, further studies (Trounson et al., 1998; Mikkelsen et al., 1999) did not demonstrate any difference in the number of oocytes obtained and the maturation rate. Therefore, previous studies of FSH priming have given conflicting results.

Chian and colleagues (Chian et al., 2000; Chian, 2004) reported that rates of maturation and pregnancy of immature oocytes retrieved from women with polycystic ovarian syndrome (PCOS) were improved by HCG priming. However, another study did not demonstrate any difference (Chung et al., 2000; Söderström-Antilla et al., 2005).

Therefore, it is unclear whether priming with gonadotrophins in unstimulated ovaries could improve the rates of oocyte
maturation as well as the developmental capacity. This study analysed whether the developmental potential of oocytes may be improved by low-dose HMG or HCG priming before oocyte collection in patients at high risk of developing OHSS undergoing IVM cycles. In addition, differences in embryological aspects were also compared among these three groups.

Materials and methods

The IVF programme was approved by the Institutional Review Board of the Maria Infertility Hospital.

Patients

This study was performed retrospectively. All patients (n = 56) had experienced OHSS in previous ovarian stimulation cycles. The patients were selected by a computerized method based on antral follicle count (≥15), age, and endometrial thickness at the time of embryo transfer (>9 mm) among patients who underwent IVM randomly in the same period. The patients were primed with nothing (group 1: 23 patients, 27 cycles), low-dose HMG for 2 days (150 IU per day, days 3–4 of the cycle) (group 2: 12 patients, 12 cycles), or 10,000 IU of HCG (group 3: 21 patients, 23 cycles) before oocyte retrieval. Priming with HCG occurred on the day when endometrial thickness was >6 mm, and according to the patient’s cycle length. When following this criterion, the largest follicle at the time of gonadotrophin administration was 7–11 mm. The types of infertility in each group are listed in Table 1. Patients with anovulatory cycles received intramuscular injection of progesterone (Progest; Samil Pharmacology, Seoul, Korea). Withdrawal bleeding occurred within 10 days after the last dose.

Oocyte collection

The oocytes were collected between cycle days 9 and 17 based on the patient’s cycle length and endometrial thickness of approximately 6 mm in the three groups. A transvaginal ultrasound machine (Aloka, Tokyo, Japan) with 19-gauge aspiration needle (Cook, Eight Mile Plains, Queensland, Australia) was used to aspirate follicles. A portable aspiration pump was used with a pressure between 80 and 100 mmHg. The aspirates were collected in tubes containing prewarmed heparinized Ham’s F-10 medium that contained bicarbonate and HEPES buffers supplemented with 0.3% human serum albumin. Follicular aspirates were filtered using 70 μm mesh in hole size (Falcon, Becton Dickinson and company, NJ, USA), washed three times, and the oocytes were isolated under a stereomicroscope (Yoon et al., 2001a).

In-vitro maturation

The collected oocytes were classified as follows: atretic, dispersed cumulus, compacted cumulus, or sparse cumulus. The atretic oocytes were discarded and the maturity of oocytes at the time of retrieval was evaluated using the sliding method (Son et al., 2005). Mature oocytes on collection day were inseminated on the same day and the immature oocytes were cultured in IVM medium consisting YS medium (a glucose-free standard medium, Yoon et al., 2001a) supplemented with 1 IU/ml FSH, 10 IU/ml HCG and 10 ng/ml recombinant human epidermal growth factor (Daewoong Pharmaceutical Co., Korea) (Son et al., 2002). After culture for 24–30 h (day 1), the oocytes were denuded of cumulus cells with 0.003% hyaluronidase (Sigma Chemical Co., St Louis, MO, USA) and mechanical pipetting. The pipetting was stopped when germinial vesicle (GV)-stage oocytes were found during the denuding of cumulus cells. However, for metaphase I (MI) and metaphase II (MII) stage oocytes, cumulus cells were completely removed. Following examination, immature oocytes remaining at GV or MI stage were further cultured in the same medium and the meiotic status was re-examined on day 2 (48–52 h culture).

IVF, in-vitro development (IVD), and embryo transfer

Matured oocytes were inseminated by intracytoplasmic sperm injection (ICSI) using the partner’s spermatozoa. Fertilization was assessed 17–19 h after insemination for the appearance of two distinct pronuclei and two polar bodies. The zygotes were co-cultured with cumulus cells in 10 μl YS medium supplemented with 10% human follicular fluid (hFF; Yoon et al., 2001b). The cumulus cells for co-culture were prepared as described previously (Yoon et al., 2001b). Briefly, only corona radiata masses from the cleaned cumulus-oocyte complexes (COC) were excised.

Table 1. Characteristics of patients receiving no priming, human menopausal gonadotrophin (HMG) priming, or human chorionic gonadotrophin (HCG) priming during in-vitro maturation treatment (groups 1, 2 and 3 respectively).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group 1 (non-primed)</th>
<th>Group 2 (HMG-primed)</th>
<th>Group 3 (HCG-primed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients (mean age in years ± SD)</td>
<td>23 (33.0 ± 2.8)</td>
<td>12 (32.0 ± 3.3)</td>
<td>21 (33.6 ± 3.2)</td>
</tr>
<tr>
<td>Duration of infertility (mean years ± SD)</td>
<td>3.8 ± 0.4</td>
<td>3.7 ± 0.3</td>
<td>3.9 ± 0.6</td>
</tr>
<tr>
<td>Diagnosis of infertility</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCO or PCOS</td>
<td>6</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Tubal factor</td>
<td>5</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Male factor</td>
<td>5</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Ovarian factor</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>5</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

PCO = polycystic ovaries; PCOS = polycystic ovary syndrome.
using two pieces of 30-gauge needle. The corona radiata masses were treated with 100–150 μl YS medium containing 10% hFF and 0.003% hyaluronidase for lyses of gap junctions, resulting in change of the corona radiata masses to a pool containing many single cells. The cumulus cell layers used for co-culture were prepared under 10 μl droplets by seeding cumulus cells \((1 \times 10^4)\) obtained from the corona radiata of COC. After 4 h of incubation, the medium was exchanged with a pre-equilibrated culture medium to remove hyaluronidase and unattached cells, and the drop dishes containing cumulus layers were incubated overnight. All droplets for co-culture were exchanged with pre-equilibrated culture medium every morning. At each change, 5 μl was removed and an equal volume of fresh medium was added. Embryos were transferred on day 4 after oocyte retrieval by the transcervical route in standard fashion. After embryo transfer, surplus embryos were cultured until day 7 after insemination. The embryos developed to blastocyst stage were compared in the three groups and cryopreserved.

**Endometrium preparation**

For the preparation of the endometrium, patients were given 10,000 IU HCG (IVF-C; LG Chemical, Korea) at the time of oocyte retrieval. Oestradiol valerate (Progynova; Schering, Berlin, Germany) 6 mg was administered daily from the day of immature oocyte retrieval. Progesterone (100 mg) was administered daily starting on the day of ICSI. Both medications were continued until either a negative pregnancy urine test or a positive fetal heartbeat was observed.

**Statistical analysis**

Differences between treatment groups in each experiment were compared with the chi-squared test using the Statistical Analysis System (SAS Institute, Cary, NC, USA) software package.

**Results**

There was a similar distribution among all three groups for age, and duration and cause of infertility (Table 1). Figure 1 shows the COC retrieved from experimental groups under low magnification (×200). There was a different COC pattern between group 3 and the other two groups. There was a similar appearance of the COC between non-primed and HMG-primed groups (Figure 1a). Oocytes that were classified as having dispersed, compacted, and sparse cumulus cell appearance were used for culture (Figure 2). Figure 3 shows the percentage of oocytes classified according to their cumulus appearance at the time of oocyte retrieval in three groups. This figure shows that oocytes with dispersed cumulus cells only appeared in the HCG-primed group. The cumulus cell appearance of oocytes collected from group 1 was similar to those collected from group 2 (Figures 1 and 3). In group 3, 11% (41/360) of oocytes were at MII stage at the time of retrieval. All the MII stage oocytes collected in the HCG-primed group showed a dispersed cumulus cell appearance (Figure 4) as well as different cumulus expansion compared with MII stage oocytes collected from ovarian stimulation cycles (Figures 4 and 5). In contrast, no MII oocytes occurred in groups 1 and 2.

Comparisons of the time course of maturation to MII in the three groups are shown in Figure 6. After culture until day 1, oocyte maturation in vitro in the HCG-primed group (51.4%; 164/319) was faster than that of group 1 (45.0%; 191/424) and group 2 (40.8%; 71/174) \((P < 0.05)\). The oocytes matured in vitro until day 2 were comparable among three groups (74.8, 70.7 and 73.0% respectively). Table 2 summarizes the outcomes in each group, in terms of number of oocytes collected, maturation rate to MII stage, fertilization and cleavage rates. The mean number of the collected oocytes was comparable in the three groups (18.0 ± 4.9, 16.6 ± 4.9, and 17.5 ± 5.3). No difference was found in the rates of total maturation, normal fertilization and cleavage in these three groups. After embryo transfer, the blastocyst development rate of residual embryos in group 3 (20.0%; 21/105) was significantly higher than those of group 1 (7.8%, 8/103) and group 2 (7.7%, 3/39), \((P < 0.05)\) (Table 3). There were six clinical pregnancies (22.2%; 6/27) in group 1, three pregnancies (25.0%, 3/12) in group 2, and nine pregnancies (39.1%, 9/23) in group 3 (Table 2). The implantation rates were 6.5% (9/138), 6.8% (4/59) and 12.2% (14/115) in groups 1, 2 and 3 respectively (Table 2). Multiple pregnancy rates were 50% (3/6), 33.3% (1/3) and 55.6% (5/9) in groups 1, 2 and 3 respectively, but there was no triplet pregnancy in any of the groups.

**Figure 1.** Cumulus–oocyte complex (COC) retrieved from non-primed, human menopausal gonadotrophin (HMG)-primed or human chorionic gonadotrophin (HCG)-primed in-vitro maturation (IVM) cycles. (a) Oocytes just after retrieval in non-primed or HMG-primed IVM cycles. (b) Oocytes just after retrieval in HCG-primed IVM cycles. Original magnification ×200.
Figure 2. Human immature oocytes at the time of retrieval. (a) A germinal vesicle (GV)-stage oocyte with dispersed cumulus cell appearance. (b) A GV-stage oocyte with compacted cumulus cell appearance. (c) A GV-stage oocyte with sparse cumulus cell appearance. Original magnification ×400.

Figure 3. The incidence of oocytes with cumulus cell appearances of dispersed (mid-grey bars), compacted (dark grey bars), sparse (white bars), or degenerated (light grey bars). HMG = human menopausal gonadotrophin; HCG = human chorionic gonadotrophin.
**Figure 4.** Cumulus cell expansion of metaphase II (MII) stage oocytes at the time of retrieval. (a) MII-stage oocyte retrieved from human chorionic gonadotrophin-primed in-vitro maturation cycle. (b) MII-stage oocyte retrieved from ovarian stimulation cycle. Original magnification ×400. PB = 1st polar body.

**Figure 5.** Cumulus cell expansion of metaphase II (MII)-stage oocytes retrieved from human chorionic gonadotrophin-primed in-vitro maturation cycles. (a) MII-stage oocyte with clumped corona radiata. (b) MII-stage oocyte with little expanded corona radiata. (c) MII-stage oocyte with moderately expanded corona radiata. (d) MII-stage oocyte without corona radiata. Original magnification ×400. PB = 1st polar body.
Figure 6. The time course of human oocyte maturation during culture in vitro in non-primed (group 1, white squares), human menopausal gonadotrophin-primed (group 2, black triangles), and human chorionic gonadotrophin-primed (group 3, black squares). Bars indicate standard error of the mean (SEM).

Table 2. Maturation, fertilization, development and pregnancy rates using in-vitro matured human oocytes from the three study groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1 (non-primed)</th>
<th>Group 2 (HMG-primed)</th>
<th>Group 3 (HCG-primed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. cycles</td>
<td>27</td>
<td>12</td>
<td>23</td>
</tr>
<tr>
<td>No. oocytes collected (mean ± SD)</td>
<td>486 (18.0 ± 4.9)</td>
<td>199 (16.6 ± 4.9)</td>
<td>403 (17.6 ± 5.3)</td>
</tr>
<tr>
<td>No. viable oocytes</td>
<td>424</td>
<td>174</td>
<td>360</td>
</tr>
<tr>
<td>No. mature oocytes at retrieval (%)</td>
<td>0</td>
<td>0</td>
<td>41 (11.4)</td>
</tr>
<tr>
<td>No. oocytes cultured in vitro</td>
<td>424</td>
<td>174</td>
<td>319</td>
</tr>
<tr>
<td>No. MII oocytes matured in vitro (%)</td>
<td>317 (74.8)</td>
<td>123 (70.7)</td>
<td>253 (73.0)</td>
</tr>
<tr>
<td>Total no. MII oocytes</td>
<td>317</td>
<td>120</td>
<td>274</td>
</tr>
<tr>
<td>No. 2PN oocytes (%)</td>
<td>241 (76.0)</td>
<td>98 (79.7)</td>
<td>220 (80.3)</td>
</tr>
<tr>
<td>No. oocytes cleaved (%)</td>
<td>215 (89.2)</td>
<td>84 (85.7)</td>
<td>153 (94.5)</td>
</tr>
<tr>
<td>No. embryos transferred (mean ± SD)</td>
<td>138 (5.1 ± 1.2)</td>
<td>59 (4.9 ± 1.4)</td>
<td>115 (5.0 ± 1.4)</td>
</tr>
<tr>
<td>No. implantations (%)</td>
<td>9 (6.5)</td>
<td>4 (6.8)</td>
<td>14 (12.2)</td>
</tr>
<tr>
<td>No. clinical pregnancies (%)</td>
<td>6 (22.2)</td>
<td>3 (25.0)</td>
<td>9 (39.1)</td>
</tr>
<tr>
<td>No. live births (%)</td>
<td>5 (18.5)</td>
<td>2 (16.7)</td>
<td>7 (30.4)</td>
</tr>
</tbody>
</table>

HMG = human menopausal gonadotrophin; HCG = human chorionic gonadotrophin, MII = metaphase II; PN = pronucleate.
Table 3. Development to blastocyst of residual embryos from the three study groups.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (non-primed)</th>
<th>Group 2 (HMG-primed)</th>
<th>Group 3 (HCG-primed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of 2PN oocytes</td>
<td>241</td>
<td>98</td>
<td>220</td>
</tr>
<tr>
<td>No. of embryos transferred</td>
<td>138</td>
<td>59</td>
<td>115</td>
</tr>
<tr>
<td>No. of residual embryos</td>
<td>103</td>
<td>39</td>
<td>105</td>
</tr>
<tr>
<td>No. of blastocysts developed (%)</td>
<td>8/103 (7.8)</td>
<td>3/39 (7.7)</td>
<td>21/105 (20.0)</td>
</tr>
</tbody>
</table>

HMG = human menopausal gonadotrophin; HCG = human chorionic gonadotrophin; PN = pronucleate.

*p < 0.05.

Discussion

The results of the present study indicate that the COC morphology in HCG-primed IVM cycles is different compared with non-primed or HMG-primed groups, and MII-stage oocytes can be obtained at the time of retrieval in the HCG-primed group. In addition, the in-vitro maturation time of oocytes is hastened by HCG priming compared with no and low-dose HMG stimulation, and HCG priming before collection of immature oocytes may improve the developmental competence.

Significant numbers of human immature oocytes can be matured to MII, with subsequent fertilization, cleavage and development to viable offspring according to variable sources (Trounson et al., 1994; Nagy et al., 1996; Russell et al., 1997). However, investigators have also reported lower pregnancy and implantation rates (Barnes et al., 1996; Trounson et al., 1998). First of all, the low success rate might be attributed to asynchrony in the cytoplasmic and nuclear maturation of the oocyte. Cytoplasmic maturity might not be complete in spite of a mature nucleus (Mikkelsen, 2005). Aberrations in cytoplasmic maturation are more likely to be apparent as failure in later stages of development (Moor et al., 1998). Another possible explanation could be that matured oocytes are obtained in relatively low numbers in IVM cycles compared with conventional IVF cycles. To increase cytoplasmic and nuclear maturation of the immature oocytes, the IVM medium was supplemented with gonadotrophins and epidermal growth factor (EGF). EGF has been demonstrated to increase cytoplasmic maturation of immature oocyte. In addition, Son et al. (1997) have reported the presence of EGF receptor in oocytes (Son et al., 1997), including GV-stage oocytes. Therefore, the EGF in IVM medium can help immature oocytes denuded of cumulus cells to achieve oocyte maturity after culture. The concentrations used in the present study were chosen on the basis of previous experience in IVM (Son et al., 2002).

To achieve a substantial improvement in the number and developmental competence of oocytes, several investigators have attempted pretreatment with gonadotrophins. Wynn and colleagues (1998) administrated 600 IU recombinant human FSH (rhFSH) to women for 5 days prior to collection of immature oocytes, and the mean number of oocytes collected and maturation to MII were higher in rhFSH treated women compared with untreated women (Wynn et al., 1998). Hence, they asserted that more MII oocytes could be obtained after rhFSH treatment. However, two studies of the treatment of women with FSH found no improvement in oocyte recovery, IVM, fertilization and development (Trounson et al., 1998; Mikkelsen et al., 1999). The present study confirmed the latter finding with oocytes obtained after treatment with HMG (150 IU per day) for 2 days in the early follicular phase. Therefore, it was demonstrated that there is no substantial improvement in the number and developmental competence of oocytes following pretreatment with low-dose gonadotrophins in the early follicular phase.

Chian et al. (2000) reported good pregnancy rates following HCG priming (10,000 IU) for polycystic ovarian syndrome (PCOS) patients with irregular menstrual cycles. A similar approach was adopted in this study for patients with a high risk of OHSS. At the time of oocyte retrieval, the oocytes with dispersed cumulus cell appearance only appeared in the HCG-primed group (group 3). The presence of dispersed cumulus in the HCG-primed group implies that even though there is no direct evidence when LH receptors in folliculogenesis appear, some follicles at the time of HCG-priming in patients might have LH receptors, resulting in retrieved oocytes with dispersed cumulus cell appearance. LH receptor expression has been confirmed in dispersed cumulus cells of oocytes generated from HCG-primed IVM cycles (Yang et al., 2005). Jin and colleagues (2005) have reported that there are LH receptors on granulosa cells and cumulus cells derived from follicles of ≥5.0 mm in diameter and these small follicles in cattle can respond to HCG injection (Jin et al., 2005). Therefore, the reason that eggs were collected with dispersed cumulus cells at retrieval in HCG-primed IVM cycles could be due to the presence of LH receptor in follicles ≥5 mm in humans. Furthermore, 11% of MII oocytes were collected at the time of oocyte retrieval in the HCG-primed group and they had dispersed cumulus cells. In fact, sometimes an MII-stage egg can be found from a follicle as small as 8 mm, and very often from 10–12 mm follicles in HCG-primed IVM cycles. Previously, it was reported that mature oocytes could only be collected from unstimulated ovaries during in-vivo HCG priming, and a clinical pregnancy established by transfer of blastocysts derived from these mature oocytes (Son et al., 2002). In comparison, Chian et al. (2000) observed GV breakdown oocytes but did not observe any MII oocytes at the time of oocyte retrieval after HCG priming. This might be due to evaluating oocytes maturity without denuding cumulus cells. Another possible explanation could be that oocytes were collected from smaller-sized follicles than that of the present study. As shown in Figure 2, different cumulus cell expansion...
in COC appeared between MII stage oocytes retrieved from HCG-primed IVM and ovarian stimulation cycles. Therefore, it is difficult to assess the exact maturity unless an experienced embryologist can assess the maturity with high magnification using the sliding method (Son et al., 2005). Therefore, some groups that reported the HCG-priming effect in IVM cycles did not mention MII-stage oocytes on day of oocyte retrieval. It was reported that the oocytes reaching MII stage in vivo in HCG-primed IVM cycles have better embryonic developmental competence (Son et al., 2005). If an embryologist overlooks MII stage oocytes retrieved on collection day in the HCG-primed IVM cycles, the oocytes will be arrested in MII-stage for more than 24 h. The developmental competence of oocytes in prolonged arrest might therefore be reduced. However, further work is needed to evaluate the optimal interval between MII stage and insemination.

It was also observed that 30% of oocytes from the HCG-primed group had compacted cumulus cells. Oocytes that have compact cumulus cells after administration of large doses of HCG (10,000 IU) may have insufficient LH receptors to induce the cumulus cell response in vivo. It was postulated that follicle sizes at the time of HCG priming are so different that varying appearance of cumulus cells might be observed at the time of oocyte retrieval. It has also been confirmed that there is much lower expression of LH receptors in compact cumulus cells than that of dispersed cumulus cells in HCG-primed IVM cycles (Yang et al., 2005).

There was no difference in total maturation to day 2 among the three groups. However, the speed of oocyte maturation was different among oocytes retrieved from non-primed, HMG-stimulated and HCG-stimulated ovaries. The rate of oocyte maturation during the day of collection was increased following HCG priming before oocyte retrieval, as suggested in Chian et al. (2000). However, the speed of oocyte maturation in the HMG-stimulated group was similar to the non-primed group. Barnes et al. (1996) reported that those oocytes first reaching MII were shown to be the most competent to develop into blastocysts. This result has also been reported in IVM cycles by Son and colleagues (2005), who showed that rapidly matured oocytes could produce more good quality embryos (Son et al., 2005). The present study, therefore, did not evaluate the difference in embryo developmental quality at cleavage stage according to maturation day. In addition, there is a high chance of retrieving in-vivo-matured oocytes depending on follicle size on the day of retrieval in the HCG-primed group, as mentioned before. Therefore, it was postulated that the fast oocyte maturation in the HCG-primed group could improve the developmental competence of these embryos. As shown in Table 3, 20.0% (21/105) of the residual embryos developed from two pronucleate (2PN) to blastocyst stage. This indicates that HCG priming can produce high-quality human embryos, which may potentially increase the pregnancy rate.

To further clarify the HCG priming effect in IVM cycles, this study examined whether HCG priming prior to oocyte collection in the same patients who failed previously to become pregnant in non-priming IVM cycle had any influence on oocyte maturation and embryo developmental competence in vitro (unpublished data). The reason for comparing the same patients was due to variable factors between patients, and the same result was obtained; that HCG priming can influence IVM, maturation time in vitro and embryo quality in IVM cycles.

There have been no reports regarding chromosomal abnormalities of embryos generated by IVM in humans. However, Park and colleagues (1997) have reported 31.8% chromosomal abnormality in IVM MII stage oocytes (Park et al., 1997). This percentage was comparable with MII stage oocytes generated from ovarian stimulation. Up to now, more than 250 normal babies have been born in the authors’ hospital after IVM cycles. Therefore, these results imply that IVM of human oocytes itself may not increase the chromosomal abnormality in their embryos.

In conclusion, HCG priming before collection of immature oocytes allows MII stage oocytes to be obtained on the day of retrieval, hastens oocyte maturation and improves developmental competence. However, further research is needed to define the best conditions for both clinical and laboratory procedures such as the timing of immature oocyte collection, culture conditions, and the endometrium.

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