Combination of FSH priming and hCG priming for in-vitro maturation of human oocytes

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BACKGROUND: The purpose of this study was to determine if there is any additional benefit from FSH priming in addition to hCG priming on in-vitro maturation (IVM) programmes. METHODS: Sixty women with polycystic ovary syndrome (PCOS) who underwent 68 IVM cycles were randomized by computer-generated numbers to receive FSH stimulation or not. Thirty-five cycles were pretreated with 75 IU rFSH for 6 days, and 33 cycles were not. Every cycle was given hCG 10 000 IU 36 h before oocyte retrieval. Immature oocytes were matured in vitro and fertilized by ICSI, and the resulting embryos were replaced on day 2 or 3. RESULTS: A total of 1528 immature oocytes were recovered. The overall maturation and fertilization rates were 74.2 and 72.8% respectively. After embryo transfer, 23 pregnancies resulted (33.8%). The oocyte numbers and endometrial thickness were similar between FSH-primed and non-FSH-primed groups. Serum estradiol level on the day of hCG injection was significantly higher in the FSH-primed group than in the non-FSH-primed group (377.2 pmol/l versus 143.8 pmol/l, \( P = 0.001 \)). The maturation rate, fertilization rate and pregnancy rate were 76.5, 75.8 and 31.4% respectively for FSH-primed group, and 71.9, 69.5 and 36.4% respectively for non-FSH-primed group (all not significant).

CONCLUSIONS: IVM is a feasible treatment for women with PCOS. FSH priming has no additional beneficial effect on IVM.

Key words: ICSI/in-vitro maturation/PCOS/randomized controlled trials

Introduction

Modern assisted reproductive technologies (ART) rely heavily on controlled ovarian stimulation to increase the number of available oocytes and embryos, and therefore the pregnancy rate. However, the high cost of ovarian stimulation drugs, together with the need for frequent hospital visits limit the affordability of ART. Also, ovarian stimulation is associated with side effects such as nausea, abdominal pain, mood swings, menopausal symptoms, ovarian hyperstimulation syndrome (OHSS), and potential cancer risk. Thus, the recovery of immature oocytes followed by in-vitro maturation (IVM) and fertilization is an attractive alternative. In comparison with conventional ART, IVM is associated with lower cost, fewer hospital visits, and fewer complications.

Since Cha et al. reported the first pregnancy resulting from IVM of oocytes from unstimulated ovaries (Cha et al., 1991), several subsequent pregnancies have been reported, including some using immature oocytes from unstimulated women with polycystic ovary syndrome (PCOS) (Trounson et al., 1994). However, the pregnancy rates were disappointingly low, and only Cha et al. reported an acceptable pregnancy rate of 27.1% (Cha and Chian, 1998; Cha et al., 2000). This low pregnancy rate is speculated to be at least partly due to abnormalities of cytoplasmic maturation.

A more recent breakthrough in IVM is hCG priming, as proposed by Chian et al. (Chian et al., 1999, 2000). They found that by giving hCG 10 000 IU 36 h before immature oocyte retrieval, the maturation process in vitro was hastened, and they achieved an impressive 40% pregnancy rate, which was better than the other studies of IVM. Following their protocol we were able to obtain similar results (unpublished data). This group even demonstrated in a case–control study that in PCOS women, IVM gave comparable pregnancy rates with IVF (Child et al., 2002).

Other researchers have proposed FSH priming, i.e. pretreatment with FSH before immature oocyte recovery, in the hope of obtaining more oocytes or enhancing oocyte maturation (Mikkelsen et al., 1999; 2001; Suikkari et al., 2000). The results, however, were conflicting, and no study has ever been performed to see if, with hCG priming, pretreatment with FSH has any additional benefit for IVM. We therefore initiated a study to evaluate if the combination of FSH priming and hCG priming would affect the number of oocytes recovered,
endometrial thickness, maturation potential, or pregnancy rate in IVM programmes.

Materials and methods
From November 1999 to December 2000, women with PCOS undergoing IVM cycles were included in the study. The study protocol was approved by our ethics committee. All patients had oligomenorrhoea or amenorrhoea with ultrasound characteristic of more than 10 small cysts (2–8 mm in diameter) around a dense stroma. The protocol of IVM was modified from the studies of Chian et al. (Chian et al., 1999; 2000). Every woman received medroxyprogesterone (Provera; Pharmacia & Upjohn, Puurs, Belgium) 10 mg per day for 10 days to induce withdrawal bleeding. On cycle day 2 or day 3, they were checked for LH, FSH, and estradiol (E2) levels, and an ultrasound scan was performed to ensure that no ovarian cysts were present.

The patients were randomized by computer-generated randomization list to FSH-primed (group A) and non-FSH-primed (group B) groups. For the patients in group A, rFSH (Gonal-F; Serono, Geneva, Switzerland) at 75 IU per day was given for 6 days from day 3. Oocyte retrieval was scheduled between days 10 and 14 of the cycle, and hCG 10 000 IU (Pregnyl; NV Organon, Oss, The Netherlands) was given 36 h before the retrieval. On the day of hCG injection, endometrial thickness was measured using an Aloca SSD-5000 unit (Aloca, Tokyo, Japan) equipped with a 5-MHz transvaginal transducer. Transvaginal oocyte retrieval was performed 36 h after hCG injection. If the follicles grew to >10 mm on the day of hCG injection or 12 mm at oocyte retrieval, the cycle would be cancelled.

After the patient was sedated with IV propofol (Diprivan; AstraZeneca, Cheshire, UK), oocyte retrieval was performed using a 17 G double-lumen aspiration needle (K-OP51-1635-ET; Cook, Brisbane, Queensland, Australia) with a reduced pressure of 7.5 kPa. We tried to puncture every visible follicle, and if no oocyte was obtained, the follicle was flushed with HEPES-buffered human tubal fluid medium (hTF; Irvine Scientific, Santa Ana, CA, USA) until an oocyte was found, or for three times at most. The oocytes were washed in TCM 199 medium (Sigma Chemical Co., St Louis, MO, USA) with 10% fetal bovine serum (FBS; Sigma Chemical Co.). The oocytes were then cultured in groups in maturation medium in 4-well dishes (Nunc; Copenhagen, Denmark) at 37°C in an atmosphere of 5% CO2 in air for up to 48 h. The maturation medium consisted of TCM 199 medium supplemented with 20% heat-inactivated patient serum, 75 mIU/ml hMG (Humegon; NV Organon, Oss, The Netherlands) was given 36 h before the retrieval. On the day of hCG injection, endometrial thickness was measured using an Aloca SSD-5000 unit (Aloca, Tokyo, Japan) equipped with a 5-MHz transvaginal transducer. Transvaginal oocyte retrieval was performed 36 h after hCG injection. If the follicles grew to >10 mm on the day of hCG injection or 12 mm at oocyte retrieval, the cycle would be cancelled.

As shown in Table I, the mean ages, day 3 serum FSH and LH levels, GV oocyte numbers, and endometrial thickness between the FSH-primed group and the non-FSH-primed group were all similar. Serum E2 level on the day of HCG injection was significantly higher in the FSH-primed group than in the non-FSH-primed group (377.2 versus 143.8 pmol/l, P = 0.001). The 24 and 48h maturation rates were similar between both groups. The fertilization rates, implantation rates, and pregnancy rates were not significantly different between the two groups.

The 68 IVM cycles resulted in 68 embryo transfers and 23 pregnancies (33.8%). The mean numbers of embryos transferred were 3.8 per cycle in each group (range: 2–6), with an implantation rate of 10.5%. Excluding two early miscarriages and one fetal death at 17 weeks gestation (all singletons), there were 20 deliveries, with a live birth rate of 29.4%. The 20 deliveries comprised 16 singletons and four sets of twins. However, one set of twins was delivered at 27 weeks and a singleton was delivered at 24 weeks, all of whom died from complications of prematurity. Therefore, there were 21 live babies who have survived to the present. All of them had normal karyotype and none of them had major or minor anomalies. Their growth and development were all within normal limits up to the time this paper was written. The mental development index and the psychomotor develop-
ment index of the Bayley scales were also all within normal limits.

Discussion

This study supports the findings of Chian et al. that hCG priming 36 h before immature oocyte retrieval gave favourable outcomes in IVM cycles (Chian et al., 1999, 2000). Our overall pregnancy rate of 33.8% was similar to the previous reports using hCG priming (Chian et al., 1999, 2000; Child et al., 2001). FSH priming, however, makes no differences to oocyte recovery, maturational and developmental potential, fertilization rate, and pregnancy rate.

Although Cha et al. reported the first birth using immature oocyte donation from oophorectomy specimens in 1991 (Cha et al., 1991) and Trounson et al. reported the first delivery after IVM from an unstimulated PCOS patient in 1994 (Trounson et al., 1994), the pregnancy rate from human IVM is still very low. Although Cha et al. achieved a 27.1% pregnancy rate after IVM from PCOS women (Cha et al., 2000), this high pregnancy rate required more embryos to be replaced (an average of 6.3 embryos per patient) since the implantation rate was still very low (6.9%).

In 1999 Chian et al. reported that giving hCG 10 000 IU 36 h before oocyte retrieval improved the maturation rate of immature oocytes from PCOS women (Chian et al., 1999). Furthermore, in a prospective randomized study on PCOS women, they found that hCG priming not only improved the percentage of oocytes achieving maturation at 48 h (84.3 versus 69.1% in the non-hCG-primed group), but also hastened the maturation process (Chian et al., 2000). Although there were no significant differences in the rates of oocyte fertilization and cleavage in the two groups, the 38.5% pregnancy rate in the hCG-primed group exceeded other studies on IVM.

FSH acts on cumulus cells and promotes cumulus cell steroid production, oocyte RNA and protein synthesis (McGee et al., 1997). Thus, increasing FSH concentrations within the follicles are coincident with the generation of a positive signal necessary to complete in-vivo oocyte maturation (Gomez et al., 1993). It has been postulated that FSH stimulation before oocyte retrieval might increase either the number of immature oocytes recovered or the maturational potential and developmental competence of the oocytes. In a study performed on rhesus monkeys, FSH priming for 6–7 days enhanced nuclear and cytoplasmic maturation of oocytes in vitro (Schramm and Bavister, 1994). In comparison with the non-stimulated monkeys, greater percentages of oocytes completed meiotic maturation (74 versus 41%), fertilized (85 versus 61%), and cleaved from the 2–4 cell (79 versus 38%) in the FSH-primed monkeys. However, the effects of FSH priming on human IVM were contradictory. Suikkari et al. proposed commencing low-dose (37.5 IU) recombinant FSH from previous luteal phase until the leading follicle reached 10 mm. An average of 11.5 oocytes were obtained, but no pregnancies were achieved (Suikkari et al., 2000). Wynn et al. gave a truncated course of 600 IU FSH to normal women; after which, significantly greater percentages of oocytes completed meiotic maturation in vitro (71.1 versus 43.5%) and higher serum E2 concentrations on the day of oocyte retrieval (1049 ± 241 versus 154 ± 17 pg/ml) were found after FSH treatment. Immature oocyte numbers and endometrial thickness were not significantly different (Wynn et al., 1998). In contrast, Trounson et al. found no significant differences in the number of oocytes recovered, maturation rate, fertilization rate, and embryo development in patients pretreated with 1 day or 3 days of 150 IU recombinant FSH compared with no treatment with FSH (Trounson et al., 1998).

Mikkelsen et al. used pre-treatment with FSH at 150 IU per day before oocyte retrieval. In women with regular cycles, pre-treatment with FSH for 3 days or until follicles reached 10 mm did not increase the number of oocytes recovered. Nor did FSH priming improve on oocyte maturation, cleavage rate, or embryo development (Mikkelsen et al., 1999). However, in another similar study of theirs on PCOS woman, FSH priming improved the maturational potential of the oocytes (59 versus

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Table I. Clinical variables and outcome of FSH-primed (Group A) and non-FSH-primed (Group B) groups

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>No. of cycles</td>
<td>35</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>30.1 ± 2.8</td>
<td>31.3 ± 4.1</td>
<td>0.147</td>
</tr>
<tr>
<td>Day 3 FSH (mIU/ml)</td>
<td>5.10 ± 1.43</td>
<td>5.54 ± 1.55</td>
<td>0.238</td>
</tr>
<tr>
<td>Day 3 LH (mIU/ml)</td>
<td>12.46 ± 7.37</td>
<td>11.63 ± 6.61</td>
<td>0.635</td>
</tr>
<tr>
<td>Estradiol on day of hCG (pmol/l)</td>
<td>377.2 ± 361.8</td>
<td>143.8 ± 53.3</td>
<td>0.001</td>
</tr>
<tr>
<td>Endometrial thickness on day of hCG (mm)</td>
<td>8.09 ± 1.49</td>
<td>7.77 ± 1.03</td>
<td>0.318</td>
</tr>
<tr>
<td>Total number of immature oocytes</td>
<td>766</td>
<td>762</td>
<td></td>
</tr>
<tr>
<td>Mean no. of oocytes per patient</td>
<td>21.9 ± 9.4</td>
<td>23.1 ± 11.0</td>
<td>0.630</td>
</tr>
<tr>
<td>Mean MII no.</td>
<td>16.7 ± 7.6</td>
<td>16.6 ± 6.8</td>
<td>0.938</td>
</tr>
<tr>
<td>Maturation rate at 24 h (%)</td>
<td>43.2</td>
<td>39.2</td>
<td>0.470</td>
</tr>
<tr>
<td>Maturation rate at 48 h (%)</td>
<td>76.5</td>
<td>71.9</td>
<td>0.280</td>
</tr>
<tr>
<td>2PN oocytes per patient</td>
<td>12.7 ± 6.2</td>
<td>11.6 ± 4.6</td>
<td>0.389</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>75.8</td>
<td>69.5</td>
<td>0.197</td>
</tr>
<tr>
<td>Cleavage rate (%)</td>
<td>89.4</td>
<td>88.1</td>
<td>0.855</td>
</tr>
<tr>
<td>No. of transferred embryos</td>
<td>3.8 ± 1.0</td>
<td>3.8 ± 0.9</td>
<td>0.761</td>
</tr>
<tr>
<td>Pregnancy rate (%)</td>
<td>31.4</td>
<td>36.4</td>
<td>0.799</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>9.7</td>
<td>11.3</td>
<td>0.840</td>
</tr>
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higher E2 level, however, did not increase endometrial growth in IVM cycles (Requena et al., 1993). However, the 21 live babies in our study all had normal karyotypes and had no congenital malformations. Their growth and development were both normal until the time of follow-up. Similarly, Cha et al. reported 20 normal infants (Cha et al., 2000) and Child et al. reported 25 normal infants (Child et al., 2002) from IVM with no congenital anomalies. Thus, from the present information, it appears that IVM is a safe procedure.

In the current study, we tended to transfer more embryos in IVM cycles because from several studies and from our previous experience it had been shown that the implantation rates of IVM cycles were relatively low compared with IVF cycles (Cha and Chian, 1998; Trounson et al., 1998; Cha et al., 2000). Furthermore, Suikkari et al. found that the cryosurvival of the in-vitro matured zygotes and cleaved embryos was very poor compared with embryos generated from in-vivo matured oocytes (Suikkari et al., 2000). In their study, 59% of the zygotes and cleaved embryos degenerated after thawing, indicating that cryopreservation of in-vitro matured embryos might not be an optimal procedure. Although we transferred more embryos, we obtained only four sets of twins, and no higher order pregnancy.

There has been concern about the normality of in-vitro matured oocytes. For example, Nogueira et al. found 78.5% chromosomal anomalies in in-vitro matured oocytes from stimulated cycles (Nogueira et al., 2000). However, the immature oocytes were obtained from stimulated cycles where dominant follicles have formed and have undergone atresia, and they may either be of poorer quality or have been exposed to an inadequate intrafollicular environment. Therefore the result may not be extrapolated to immature oocytes from natural cycles. Two additional reports have demonstrated that the rates of aneuploidy or chromosome aberration in in-vitro matured oocytes from unstimulated cycles were ~20% (Gras et al., 1992; Racowsky and Kaufman et al., 1992). Similar rates have been reported for in-vivo matured oocytes after gonadotrophic stimulation in IVF cycles (Gras et al., 1992; Munné et al., 1993). However, the 21 live babies in our study all had normal chromosomal karyotypes and had no congenital malformations. Their growth and development were both normal until the time of follow-up.

In the current study, we did not find any benefit from FSH priming. The oocyte numbers, 24 and 48 h maturation rates, fertilization rates, and pregnancy rates were all similar between the FSH-primed and non-FSH-primed groups. The only significant difference was a higher E2 level in the FSH-primed group. This may have been because the cumulus cells responded to FSH stimulation and produced more E2. This higher E2 level, however, did not increase endometrial thickness or the maturational potential of the oocytes. Mikkelsen et al. also demonstrated that endometrial thickness did not differ between FSH-primed and non-stimulated groups (Mikkelsen et al., 2001).

Many PCOS women have a thin endometrium on the day of oocyte retrieval or embryo transfer, and a thin endometrium is associated with a reduced pregnancy rate. In IVF cycles, it has been demonstrated that the mean endometrial thickness for those not conceiving was 7.1 mm, in contrast with 8.6 mm for those achieving pregnancies (Gonen and Casper, 1990). Noyes et al. found that endometrial thickness >9 mm as well as ring pattern (triple line) and intermediate patterns denoted a more favourable prognosis for pregnancy (Noyes et al., 1995). Child et al. (2003) found that by the day of embryo transfer, the endometrial thickness was significantly greater in pregnancy compared with non-pregnancy cycles (10.2 versus 9.4 mm), although the endometrial thicknesses on the day of oocyte retrieval were comparable. In our study, the endometrial thicknesses on the day of hCG injection were 8.1 and 7.8 mm respectively, in FSH-primed and non-FSH-primed groups, and 8.2 and 7.8 mm respectively in pregnancy and non-pregnancy groups (not significant). In IVM cycles, serum estrogen levels may be inadequate to promote endometrial growth. Requena et al. showed that after immature oocyte retrieval, there was disruption of endocrinology, including rises in FSH and LH and a significant drop in E2, which might be harmful to endometrial development. They concluded that exogenous hormonal administration might be necessary to achieve correct endometrial growth in IVM cycles (Requena et al., 2001). The low implantation rate in IVM cycles could be partly due to inadequate endometrium. Further study is necessary to synchronize endometrium with embryo development.

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There has been concern that anesthesia might adversely affect oocyte maturation, fertilization and cleavage in vitro. Child et al. found in IVM programmes that the use of i.v. anesthesia (midazolam and fentanyl) with a paracervical block of lidocaine was associated with a significantly lower maturation rate than when either an i.v. anesthesia was used without a paracervical block or when spinal anesthesia was used (Child et al., 2001). Previous studies on IVM used spinal anesthesia (Chian et al., 1999, 2000), i.v. sedation with fentanyl (Cha et al., 2000), individually or in combination with a paracervical block (Mikkelsen et al., 2001). In our center, oocyte retrieval is routinely carried out under i.v. anesthesia with propofol. Propofol has been demonstrated to be detrimental to in-vitro maturation, fertilization and cleavage of mouse oocytes (Alsalili et al., 1997). Propofol has also been shown to accumulate in the follicular fluid, with increasing concentration throughout the procedure (Coetsier et al., 1992). There is, however, no study to demonstrate that propofol has any detrimental effect on human IVM; and in our study the maturation rate, fertilization rate, and pregnancy rate are similar to previous studies using hCG priming and different anesthesia methods (Chian et al., 1999, 2000; Child et al., 2001). Thus, further study is needed to evaluate if different anaesthetic modes during oocyte retrieval affect the outcome of IVM.

We used the traditional aspiration needle for mature oocytes instead of the aspiration needle designed by Trounson et al. for immature oocyte recovery (Trounson et al., 1994). The immature oocyte needle has a shorter bevel and is more rigid so that it does not bend easily when it penetrates the thickened tunica and dense stroma of PCOS ovaries. However, this needle has only a single lumen and cannot flush the follicles. Consequently, we prefer to use the double-lumen needle in order to flush every follicle in the hope of obtaining more oocytes. We found that some immature oocytes attached firmly to the follicles and were not easily aspirated out. Indeed many immature oocytes were recovered after flushing. Trounson et al. obtained an average of 15.3 oocytes from PCOS patients using
his special aspiration needle, with a maturation rate and fertilization rate of 81 and 34% respectively (Trounson et al., 1994). In our study, we obtained 21.9 and 23.1 oocytes per patient in the FSH-primed, and non-FSH-primed groups respectively. These numbers far exceed the reports of Chian et al. 2000 (7.8 oocytes), Child et al. 2001 (11.3 oocytes), and Cha et al. 2000 (13.6 oocytes), and Mikkelsen et al.’s report using FSH priming (7.5 oocytes) (Mikkelsen et al., 2001). It has been shown in IVF cycles that aspiration alone and aspiration plus flushing obtained comparable oocyte numbers, and the oocytes obtained by flushing were of poor quality and had lower fertilization rates (Tan et al., 1992). However, it remains unknown whether the flushed-out immature oocytes are of inferior quality or have poorer developmental potential. In view of the favourable outcome in our study, it seems worthwhile to spend more time to flush the follicles.

In conclusion, women with PCOS are at risk of OHSS, and IVM is a feasible treatment for these women. This study supports the beneficial effect of hCG priming on IVM. FSH priming in combination with hCG priming, however, has no additional benefit, although it increases both cost and inconvenience for the patients.

**References**


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