The use of metformin for women with PCOS undergoing IVF treatment

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BACKGROUND: Metformin appears to improve reproductive function in some women with polycystic ovary syndrome (PCOS). We wished to explore the effect of metformin in women with PCOS undergoing IVF. METHODS: A randomized, placebo-controlled, double-blind study was carried out between 2001 and 2004. Patients with PCOS undergoing IVF/ICSI treatment using a long GnRH agonist protocol were randomized to receive metformin (MET), 850 mg, or placebo (PLA) tablets twice daily from the start of the down-regulation process until the day of oocyte collection. The primary outcome was to be an improvement in the overall fertilization rate. RESULTS: One-hundred and one IVF/ICSI cycles were randomized to receive metformin (52) or to receive placebo (49). There was no difference in the total dose of rFSH required per cycle (median dose: MET = 1200 U, PLA = 1300 U; \( P = 0.937 \)). The median number of oocytes retrieved per cycle (MET = 17.2, PLA = 16.2; \( P = 0.459 \)) and the overall fertilization rates (MET = 52.9%, PLA = 54.9%; \( P = 0.641 \)) did not differ. However, both the clinical pregnancy rates beyond 12 weeks gestation per cycle (MET = 38.5%, PLA = 16.3%; \( P = 0.023 \)) and per embryo transfer (MET = 44.4%, PLA = 19.1%; \( P = 0.022 \)) were significantly higher in those treated with metformin. Furthermore, a significant decrease in the incidence of severe ovarian hyperstimulation syndrome (OHSS) was observed (MET = 3.8%, PLA = 20.4%; \( P = 0.023 \)), and this was still significant after adjustment for BMI, total rFSH dose and age (OR = 0.15; 95% CI: 0.03, 0.76; \( P = 0.022 \)). CONCLUSION: Short-term co-treatment with metformin for patients with PCOS undergoing IVF/ICSI cycles does not improve the response to stimulation but significantly improves the pregnancy outcome and reduces the risk of OHSS.

Key words: IVF/metformin/OHSS/polycystic ovary syndrome/pregnancy

Introduction

Polycystic ovary syndrome (PCOS) is the commonest endocrine disorder in women (Bal
c
en and Michelmore, 2002), and it accounts for approximately 80% of cases of anovulatory infertility. The current first-line therapy is weight loss (Clark et al., 1995, 1998) through lifestyle modification in the obese group of women and then ovulation induction treatment with clomiphene. Approximately 80% of women respond to clomiphene treatment, and the cumulative pregnancy rates after six months of treatment is between 40 and 50% (Bal
c
en et al., 1994; National Institute for Clinical Excellence Guidelines, 2004). Gonadotrophin ovulation induction therapy is usually offered to those patients who have failed to respond to clomiphene.

IVF treatment is an effective therapy for women with PCOS who are refractory to standard ovulation induction therapies or who have co-existing infertility factors (Homburg et al., 1993; Buyalos and Lee, 1996). However, the response of women with PCOS to IVF treatment is often different from women with normal ovaries. Dor et al. (1990) showed that significantly more oocytes were recovered per cycle in the PCOS group compared with the women with tubal factor infertility (19.4 versus 5.4, \( P < 0.005 \)), but this was associated with lower fertilization rates (40.4 versus 67.6%, \( P < 0.001 \)). Similar findings were observed in subsequent reports of women with PCOS (Dale et al., 1991; Urman et al., 1992; Homburg et al., 1993; MacDougall et al., 1993; Kodama et al., 1995). Despite the fact that they often require a lower total dose of FSH during stimulation (Engmann et al., 1999) compared with women with normal ovaries, women with PCOS are at a greater risk of developing moderate-to-severe ovarian hyperstimulation syndrome (OHSS), quoted at 10–18% versus 0.3–5% (MacDougall et al., 1993; Kodama et al., 1995; Jacobs and Agrawal, 1998; Engmann et al., 1999; Ludwig et al., 1999). Kodama et al. (1995) also demonstrated a significantly higher incidence of cancellation of embryo transfer in the PCOS group due to failure of fertilization and the risk of OHSS. These findings were also supported in a recent metaanalysis (Heijnen et al., 2006).
Although most retrospective data indicated that the pregnancy rates per transfer were comparable to the controls (Dor et al., 1990; Urman et al., 1992; Homburg et al., 1993; MacDougall et al., 1993), the miscarriage rates following IVF treatment were increased in women with PCOS (Balen et al., 1993). The unfavourable outcomes are related to their high BMI (Fedorcsak et al., 2000, 2001), the increased waist–hip ratio (Wass et al., 1997) and insulin resistance (Dale et al., 1998). Furthermore, a consequence of obesity among women with PCOS is an increased requirement of FSH stimulation (Fedorcsak et al., 2000, 2001). Therefore, they may not respond to a low-dose stimulation regimen. However, once the dose is increased and the threshold reached, the subsequent response can be explosive, with an increasing risk of OHSS.

Over the past 15 years, it has become increasingly recognized that insulin resistance is central to the pathogenesis of the PCOS (Tsilchorozidou et al., 2004). Metformin, a biguanide insulin-lowering agent, has been extensively investigated in the management of PCOS. Two recent systematic reviews (Costello and Eden, 2003; Lord et al., 2003) demonstrated that metformin improves reproductive function of some women with PCOS. Metformin also appeared to improve the outcomes of ovulation induction therapies when combined with clomiphene and gonadotrophin (Nestler et al., 1998; De Leo et al., 1999; Vandermolen et al., 2001; Costello and Eden, 2003; Lord et al., 2003). At the time of planning this study, there was only one retrospective study reported (Stadtmauer et al., 2001) on the use of metformin in IVF. We therefore set out to perform a randomized, placebo-controlled, double-blind study (RCT) to explore the potential benefits of using metformin during IVF treatment.

Materials and methods

Women with PCOS and a normal FSH level, aged between 20 and 39 years and undergoing IVF/ICSI cycles, were recruited from a single infertility unit. PCOS was defined as the presence of polycystic ovaries on transvaginal ultrasound scan (TVUS), more than 10 cysts, 2–8 mm in diameter, usually combined with increased ovarian volume >10 cm³ (after the transabdominal ultrasound criteria of Adams et al., 1985), together with either oligo/amenorrhea or clinical/ biochemical hyperandrogenism. Anovulation was defined as the presence of amenorrhea or oligomenorrhea (cycle length greater than 35 days) (Munster and Schmidt Lone Helm, 1993; Berek et al., 1996). This criterion would also meet the recent consensus (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). Although BMI was not an entry criterion, all women with BMI over 30 kg/m² would have been advised to lose weight 6–12 months prior to the treatment through lifestyle modification. Pretreatment inclusion criteria also included serum testosterone concentration <5.0 nmol/l, normal prolactin concentration, thyroid, renal and haematological indices. No participant had received metformin treatment within the 3 months prior to recruitment.

Exclusion criteria included concurrent hormone therapy within the previous 6 weeks, any chronic disease that could interfere with the absorption, distribution, metabolism or excretion of metformin and renal or liver disease. Patients with significant systemic disease or diabetes (type 1 or 2) were excluded. Patients with irregular menstrual bleeding were thoroughly assessed to exclude pathology of the genital tract other than PCOS.

Protocol

A local research ethics committee approval (01/112) and the permission for the use of metformin from the Government Medicine Control Agency, UK, were obtained before the study started.

The suitable patients were identified when they attended the unit for a routine pretreatment assessment 1 month prior to the commencement of IVF cycle. They would have a routine baseline TVUS, and the subjects who met the above criteria were invited to take part. A separate appointment was arranged to obtain the consent and the baseline serum for fasting glucose and insulin, sex hormone-binding globulin (SHBG) and testosterone levels before the IVF cycle.

Following a spontaneous or withdrawal bleed (after a 5-day course of 10 mg Provera, Pharmacia, Surrey, UK), the subjects received a 2-week course of combined oral contraception pills (Microgynon 30, Schering Health, West Sussex, UK) from day 2 of menstruation. This was followed by down-regulation with GnRH agonist (Nafarelin, Synarel, 200 μg, three times daily, Pharmacia, Surrey, UK) for a duration of 2–3 weeks.

The subjects were randomized to either receive metformin or placebo. The randomization process was carried out by the clinical trial office in the pharmacy department and blinded to patients and investigators. A block of four randomization techniques was performed using random tables from Linder et al. (1970). The code was kept in the trial office until the last patient completed the study. Placebo tablets for metformin were identical in appearance (size and colour) to metformin and were supplied by Penn Pharmaceuticals (Tredegar, Gwent, UK). One tablet (metformin 850 mg or placebo) was prescribed to be taken 12 hourly from the first day of down-regulation to the day of egg retrieval.

Stimulation was commenced when the measurement of endometrial thickness was <3 mm by TVUS. A low-dose step-up regimen was used with a starting dose of 100 U rFSH (Puregon, Organon Laboratories, Cambridge, UK). Women returned at day 7 of the stimulation for further assessment with TVUS. When there was no ovarian response (no follicle beyond 13 mm in diameter), the dose of rFSH was increased to 150 or 200 U for patients with BMI <30 kg/m² and >30 kg/m², respectively. All women were reviewed on alternate days until the lead follicle(s) was over 15 mm in diameter and were seen daily thereafter. When there was still no response by day 11 of the stimulation, the dose was increased by a further 100 U. The cycle was abandoned at day 14 of the stimulation when the response was poor (less than two lead follicles at 17 mm in diameter).

About 10 000 U of HCG (Profasi, Serono Pharmaceuticals, Middlesex, UK) was administered in the evening when there were more than three follicles over 17 mm in diameter. A serum sample was also obtained on the same day for estradiol, testosterone and SHBG concentrations. The subjects were scheduled to have egg retrieval procedures 36–38 h after HCG administration.

At the day of egg retrieval, serum samples were obtained for fasting glucose, insulin and vascular endothelial growth factor (VEGF). Follicular fluids (free of contamination from saline flushing) were collected from the follicles with a diameter between 17 and 19 mm. The fluids were frozen within 4 h for future analysis. All follicles with a diameter over 14 mm were aspirated and were flushed twice with normal saline when the oocytes were not found in the first aspirate.

All oocytes were inseminated or injected (ICSI) 4 h after the egg retrieval. The culture and flushing medium used in the laboratory were supplied by Medicult, UK. Fertilization checks were carried out 16–20 h post-insemination. Only those with two pronuclei indicated fertilization.

Embryo replacement was carried out two days after egg retrieval. A maximum of two embryos were transferred under abdominal USS guidance. A Wallace embryo replacement catheter was used, and the tip of catheter was placed at 1 cm below the fundus. Two of the
best-quality of embryos were selected for embryo transfer. The transferred embryos were graded into a scale of 5, according to their morphology and the degree of fragmentation. The embryo score (the grade of the embryo multiplied by the number of blastomeres) was derived from the method described by Steer et al. (1992). The average embryo score was calculated from the total score of the embryo(s) being replaced divided by the number of transferred embryo(s).

A daily Cyclogesist pessary (400 mg, Alpharma, Devon, UK) was used for luteal phase support until the day of pregnancy test. The pregnancy test was carried out at day 14 after egg retrieval and was classified as positive when the serum beta-HCG level was over 5 IU/l. All women with a positive result were offered an early TVUS at 4 weeks after embryo transfer. A clinical pregnancy was defined as a viable pregnancy beyond 12 weeks gestation, whilst a baby born after 24 weeks gestation was classified as a live birth.

All women who were at risk of developing (more than 30 oocytes retrieved) or symptomatic of severe OHSS were offered embryo cryopreservation (at pronuclei stage) for future transfers. These embryos were not graded by the method described above. The classification of OHSS was based on the criteria described by Golan et al. (1989). A coating regime was not employed to manage the patients at risk of developing OHSS.

Outcomes measures
The primary outcome measure was the improvement on the overall fertilization rates, which was defined as a normal fertilization with two pronuclei stage embryo divided by the number of oocytes retrieved per cycle. The main secondary measures were the effects on the pregnancy and the severe OHSS rates (see above for the definition).

Power calculation
At the time when the study was planned, no RCT had been carried out to investigate the use of metformin during IVF treatment. Therefore, the power calculation was based on the data from retrospective studies (Dor et al., 1990; Urman et al., 1992; Homburg et al., 1993; MacDougall et al., 1993; Kodama et al., 1995). The common findings for patients with PCOS undergoing IVF were lower fertilization rates, more oocytes retrieved and a higher risk of developing OHSS compared with the controls. However, apart from the fertilization rates, the figures of the reported number of oocytes retrieved and the OHSS rates varied a lot among all the studies. The studies from MacDougall et al. (1993) and Homburg et al. (1993) were among the largest series addressing the IVF outcomes in patients with PCOS with similar fertilization rates (52.8 and 57.3%).

Recent evidence demonstrated that metformin reduces serum testosterone one concentrations in women with PCOS (Tsilchorozidou et al., 2004). Teissier et al. (1999, 2000) also suggested that a high level of testosterone may affect oocyte maturity and fertilization potential. We therefore hypothesized that metformin improved the fertilization rates through its effects on testosterone production. Hence, we chose the fertilization rate as our primary power calculation. The fertilization rates from the study by MacDougall et al. (1993) were 52.8 ± 3.4% and 66.1 ± 3.4% for patients with PCOS and non-PCOS, respectively. With an improvement of 20%, the calculated standardized difference (d) would be 0.67. The chosen power in the study was 90%, with a type I error of 0.05. From the power table (Machin and Campbell, 1987), when d = 0.67 and the power = 0.90, the projected sample size was 100 with 50 subjects in each arm.

Biochemical assays
All the samples were stored at −20°C and were analysed in the biochemistry department of the study centre. The analyses were as previously described (Wijeyaratne et al., 2002). Plasma glucose was measured using an enzymatic colorimetric assay (Hitachi, Roche Diagnostic, Lewes, UK), with intra-assay coefficients of variation (CV) of 3.0% at 2.4 mmol/l and 1.9% at 20.2 mmol/l. Serum insulin was measured by an immunoluminometric assay (ADVIA Centaur, Bayer) with CV of 4.5% at 17.39 mU/l and 10.1% at 124.83 mU/l. Serum SHBG was measured by an immunoluminometric assay (Euro DPC Immulite), with intra-assay CV of 6.9% at 4.5 nmol/l and 4.1% at 64 nmol/l and inter-assay CV of 13% at 6.0 nmol/l and 7.5% at 71 nmol/l. Serum testosterone was measured by using stable isotope-dilution liquid chromatography tandem mass spectrometry, as described previously (Caswell et al., 2005). Free androgen index (FAI) was derived from the ratio of the total testosterone concentration (nmol/l) to the concentration of SHBG (nmol/l) times 100.

Vascular endothelial growth factor (VEGF) profile was determined by fluid-phase immunoassay, as previously described (Powell et al., 2004), using custom kits (Upstate, Milton Keynes, UK) on a Luminex 100 cytometer (Luminex, Austin, TX, USA) equipped with StarStation 2 software (Applied Cytometry Systems, Dinnington, UK). All samples were subjected to a 1 : 3 dilution prior to analysis following the manufacturer’s recommendation.

Data analysis and statistics
Data were analysed on the basis of intention to treat. All the subjects who had their embryos cryopreserved because of the risk of developing severe OHSS were included in the group of patients who developed symptomatic severe form of OHSS during the analysis.

The insulin sensitivity (IS) was calculated from the method, Quantitative Insulin Sensitivity Check Index (QUICKI), described by Katz et al. (2000). QUICKI = 1/[log(I) + log(G)], with I (fasting insulin concentrations) in μU/ml and G (fasting glucose concentrations) in mmol/l (conversion from mmol/l to mg/l = x, a factor of 18.0). The reason to use this method has been discussed in our previous paper (Tang et al., 2006).

For parametric data, the assumption of normal distribution was assessed by a normal plot and the Kolmogorov–Smirnov test. The assumption of the two groups having the same variances was tested by using the F-test. Paired t-test or two-sample t-test was applied as indicated. When the data did not meet the above assumptions, a log10 transformation of the data was carried out. If the transformed data were still not meeting the assumptions, non-parametric methods, Wilcoxon signed rank test or Mann–Whitney test, were applied. A P-value <0.05 was considered to be statistically significant. The z-test was used to analyse the two proportions with Yates correction.

In the multiple linear regression analysis, the same normality test was used as in the t-test, and the test for constant variance was computed by using the Spearman rank correlation between the absolute values of the residuals and the observed value of the dependent variable. When the criteria of normality or constant variance were not met, a log10 transformation of the data was performed. Durbin–Watson statistic was used to test residuals for their independence of each other.

In the logistic regression analysis, the regression coefficients computed by minimizing the sum of squared residuals in multiple logistic regression are also the maximum likelihood estimates. P is the value calculated for the Wald statistic, which is the regression coefficient divided by the standard error. All the statistical analyses were performed using SigmaStat, version 2.

Results
Recruitment progress
During a 3-year period, between 2001 and 2004, 94 women were screened and underwent 101 consecutive IVF/ICSI cycles (Figure 1). All our subjects also met the Rotterdam consensus
The baseline characteristics of the subjects in metformin and placebo groups (Table I), with the number of women who stopped the medication before completion of the cycle did not differ (MET = 5.9%, PLA = 2.0%). One cycle in the metformin group and five cycles in the placebo group had all the embryos cryopreserved for subsequent transfers (freeze-all) due to the risk of OHSS. One IVF cycle in the metformin arm did not undergo embryo transfer due to failed fertilization.

There were no significant differences in the duration of stimulation (12 versus 12 days, \( P = 0.627 \)) and the total dose of rFSH required per cycle (MET = 1200 U, PLA = 1300 U; \( P = 0.937 \)). Multiple linear regression analysis showed that only BMI was significantly correlated with the total dose of rFSH required in the treatment (coefficient = 0.011, \( P < 0.001 \)), but not the age of the participants or the use of metformin.

The number of follicles aspirated and the number of oocytes retrieved were also similar between the groups (Table II). Multiple linear regression showed that the total dose of rFSH used, BMI, age or the use of metformin have any effects on the number of oocytes recovered (adjusted \( R^2 = 0.00, P = 0.608 \)). During the study period, the mean number of oocytes retrieved (95% CI 15.3–18.2, \( P < 0.001 \)) was higher than the average number (12.9) from the women without PCOS who underwent IVF/ICSI cycles in the unit.

Metformin did not improve the overall fertilization rates (52.9 versus 54.9%, \( P = 0.641 \)), and the cleavage rates were also similar (Table II). The findings were still insignificant after adjustment for age, IVF or ICSI treatment, number of eggs retrieved and BMI. The average fertilization rate of women without PCOS in the same period of time was 61.9%.

**Table I.** The baseline characteristics of the subjects in metformin and placebo groups

<table>
<thead>
<tr>
<th></th>
<th>Metformin (52)</th>
<th>Placebo (49)</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>31.3 (4.0)</td>
<td>31.1 (4.0)</td>
<td>0.850</td>
</tr>
<tr>
<td>Menses in the preceding 6 months</td>
<td>4.0 (1.8)</td>
<td>4.1 (1.7)</td>
<td>0.776</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.5 (17.0)</td>
<td>70.4 (14.1)</td>
<td>0.117</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.9 (5.6)</td>
<td>26.9 (4.8)</td>
<td>0.330</td>
</tr>
<tr>
<td>Duration of infertility (years)</td>
<td>4.5</td>
<td>4.0</td>
<td>0.187</td>
</tr>
<tr>
<td>Nulliparity (%)</td>
<td>57.7</td>
<td>63.3</td>
<td>0.710</td>
</tr>
<tr>
<td>ICSI cycles (%)</td>
<td>40.4</td>
<td>51.0</td>
<td>0.385</td>
</tr>
<tr>
<td>Patients who had previous IVF cycle (%)</td>
<td>28.8</td>
<td>28.6</td>
<td>0.843</td>
</tr>
<tr>
<td>Asian women (%)</td>
<td>17.3</td>
<td>8.2</td>
<td>0.283</td>
</tr>
<tr>
<td>Subjects who developed OHSS in previous cycle (%)</td>
<td>13.5</td>
<td>4.1</td>
<td>0.192</td>
</tr>
<tr>
<td>Women with ovarian volume ( &gt;10 \text{ cm}^3 ) (%)</td>
<td>52.8</td>
<td>34.3</td>
<td>0.197</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>6.7 (3.6)</td>
<td>6.8 (5.0)</td>
<td>0.904</td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>5.0 (1.5)</td>
<td>5.0 (1.4)</td>
<td>0.929</td>
</tr>
<tr>
<td>Testosterone (nmol/l) (^b)</td>
<td>2.03</td>
<td>2.18</td>
<td>0.501</td>
</tr>
<tr>
<td>SHBG (nmol/L) (^b)</td>
<td>44.5</td>
<td>44.8</td>
<td>0.968</td>
</tr>
<tr>
<td>Free androgen index (^b)</td>
<td>4.61</td>
<td>4.88</td>
<td>0.768</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>3.19 (1.26)</td>
<td>3.26 (1.45)</td>
<td>0.826</td>
</tr>
<tr>
<td>Fasting insulin (mU/l) (^b)</td>
<td>7.53</td>
<td>6.57</td>
<td>0.331</td>
</tr>
<tr>
<td>Insulin sensitivity (QUICKI) (^d)</td>
<td>0.377</td>
<td>0.386</td>
<td>0.316</td>
</tr>
</tbody>
</table>

\(^a\)Median.
\(^b\)Geometric means.
\(^c\)Quantitative Insulin Sensitivity Check Index (QUICKI) method = 1/ \([\log(f_0)+\log(G_0)]\).
\(^d\)Fasting insulin levels in \( \mu U/ml \) (factor of 18.0).

The progress of the subjects through the study.

Withdrew = 5 (due to poor response)
Withdrew = 2 (due to poor response)
Completed = 47
Completed = 47

**Figure 1.** The progress of the subjects through the study.

Demographic data

There were no significant differences in the baseline characteristics of the women between the two groups (Table I), with the mean age (MET = 31.3 years, PLA = 31.1 years; \( P = 0.850 \)), the mean BMI (MET = 27.9 kg/m², PLA = 26.9 kg/m²; \( P = 0.330 \)) and the median duration of infertility (MET = 4.5 years, PLA = 4.0 years; \( P = 0.187 \)). The menstrual frequencies in the preceding 6 months were also not different (MET = 4.0, PLA = 4.1; \( P = 0.776 \)).

Similar to our previous study (Tang et al., 2006), there was a negative correlation between the baseline insulin sensitivity, SHBG and BMI (data not shown).

IVF and stimulation

The duration of taking metformin tablets in the study was similar to those in the placebo group, 28 versus 28 days (\( P = 0.627 \)). Despite the fact that significantly more women experienced the gastrointestinal side effects from metformin (Table II), the number of women who stopped the medication before completion of the cycle did not differ (MET = 5.9%,
The clinical outcomes in the metformin and the placebo groups

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Metformin (52)</th>
<th>Placebo (49)</th>
<th>P-value</th>
<th>95% CI for difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle cancellation (%)</td>
<td>9.6</td>
<td>4.1</td>
<td>0.487</td>
<td>-0.044 to 0.154</td>
</tr>
<tr>
<td>Days of stimulation</td>
<td>12</td>
<td>12</td>
<td>0.627</td>
<td></td>
</tr>
<tr>
<td>Total dose of rFSH (U)</td>
<td>1200</td>
<td>1300</td>
<td>0.937</td>
<td></td>
</tr>
<tr>
<td>Large (&gt;17 mm) to small (=14 mm) follicle ratio</td>
<td>0.388</td>
<td>0.449</td>
<td>0.262</td>
<td></td>
</tr>
<tr>
<td>Number of follicles entered at egg retrieval</td>
<td>22</td>
<td>20</td>
<td>0.318</td>
<td></td>
</tr>
<tr>
<td>Number of eggs</td>
<td>17.3 (7.4)</td>
<td>16.2 (7.0)</td>
<td>0.459</td>
<td>-1.851 to 4.064</td>
</tr>
<tr>
<td>Overall fertilization rate (%)</td>
<td>52.9 (21.8)</td>
<td>54.9 (19.1)</td>
<td>0.641</td>
<td>-10.4 to 6.4</td>
</tr>
<tr>
<td>Cevage rate (%)</td>
<td>96.0 (11.9)</td>
<td>92.2 (18.1)</td>
<td>0.240</td>
<td>-2.626 to 10.35</td>
</tr>
<tr>
<td>Freeze-all cycles (%)</td>
<td>1.92</td>
<td>10.2</td>
<td>0.181</td>
<td>-17.5 to 0.009</td>
</tr>
<tr>
<td>Number of embryo transferred</td>
<td>2</td>
<td>2</td>
<td>0.695</td>
<td></td>
</tr>
<tr>
<td>Average embryo score of the transferred embryos</td>
<td>17</td>
<td>16</td>
<td>0.259</td>
<td></td>
</tr>
<tr>
<td>Positive pregnancy rate per cycle (%)</td>
<td>48.1</td>
<td>34.7</td>
<td>0.245</td>
<td>-5.8 to 32.6</td>
</tr>
<tr>
<td>Clinical pregnancy rate (beyond 12 weeks) per cycle (%)</td>
<td>38.5</td>
<td>16.3</td>
<td><strong>0.023</strong></td>
<td>4.7 to 39.7</td>
</tr>
<tr>
<td>Live birth rate (beyond 24 weeks) per cycle (%)</td>
<td>32.7</td>
<td>12.2</td>
<td><strong>0.027</strong></td>
<td>4.2 to 36.9</td>
</tr>
<tr>
<td>Positive pregnancy rate per transfer (%)</td>
<td>55.6</td>
<td>40.5</td>
<td>0.233</td>
<td>-5.9 to 36.1</td>
</tr>
<tr>
<td>Clinical pregnancy rate (beyond 12 weeks) per transfer (%)</td>
<td>44.4</td>
<td>19.1</td>
<td><strong>0.022</strong></td>
<td>5.65 to 44.9</td>
</tr>
<tr>
<td>Live birth rate (beyond 24 weeks) per transfer (%)</td>
<td>37.8</td>
<td>14.3</td>
<td><strong>0.025</strong></td>
<td>4.9 to 42.0</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>32.6</td>
<td>23.5</td>
<td>0.252</td>
<td>-4.5 to 22.7</td>
</tr>
<tr>
<td>Subjects with surplus of good grade embryos for freezing after ET (%)</td>
<td>31.1</td>
<td>19</td>
<td>0.293</td>
<td>-6.2 to 30.4</td>
</tr>
<tr>
<td>Severe OHSS that required hospitalization (%)</td>
<td>3.8</td>
<td>20.4</td>
<td><strong>0.023</strong></td>
<td>-29.2 to 4.0</td>
</tr>
<tr>
<td>Subjects who experienced side effects (%)</td>
<td>45.1</td>
<td>8.2</td>
<td>&lt;0.001</td>
<td>0.195 to 0.543</td>
</tr>
<tr>
<td>Subjects who stopped the medication prematurely (%)</td>
<td>5.9</td>
<td>2.0</td>
<td>0.628</td>
<td>-0.038 to 0.116</td>
</tr>
</tbody>
</table>

*Median.

which was higher than the subjects in the placebo group (95% CI 49.3—60.5%, P = 0.012) and in the metformin group (95% CI 46.5—59.3%, P = 0.005). In addition, subgroup analysis did not reveal any differences between IVF and ICSI cycles, within and between groups, in terms of their baseline characteristics and the clinical outcomes including fertilization rates (data not shown).

Women in both groups received a similar number and quality of embryos. The majority of women (95.4%) had two embryos replaced. One women in the metformin group and three in placebo group received only one embryo due to poor fertilization (P = 0.567). The endometrial thickness at the day of HCG administration was not different (MET = 11.0 mm, PLA = 10.7; P = 0.495). The positive pregnancy rates per cycle and per transfer were not significantly different (Table II). However, the clinical pregnancy rates per cycle (38.5 versus 16.3%, P = 0.023) (ITT analysis) and per transfer (44.4 versus 19.1%, P = 0.022) (per protocol analysis) were significantly better in the metformin group. The clinical pregnancy rate per transfer in women who received two embryos was still significantly higher in the metformin group (45.5 versus 20.5%, P = 0.031). Similarly, the live birth rate per cycle (32.7 versus 12.2%, P = 0.027) and per transfer (37.8 versus 14.3%, P = 0.025) was also higher for subjects taking metformin (Table II). The differences were still significant after adjustment for BMI and the age of the patient (coefficient = 1.21, OR = 3.35, 95% CI 1.16–9.64, P = 0.025). The median gestations at birth were 39 weeks (range 34–43) and 40 weeks (range 34–43) in the metformin and the placebo groups, respectively (P = 0.446). There were no significant differences in the twinning rates (MET = 6.7%, PLA = 9.5%; P = 0.928) or the implantation rates (MET = 32.6%, PLA = 23.5%; P = 0.252).

Biochemical results

There were no significant changes in the fasting serum glucose levels between baseline and at the day of oocyte retrieval in both groups (Table III). However, metformin significantly reduced the fasting insulin levels after 4 weeks of medication.
compared with the placebo group ($P = 0.05$). The magnitude of the reduction of insulin concentrations was negatively influenced by BMI (coefficient = $-0.031$, $P = 0.006$, adjusted $R$-square = 0.173). We did not observe any changes in insulin sensitivity in both groups.

After the adjustment for the total rFSH dose and number of follicles, metformin reduced the estradiol concentrations at the day of HCG administration (Table IV).

Although there were no significant changes in testosterone levels in the metformin group (baseline geometric mean = 1.99 nmol/l, geometric mean at the day of HCG administration = 1.97 nmol/l, $P = 0.892$), there was a significant increase in the placebo group (baseline geometric mean = 2.06 nmol/l, geometric mean at the day of HCG administration = 2.52 nmol/l, $P = 0.040$). Both testosterone concentrations and FAI were lower in the metformin group at the day of HCG administration (Table V). The findings were still significant after adjustment for age, number of follicles, BMI and total dose of rFSH (data not shown). The SHBG levels were similar in both groups.

In addition, there was a negative correlation between FAI and the number of follicles, estradiol levels, age and fasting insulin concentration, serum VEGF levels were significantly lower in the metformin group (coefficient = $-35.6$, $P = 0.048$).

### Table IV. Multiple linear regression analysis of the log$_{10}$ estradiol levels on the number of follicles, total rFSH dose and the use of metformin

<table>
<thead>
<tr>
<th></th>
<th>Coefficient</th>
<th>Standard error</th>
<th>$t$</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>3.58</td>
<td>0.097</td>
<td>37.1</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Number of follicles</td>
<td>0.016</td>
<td>0.003</td>
<td>2.68</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Total rFSH dose</td>
<td>0.00</td>
<td>0.00</td>
<td>-0.470</td>
<td>0.639</td>
</tr>
<tr>
<td>Metformin</td>
<td>-1.08</td>
<td>0.052</td>
<td>-2.09</td>
<td>0.010</td>
</tr>
</tbody>
</table>

Adjusted $R$-square = 0.282. The analysis of variance for the regression: $F$-value = 12.4, $P$-value $<0.001$, residual SD = 2.21.

### Table V. The androgen levels in the metformin and placebo groups at the day of HCG administration

<table>
<thead>
<tr>
<th></th>
<th>Metformin (47)</th>
<th>Placebo (47)</th>
<th>$P$-value</th>
<th>95% CI for difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone (nmol/l)$^a$</td>
<td>1.96</td>
<td>2.52</td>
<td>0.029</td>
<td>0.62 to 0.97</td>
</tr>
<tr>
<td>Sex hormone-binding globulin (nmol/l)</td>
<td>91.2 (41.0)</td>
<td>83.8 (34.8)</td>
<td>0.361</td>
<td>$-8.66$ to 23.5</td>
</tr>
<tr>
<td>Free androgen index$^a$</td>
<td>2.43</td>
<td>3.34</td>
<td>0.004</td>
<td>0.58 to 0.71</td>
</tr>
</tbody>
</table>

$^a$Geometric means, mean ratio (metformin/placebo) and the corresponding 95% CI were reported after the results were back-transformed.

### Discussion

This is the largest RCT reported on the effects of co-treatment with metformin in women with PCOS undergoing IVF. Trails to date have been a retrospective study by Stadtmauer (2001), with 60 patients, an open-label study by Fedorcsak et al. (2003), with 17 patients, and the first RCT by Kjotrod et al. (2004), with 73 subjects.

We demonstrated that a short course of 28 days of metformin during the IVF cycle improved the pregnancy outcome and reduced the risks of OHSS, despite the fact that it neither enhanced the response to the stimulation nor improved the fertilization rate. Additionally, metformin also reduced the serum estradiol, androgen, fasting insulin and VEGF concentrations. The regimen was also well tolerated with a low rate of withdrawal.

It has been known that a high LH level stimulates ovarian androgen production (Abbott et al., 2002, 2005). The GnRH agonist (Nafarelin) significantly suppresses LH levels, and the levels remain low during the stimulation with rFSH (Cheung et al., 2002). The LH levels are also lower in the patients treated with rFSH compared with the women receiving human menopausal gonadotrophin (Teissier et al., 1999; Filicori et al., 2003). We therefore employed a long GnRH (Nafarelin) protocol and used rFSH for stimulation to minimize the stimulatory effects from LH.

The calculated study sample size was based on the improvement of fertilization rates (primary outcome). To improve the reliability on the findings of the secondary outcome measures (the pregnancy and OHSS rates), we minimized the number of variables in the study protocol such that all subjects received the same starting dose of rFSH, the same dose of HCG, there was no coasting period for women at risk of OHSS and two variables in the study protocol such that all subjects received the same starting dose of rFSH, the same dose of HCG, there was no coasting period for women at risk of OHSS and two embryos transferred when available. In addition, over 95% of the clinical procedures (scanning, egg retrieval, embryo transfer) were carried out by a single researcher.

We were unable to demonstrate that metformin improved the response to stimulation, the number of oocytes retrieved and the cleavage rates or the embryo quality (Table II). The findings were consistent with the other prospective studies (Fedorcsak et al., 2003; Kjotrod et al., 2004), but contradicted the findings of Stadtmauer et al. (2001). This may be partly explained by the fact that Stadtmauer et al. (2001) compared the outcomes with their historic data.

Although the fertilization rates were not significantly different between the metformin and the placebo groups, they were significantly lower than the non-PCOS group who underwent IVF treatments during the same period of time. Teissier et al. (2000) suggested that the follicular endocrine microenvironment is related to oocyte quality in women undergoing IVF, and an excess follicular androgen concentration may affect oocyte quality. Our study showed that 4 weeks co-treatment with metformin reduced serum testosterone and FAI levels. Therefore, a more prolonged treatment with metformin before starting the IVF cycle might be expected to improve oocyte quality since the duration of maturation from primary follicles to antral follicles stage is more than 3 months (Gougeon, 1996). However, Kjotrod et al. (2004) did not find that pretreatment with metformin for at least 4 months improved the...
fertilization rates. A potential advantage of using a short course of metformin as in our study is that it improves the compliance and reduces the withdrawal rate.

The clinical pregnancy and live birth rates were significantly better in the metformin group. We postulate the mechanism that metformin improves the pregnancy outcome through its capability to reduce androgen production (Table V). The serum androgen levels rise during the ovarian stimulation in IVF cycles (Fanchin et al., 2000), and the levels tend to be higher in patients with PCOS (Kodama et al., 1995). Takeuchi et al. (1993), Check et al. (1995) and Kodaman and Taylor (2004) suggested that high androgen levels negatively affected the pregnancy outcome. In this study, not only were we able to demonstrate that both the total testosterone and FAI levels were significantly lower in the metformin group, but we also observed a negative correlation between FAI and serum HCG concentrations at day 12 after embryo transfer. Since day 12 serum HCG concentration has been reported to be a reliable predictor for pregnancy outcome (Poikkeus et al., 2002), our finding suggested that a high testosterone level posed a negative effect on pregnancy outcome.

Furthermore, Okon et al. (1998) showed that there was a negative correlation between androgen concentrations and uterine placental protein 14 (PP14) levels in women with PCOS with a history of recurrent miscarriage. PP14 is a marker of endometrial receptivity (Westergaard et al., 1998; Sunder and Lenton, 2000), and serum PP14 levels were found to be increased in the conception compared with non-conception IVF cycles (Suzuki et al., 2000; Westergaard et al., 2004). On the basis of the above evidence, high androgen levels may have detrimental effects on endometrial receptivity (Rose et al., 1988; Kodaman and Taylor, 2004) and consequently affect the outcomes of pregnancy.

In contrast to the study by Kjotrod et al. (2004), we demonstrated that metformin reduced the estradiol levels at the day of HCG administration. One possible explanation is that Kjotrod’s analysis was not adjusted for other confounding factors that may influence oestrogen production such as the total dose of rFSH and the number of follicles. Serum estradiol levels have been consistently found to be higher in women with PCO undergoing IVF treatment in comparison with controls (Dor et al., 1990; Urman et al., 1992; MacDougall et al., 1993; Kodama et al., 1995). These observations may be explained by the increase in levels of androgen substrates (Tsilechorozidou et al., 2004) and in aromatase activity (Andreani et al., 1994; la Marca et al., 2002). Furthermore, both insulin and insulin-like growth factor I (IGF-I) augment the stimulating effects of FSH on oestrogen production from the granulosa cells (Mason et al., 1993; Andreani et al., 1994). Therefore, the effects of metformin on the reduction in androgen and insulin levels could contribute to the decreased estradiol concentrations. There is also evidence to suggest that metformin has direct effects on human ovarian steroidogenesis. Attia et al. (2001) and Mansfield et al. (2003) demonstrated a reduction in androstenedione production from the theca cells when they were co-cultured with metformin. In addition, metformin was also found to reduce aromatase activity directly (la Marca et al., 2002). These findings may also explain the rapid biochemical improvements in the serum testosterone and estradiol concentrations observed in our study after a short course of metformin therapy. Abbott et al. (2005) suggested that hyperinsulinaemia may induce an increased pituitary secretion of LH and lead to an increased androgen production. However, Cheung et al. (2002) demonstrated that LH levels were significantly suppressed by GnRH, and the levels remained low throughout the stimulation with rFSH on patients with PCOS. The observed reduction in testosterone concentration by metformin in our study was unlikely to be resulted from the changes of LH levels, although we did not measure LH levels during the stimulation.

It has long been recognized that patients with PCOS undergoing IVF treatments are at a greater risk of developing OHSS (Delvigne et al., 1993). However, the actual incidence is difficult to quantify, with the reported rates varying between 10 and 18% (MacDougall et al., 1993; Kodama et al., 1995; Engmann et al., 1999; Ludwig et al., 1999). The incidence of severe OHSS in this study was certainly among the highest figure reported. This may be because it was not our unit policy to offer women who were at risk of OHSS a coating period before egg retrieval. Instead, they had all the embryos cryopreserved for future transfers. All these subjects were included in the ITT analysis as having severe OHSS. Conversely, the incidence of women who actually developed severe OHSS (analysis per protocol) was still significantly lower in the metformin group.

The cardinal feature of the pathogenesis of OHSS is an increase in capillary permeability (Whelan and Vlahos, 2000). VEGF is an endothelial cell mitogen with potent angiogenic properties (Ferrara and Davis-Smyth, 1997) and is thought to be a key mediator of OHSS (Lee et al., 1997; Agrawal et al., 1998, 1999; Pellicer et al., 1999). Serum VEGF levels at the day of egg retrieval are elevated in patients with PCOS compared with controls and are also increased in women who develop OHSS. Although there is a positive correlation between the serum VEGF and estradiol level at the day of HCG administration (Agrawal et al., 1999), VEGF is a better predictor for OHSS (Agrawal et al., 1999).

An in vitro study revealed that the expression of VEGF mRNA in human luteinized granulosa cells was dose and time dependently enhanced by HCG (Neulen et al., 1995). The expression was also higher in the OHSS group than the controls (Wang et al., 2002). Additionally, Miele et al. (2000) demonstrated that both insulin and IGF-I increased VEGF mRNA expression. Agrawal et al. (2002) also showed that insulin augmented both gonadotrophin and HCG on the production of VEGF from human luteinized granulosa cells. The concentrations were also higher in the PCO and the OHSS groups. Based on the above evidence, we postulate that the reduction in the incidence of OHSS in the metformin group may be attributed to its effect on reduction of insulin levels and thereby the subsequent production of VEGF. Recent evidence also suggested that metformin suppressed VEGF levels in women with PCOS in natural cycles (Fleming et al., 2005).

It is interesting also to note that OHSS is often more severe in women who conceive, and yet the rate of OHSS was lower in the metformin group despite a higher pregnancy rate.
Another hypothesis is that metformin may have direct ovarian effects similar to troglitazone. There is selective insulin resistance in target tissues in women with PCOS (Dunaif, 1997), and these are due to post-receptor defects (Dunaif, 1997; Wu et al., 2003; Tsilchorozidou et al., 2004). Thus, while the intracellular metabolic pathways are impaired, the mitogenic and the steroidogenic pathways remain intact. Wu et al. (2003) also demonstrated that similar conditions exist in the ovarian tissue from patients with PCOS. Additionally, the mitogenic effects of IGF-I were found to be greater in the cultured granulosa cells from PCO compared with normal ovaries (Wu et al., 2003). The study also showed that troglitazone, an insulin-sensitizing agent, reversed this process. A reduction in these mitogenic processes in ovarian tissue may decrease the likelihood of an exaggerated response to stimulation by gonadotrophin.

Metformin appeared to reduce estradiol levels and to reduce the risk of over stimulation (lower VEGF concentrations). The findings were also consistent with the observations in the ovulation induction therapy with gonadotrophin (De Leo et al., 1999; Yarali et al., 2002; Haas et al., 2003; Tasdemir et al., 2004).

In conclusion, we have shown that a short-term use of metformin for 4 weeks only during the down-regulation and low-dose stimulation in women with PCOS undergoing IVF achieves a significant improvement in ongoing pregnancy rate with a concomitant fall in the risk of OHSS.

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