A randomized controlled trial evaluating metformin pre-treatment and co-administration in non-obese insulin-resistant women with polycystic ovary syndrome treated with controlled ovarian stimulation plus timed intercourse or intrauterine insemination

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BACKGROUND: There are few data in the literature regarding the utility of metformin before and during gonadotrophin administration in women with polycystic ovary syndrome (PCOS). The aim of the present study was to assess the effect of the pre-treatment and co-administration of metformin in infertile PCOS women treated with controlled ovarian stimulation (COS) followed by timed intercourse (TI) or intrauterine insemination (IUI). METHODS: Seventy insulin-resistant primary infertile women with PCOS were randomized to receive metformin chloridrate (850 mg twice daily; group A) or placebo tablets (two tablets daily; group B) for 3 months. Three trials of COS using highly purified urinary FSH (hpFSH) plus TI/IUI were performed. Number of ampoules of gonadotrophin used, duration of the ovarian stimulation, cycle cancellation, ovulation, pregnancy, abortion, live birth, mono-ovulatory cycles, multiple pregnancies and ovarian hyperstimulation syndrome (OHSS) rates were assessed. RESULTS: No difference between groups was detected in ovulation, cycle cancellation, pregnancy, abortion, live birth, multiple pregnancies and OHSS rates. The mono-ovulatory cycle rates were significantly (P=0.002) more frequent in group A than in group B, whereas the days of stimulation for non-cancelled cycles and the number of vials of gonadotrophins used were significantly (P<0.001) higher in group A than in group B. CONCLUSION: In insulin-resistant women with PCOS, metformin pre-treatment and co-administration with hpFSH increases the mono-ovulatory cycles.

Key words: anovulation/gonadotrophins/infertility/metformin/PCOS

Introduction

Different criteria have been used to diagnose the polycystic ovary syndrome (PCOS) (Zawadzki and Dunaif, 1992; Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group, 2004) but the ovarian dysfunction remains probably the pivotal feature, making this syndrome the major cause of anovulatory infertility (Homburg and Insler, 2002).

The main goal for the treatment of anovulatory infertility is the induction of mono-ovulatory cycles in order to avoid multiple pregnancies (ESHRE Capri Workshop Group, 2003). Several approaches have been proposed to induce mono-ovulation in anovulatory PCOS patients (Palomba et al., 2004a). The first therapeutic option in the management of these patients is clomiphene citrate, which achieves an ovulation rate of 60–85% and a pregnancy rate of 30–40% (Hughes et al., 2000). Recently, metformin chloridrate has been shown to induce ovulatory cycles in anovulatory clomiphene citrate-resistant or non-resistant patients with PCOS and to improve ovulation also as an additional treatment in women who received clomiphene citrate (Kashyap et al., 2004). Metformin and clomiphene citrate are similarly effective in terms of ovulation rates but metformin seems to increase the pregnancy rate more significantly (Palomba et al., 2005).

Notwithstanding the effectiveness of clomiphene citrate and metformin, administered alone or associated in a sequential or combined regimen, 40% and 50% of women with PCOS remain, respectively, anovulatory or fail to conceive having ovulated on clomiphene citrate and/or metformin treatment (Hughes et al., 2000; Palomba et al., 2004b; Palomba et al., 2005). For these patients, controlled ovarian stimulation (COS)
followed by timed intercourse (TI) or intrauterine insemination (IUI) is the next therapeutic step before controlled ovarian hyperstimulation (COH) for assisted reproductive techniques (Nugent et al., 2000; Palomba et al., 2004a). For both COS and COH, the administration of gonadotrophins is related to a significant increase of multiple pregnancies and of ovarian hyperstimulation syndromes (OHSS) (Bayram et al., 2004).

To date, in English literature the data regarding the possible efficacy of metformin in minimizing the side-effects of gonadotrophin treatment are few and contrasting, probably because they have been obtained on heterogeneous patient populations (De Leo et al., 1999; Stadtmauer et al., 2001, 2002; Yarali et al., 2002; Fedorcsak et al., 2003; Kjotrod et al., 2004). Based on these considerations, we aimed to study the effects of the pre-treatment and co-administration of metformin in infertile insulin-resistant PCOS women treated with gonadotrophins.

Materials and methods

The procedures used were in accordance with the guidelines of the Helsinki Declaration on human experimentation. The study was approved by the Institutional Review Board of the University ‘Magna Graecia’ of Catanzaro. The purpose of the protocol was carefully explained to each woman, and a written consent was obtained from them before beginning the study.

Patients

Between May 2002 and June 2003, a total of 70 non-obese insulin-resistant primary infertile women with PCOS were enrolled. All patients were referred to our Department (University ‘Magna Graecia’ of Catanzaro) for induction of ovulation with gonadotrophins because they failed to ovulate or to conceive having ovulated, during six previous cycles of clomiphene citrate (150 mg/day per 5 days) alone or in co-administration with metformin (1700 mg daily).

The diagnosis of PCOS was made according to the National Institutes of Health criteria (Zawadzki and Dunaif, 1992). The insulin resistance was defined according to Legro et al. (1998).

Exclusion criteria for all subjects included: age <20 or >34 years; body mass index (BMI) >30 and <18 kg/m²; neoplastic, metabolic of health criteria (Zawadzki and Dunaif, 1992). The insulin resistance using a threshold value <4.5 mg/10⁻⁴ IU (SI: <582 pmol/l) (Legro et al., 1998). This measure provides the best combination of sensitivity (95%) and specificity (84%) as well as the best positive (87%) and negative predictive (94%) values as a screening test for predicting insulin resistance in PCOS (Legro et al., 1998; Ducluzeau et al., 2003).

Successively, in each subject an oral glucose tolerance test (OGTT) was performed. In particular, each patient received orally 75 g glucose load and further blood samples (10 ml each) were obtained at 30 min intervals for the following 2 h during the infusion period (at 30, 60, 90 and 120 min), and glucose and insulin concentrations were determined. Plasma glucose levels were determined by the glucose oxidase method on a Beckman Glucose Analyzer (Fullerton, CA, USA), with a sensitivity of 5.4 mg/dl (SI: 0.30 mmol/l), and an intra- and inter-assay coefficient of variation (CV) respectively of 1.0 and 1.2%.

Serum insulin was measured by a solid-phase chemiluminescent assay coefficient of variation (CV) respectively of 1.0 and 1.2%. BMI was measured as the ratio between the weight and the square of the height (kg/m²), WHR was calculated as ratio between the smallest circumference of torso (between the twelfth rib and the iliac crest) and the circumference of the hip (considered as the maximal extension of the buttocks). WHR was calculated with the patients in standing position with relaxed abdomen, arms at sides and joined feet.

Biochemical assays

At study entry, all subjects underwent venous blood drawn in the morning between 08:00 and 09:00 after an overnight fasting and resting in bed during the early proliferative phase (2nd to 3rd day) of the progesterone-induced withdrawal uterine bleeding (100 mg natural progesterone i.m; Protogest; Amsa, Rome, Italy). A butterfly needle was inserted into an antecubital vein and an i.v. saline infusion was given at a rate of -50 ml/h. The subjects were kept supine throughout the infusion period and were not allowed to smoke, sleep, or drink alcoholic or caffeinated beverages.

Thirty minutes after the needle insertion and resting in the supine position, basal blood samples (5 ml) were collected into tubes containing EDTA and immediately centrifuged at 4°C for 20 min at 1600 g, and the serum was stored at -80°C until analysis. All blood samples for each woman were assayed in duplicate determinations after a 3 day 300 g carbohydrate diet and a 12 h overnight fasting. The mean of two hormonal results was calculated. As previously reported (Orio jr et al., 2003), the following hormonal serum levels were measured: FSH, LH, thyroid-stimulating hormone, prolactin, 17β-estradiol (E₂), progesterone, 17OH-progesterone, total testosterone, androstenedione, dehydroepiandrosterone sulphate, and sex hormone-binding globulin (SHBG). The free androgen index was calculated using the following formula: total testosterone (nmol/l)/SHBG (nmol/l)×100 (Morley et al., 2002).

Glucose and insulin concentrations were also measured 30 min after insertion of the i.v. catheter to detect the fasting levels (time 0). Fasting glucose:insulin ratio was calculated in each woman to assess insulin resistance using a threshold value <4.5 mg/10⁻⁴ IU (SI: <582 mmol/pmol) (Legro et al., 1998). This measure provides the best combination of sensitivity (95%) and specificity (84%) as well as the best positive (87%) and negative predictive (94%) values as a screening test for predicting insulin resistance in PCOS (Legro et al., 1998; Ducluzeau et al., 2003).

Protocol

Clinical assessments

At study entry, the same operator noted for each eligible PCOS patient the age and the years of infertility, calculated the modified Ferriman–Gallwey score (Hatch et al., 1981), evaluated the patient’s daily physical activity, job, and daily activities using a semiquantitative questionnaire (Palomba et al., 2004b), performed a transvaginal ultrasonography (TV-USG) and assessed the anthropometric measurements. The anthropometric measurements included height, weight, BMI and waist:hip ratio (WHR). Body height and weight were measured without shoes and clothes, respectively. BMI was measured as the ratio between the weight and the square of the height (kg/m²). WHR was calculated as ratio between the smallest circumference of torso (between the twelfth rib and the iliac crest) and the circumference of the hip (considered as the maximal extension of the buttocks). WHR was calculated with the patients in standing position with relaxed abdomen, arms at sides and joined feet.
method described by Tai (1994) for the metabolic curves. The \( \frac{\text{AUC}_{\text{glucose}}}{\text{AUC}_{\text{insulin}}} \) ratio was also calculated in each subject.

**Randomization and treatment**

Using a computer generating randomization lists, the subjects were allocated in single blocks into two treatment groups of 35 women each (groups A and B). The random allocation sequence was concealed until the interventions were assigned. Group A was treated with metformin cloridrate (Metforal, Laboratori Guidotti, Pisa, Italy) at a dosage of 850 mg twice daily, whereas group B received placebo tablets (two tablets of poly-vitamins daily). The drug and the placebo were packaged in identical form in the pharmacy of the University of Catanzaro and labelled according to subject number. The patients were instructed to take the tablets with the meals. The duration of treatment was 3 months. After 3 months of pre-treatment with metformin or placebo, all patients underwent three trials of COS followed by TI or IUI (as below detailed). Metformin and placebo were continued during COS and they were stopped in patients who conceived (Norman et al., 2004). For the whole of the study period, operators and patients were blinded to the treatment allocation.

In all patients the COS was achieved using highly purified urinary FSH (hpFSH; Fostimon 75 IU, Amsa, Rome, Italy) in a low-dose step-up protocol (Nugent et al., 2000). Specifically, gonadotrophin administration started on the 3rd day of the progesterone-induced menstrual bleed with 75 IU of hpFSH daily for 14 days (1 vial i.m. daily). If no ovarian response was noted after 14 days of hpFSH administration (at least one follicle with maximum diameter ≥10 mm), the daily dose was increased by 37.5 IU every week until active follicular development was observed at TV-USG examination. Any further changes were made by increments of 37.5 IU daily at weekly intervals to a maximum of 225 IU daily. If a dominant follicle emerged, the dose of hpFSH was maintained until the follicle reached a diameter ≥17 mm. If not more than three leading follicles ≥17 mm were observed on TV-USG, HCG (Gonasi HP 5000; Serono, Rome, Italy) 10 000 IU was injected i.m. 24 h after the last hpFSH injection. The presence of more than three dominant follicles ≥14 mm in diameter or the absence of follicular response after 35 days of treatment were considered as indications for abandoning the cycle (cancelled cycles).

Ovulation induction monitoring was performed by serial TV-USG and serum E\(_2\) evaluation. Both TV-USG scans and E\(_2\) determinations were performed every 3 days beginning on the 7th day after starting treatment and then every 3 days. TV-USG assessments were obtained by the same experienced operator using an ultrasonic scanner (Apio; Toshiba Medical Systems, Rome, Italy) equipped with a 7.5 MHz vaginal probe. The follicular dimensions were calculated using the arithmetic mean of the two main diameters of each follicle.

PCOS women who previously failed to ovulate underwent TI, whereas those who were ovulating but did not conceive underwent IUI. In order to perform IUI, (Cohen et al., 2000) semen was collected by masturbation, washed and processed with a standardized swim-up procedure (Aboughar et al., 2001; Duran et al., 2002). A single IUI was then performed by means of the modified Sheppard catheter (Cook Ob/Gyn, Cook Group Incorporated, Bloomington, IN, USA).

Both TI and IUI were performed 35 h after ovulation induction. No drug was administered as luteal phase support.

At the end of the study, a 9 month extension of the follow-up period was done to obtain the live-birth rate for each treatment group. Throughout the study, no change in diet or physical activity was implemented. On the contrary, the subjects were instructed to follow their usual diet and physical activity.

**Main outcome measures**

For each stimulation cycle, the peak E\(_2\) levels and the number of dominant follicles on the day of HCG administration, the duration of stimulation, and the number of hpFSH vials administered were recorded.

During the study, the cycle cancellation, ovulation, pregnancy, abortion, and live-birth rates were evaluated for each woman. The rate of mono-ovulatory cycles and the incidence of multiple pregnancies and OHSS were also recorded in both groups. A cycle was defined as mono-ovulatory when ovulation was achieved in the presence of only one dominant follicle (≥17 mm). Ovulation was defined by assaying plasma progesterone levels 8 days after HCG injection (>10 ng/ml; SI: 32 nmol/l). Ovulation rate was calculated as percentage of ovulatory cycles/total non-cancelled cycles. The cycle cancellation rate was calculated as percentage of cancelled cycles/total number of cycles started. Pregnancy rate was defined as percentage of pregnancies/total cycles started and as percentage of pregnancies/total non-cancelled cycles. A rising β-HCG (the first assay was performed 15 days after TI/IUI) and sonographic evidence of intrauterine gestational sac were considered criteria to define a physiological pregnancy. Abortion rate was defined as percentage of early pregnancy losses (within the first 12 weeks of gestation)/total pregnancies. Live-birth rate was defined as percentage of women with baby alive/ women who achieve a pregnancy.

**Safety assessment**

Subjects were instructed to report on a daily diary the characteristics of the menstrual cycle and the onset of any adverse experiences (AE). For each AE reported on the daily diary, the severity, duration, and a possible cause–effect relationship with drug administrations was noted. To evaluate the compliance with the treatment and with the protocol, the number of tablets forgotten, the changes in diet, physical activity and weight were recorded in the same diary.

Standard clinical evaluations and laboratory analyses, including haematological, renal function and liver function tests, and microscopic examinations of sediment from midstream urine specimens were performed at study entry and after three cycles of treatment.

**Statistical analysis**

The primary end-point of the current study was the multiple pregnancy rate. Our secondary end-point was the mono-ovulation rate. No data currently available in the literature on patients with similar characteristics can be used for the pre-study sample size calculation. Based on these considerations, our population sample was determined according to the expected rate of primary infertile PCOS patients who referred to our Centre for ovulation induction with gonadotrophins throughout 1 year. At study end, a post-study power analysis for the main outcome measures was performed. A sample size calculation for each end-point in order to obtain a power >80% was also performed.

Data are expressed as mean ± SD. The Kolmogorov–Smirnov statistic with a Lilliefors significance level was used for testing normality, and the unpaired t-test and the Mann–Whitney U-test were applied as appropriate. For categorical variables the Pearson \( \chi^2 \)-test was performed, unless the Fisher’s exact test was required for frequency tables when >20% of the expected values were <5. \( P \leq 0.05 \) was considered significant. The Statistics Package for Social Science (SPSS 13.0 Sep 2004; SPSS Inc., Chicago, IL, USA) was used for statistical analyses. The power analysis and the sample size calculation were performed using SamplePower release 2.0.
Results

Figure 1 shows the trial design.

After randomization, no difference was detected in any clinical, hormonal, and metabolic parameter between treatment groups (Table I). At study entry, all women had polycystic ovaries at TV-USG assessment, and thus also satisfied the ESHRE/ASRM criteria (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). The two groups were also homogeneous with regard to percentage of patients who had previously failed to ovulate or to conceive having ovulated. The frequency of normal- and overweight patients for each group was also similar.

Results included in the present study were obtained from a total of 70 patients; 35 subjects for each treatment group. No woman dropped out from our study protocol. In each treatment group no pregnancy occurred during the 12 week period of metformin or placebo pre-treatment. Table II summarizes the main results.

The patients were followed for a total of 85 and 87 cycles in groups A and B, respectively. No significant difference ($P = 0.116$) in cycle cancellation rate was observed between groups. In no case was the cycle considered cancelled for absence of follicular response after 35 days of treatment. No difference between groups was observed in ovulation rate ($P = 0.453$), whereas the mono-ovulations were significantly ($P = 0.002$) more frequent in group A than in group B.

The days of stimulation for non-cancelled cycles ($P < 0.001$) and the number of vials of gonadotrophins used ($P < 0.001$) were significantly higher in group A than in group B, while the number of dominant follicles ($P = 0.019$) and the peak of $E_2$.

### Table I. Main clinical, hormonal, and metabolic data for infertile women with polycystic ovary syndrome after randomization

<table>
<thead>
<tr>
<th></th>
<th>Group A ($n = 35$)</th>
<th>Group B ($n = 35$)</th>
<th>$P$</th>
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<tbody>
<tr>
<td><strong>Blinded treatment</strong></td>
<td></td>
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<tr>
<td>Age (years)</td>
<td>26.2 ± 2.7</td>
<td>26.9 ± 2.8</td>
<td>0.291</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.5 ± 2.7</td>
<td>26.4 ± 2.5</td>
<td>0.873</td>
</tr>
<tr>
<td>Normal-weight patients (n, %)</td>
<td>10 (14.3)</td>
<td>12 (17.1)</td>
<td>0.607</td>
</tr>
<tr>
<td>Overweight patients (n, %)</td>
<td>25 (35.7)</td>
<td>23 (32.9)</td>
<td></td>
</tr>
<tr>
<td>Waist:hip ratio</td>
<td>0.87 ± 0.4</td>
<td>0.88 ± 0.5</td>
<td>0.927</td>
</tr>
<tr>
<td>Duration of infertility (months)</td>
<td>26.1 ± 4.7</td>
<td>24.8 ± 4.9</td>
<td>0.261</td>
</tr>
<tr>
<td>Patients who underwent timed intercourse (n, %)</td>
<td>8/35 (22.9)</td>
<td>10/35 (28.6)</td>
<td>0.584</td>
</tr>
<tr>
<td>Patients who underwent intrauterine insemination (n, %)</td>
<td>27/35 (77.1)</td>
<td>25/35 (71.4)</td>
<td></td>
</tr>
<tr>
<td>Modified Ferriman–Gallwey score</td>
<td>12.2 ± 2.2</td>
<td>11.3 ± 2.0</td>
<td>0.078</td>
</tr>
<tr>
<td>Physical activity score</td>
<td>1.6 ± 0.4</td>
<td>1.7 ± 0.4</td>
<td>0.299</td>
</tr>
<tr>
<td>Cigarettes smoked (n/day)</td>
<td>4.8 ± 4.4</td>
<td>5.1 ± 3.9</td>
<td>0.764</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>6.5 ± 2.7</td>
<td>7.1 ± 3.2</td>
<td>0.400</td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>17.1 ± 4.8</td>
<td>19.0 ± 5.1</td>
<td>0.113</td>
</tr>
<tr>
<td>Thyroid-stimulating hormone (mIU/ml)</td>
<td>2.7 ± 0.7</td>
<td>2.5 ± 0.6</td>
<td>0.204</td>
</tr>
<tr>
<td>Prolactin (ng/ml)</td>
<td>9.1 ± 2.7</td>
<td>9.7 ± 3.1</td>
<td>0.391</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>47.8 ± 11.1</td>
<td>51.1 ± 12.0</td>
<td>0.237</td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td>0.7 ± 0.6</td>
<td>0.5 ± 0.5</td>
<td>0.134</td>
</tr>
<tr>
<td>17OHP-Progesterone (µg/l)</td>
<td>1.7 ± 0.5</td>
<td>1.8 ± 0.8</td>
<td>0.533</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>1.0 ± 0.4</td>
<td>1.1 ± 0.4</td>
<td>0.299</td>
</tr>
<tr>
<td>Androstenedione (ng/ml)</td>
<td>1.6 ± 0.5</td>
<td>1.6 ± 0.4</td>
<td>1.000</td>
</tr>
<tr>
<td>Deidroepiandrosterone sulphate (ng/ml)</td>
<td>2499 ± 519</td>
<td>2701 ± 668</td>
<td>0.162</td>
</tr>
<tr>
<td>Sex hormone-binding globulin (nmol/l)</td>
<td>25.1 ± 6.7</td>
<td>27.9 ± 7.9</td>
<td>0.114</td>
</tr>
<tr>
<td>Free androgen index (%)</td>
<td>13.8 ± 6.6</td>
<td>13.6 ± 7.3</td>
<td>0.905</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>89.2 ± 10.1</td>
<td>93.1 ± 11.3</td>
<td>0.133</td>
</tr>
<tr>
<td>Fasting insulin (µIU/ml)</td>
<td>23.2 ± 5.2</td>
<td>22.8 ± 4.7</td>
<td>0.737</td>
</tr>
<tr>
<td>Glucose:insulin ratio (mg/10⁻⁴ IU)</td>
<td>3.9 ± 0.5</td>
<td>4.0 ± 0.4</td>
<td>0.359</td>
</tr>
<tr>
<td><strong>Oral glucose tolerance test</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>AUC</strong> (mg/dl/120 min)</td>
<td>16884 ± 5276</td>
<td>18561 ± 6892</td>
<td>0.257</td>
</tr>
<tr>
<td><strong>AUC</strong> (µIU/ml/120 min)</td>
<td>17110 ± 8752</td>
<td>18974 ± 8961</td>
<td>0.382</td>
</tr>
<tr>
<td><strong>AUC</strong> (glucose/AUC insulin ratio)</td>
<td>1.03 ± 0.99</td>
<td>1.05 ± 1.08</td>
<td>0.936</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SD.

*4* low; 2 moderate; 3 high.

The biochemical assays are reported in metric units. Conversion factors (CF) for SI: androstenedione = 3.492 (nmol/l); dehydroepiandrosterone sulphate = 0.002714 (µmol/l); estradiol = 3.671 (pmol/l); FSH = 1.0 (IU/l); fasting glucose = 0.05551 (mmol/l); fasting insulin = 7.175 (pmol/l); LH = 1.0 (IU/l); 17OHP-progesterone = 3.026 (nmol/l); progesterone = 3.180 (nmol/l); prolactin = 1.0 (µg/l); testosterone = 3.467 (nmol/l); thyroid-stimulating hormone = 1.0 (mIU/l).

AUC = area under curve.
levels ($P = 0.001$) on day of HCG administration were significantly lower in group A than in group B.

No difference in pregnancy ($P = 0.392$), abortion ($P = 0.568$) and live-birth ($P = 0.568$) rates was observed between two groups. No difference in cumulative pregnancy rate was also observed between groups ($P = 0.337$).

A trend ($P = 0.264, P = 0.195$) in multiple pregnancy rate was detected between groups. All multiple pregnancies observed in group A consisted of twin pregnancies, whereas in group B there was a case of triplet pregnancy.

No case of OHSS was observed in group A, whereas one patient in group B developed a mild OHSS during the 3rd cycle of treatment.

During the study, the two treatment schedules were generally well tolerated and the total incidence of all AE was not significantly different between the two groups. No serious AE or laboratory abnormalities were reported after the three cycles of treatment. The distribution of drug-related AE was also not significantly different between the two groups [14.3% (5/35) versus 8.6% (3/35) for groups A and B, respectively; $P = 0.707$].

Considering the present results, we calculated that our pilot study was strongly underpowered (-20%) for our primary end-point (multiple pregnancy rate), whereas it had a power of 92% for our secondary end-point (mono-ovulation cycles rate). At least 180 patients per group will be required in order to detect an effect of metformin treatment on multiple pregnancy rate with a power of 80%.

**Discussion**

Insulin exerts several effects in regulating the normal activity of the ovary in all its compartments (Poretsky et al., 1999) and insulin resistance seems to play a pivotal role in the pathogenesis of infertility in obese and non-obese women affected by PCOS (Legro et al., 2004).

Fulghesu et al. (1997) have shown that during ovarian stimulation with urinary FSH (uFSH) administrated in a conventional protocol, the E2 production and the global incidence of OHSS were significantly higher in PCOS women with raised insulin levels than in normo-insulinemic women. This increased response to exogenous gonadotrophins was considered to be caused partly by the effect of insulin on the aromatase activity of granulosa cells, as observed by a higher E2: androstenedione ratio (Fulghesu et al., 1997). Subsequently, Dale et al. (1998) have shown that, during low-dose step-up gonadotrophin treatment, insulin-resistant PCOS women have a much greater tendency to develop multiple follicles in response to gonadotrophins and, thus, a higher cycle cancellation rate in comparison with non-insulin-resistant PCOS women.

Recently, an elegant experimental study (Rice et al., 2005) has confirmed that insulin resistance in the ovary is confined to the metabolic effects of insulin, whereas the steroidogenic action of insulin remains intact. In this view, secondary hyperinsulinaemia can paradoxically enhance steroidogenesis in granulosa cells which remain normoresponsive to insulin and, thus, can be a clinical marker of high risk for ovarian hyperstimulation in PCOS patients (Fulghesu et al., 1997; Dale et al., 1998).

With this in mind, Fedorcsak et al. (2001) studied the impact of insulin resistance on the outcome of IVF/ICSI cycles in women with PCOS, showing that insulin-resistant women required higher doses of gonadotrophins and had lower E2 concentrations at ovulation induction. However, these differences disappeared after controlling for body weight (Fedorcsak et al., 2001). Furthermore, this last study had an important confounding factor consisting of pituitary suppression with buserelin and administration of gonadotrophins lacking in LH activity, i.e. recombinant FSH (rFSH).

Velazquez et al. (1994) first showed that metformin cloridrate was effective in PCOS patients in improving hormonal and metabolic pattern, and in facilitating normal menstrual cycles and pregnancy. Both observational and randomized controlled trials (Harborne et al., 2003; Kashyap et al., 2004; Palomba et al., 2004b, 2005) have successively confirmed the effectiveness of metformin in PCOS women in terms of menstrual

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**Table II.** Reproductive outcomes in insulin-resistant infertile polycystic ovary syndrome women undergoing controlled ovarian stimulation plus intrauterine insemination, treated with metformin (group A) or placebo (group B)

<table>
<thead>
<tr>
<th>Reproductive outcomes</th>
<th>Group A</th>
<th>Group B</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycles started (n)</td>
<td>85</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>Non-cancelled cycle rate (no. of non-cancelled cycles/no. of cycles, %)</td>
<td>78/85 (91.8)</td>
<td>73/87 (83.9)</td>
<td>0.116</td>
</tr>
<tr>
<td>Cycle cancellation rate (no. of cancelled cycles/no. of cycles, %)</td>
<td>7/85 (8.2)</td>
<td>14/87 (16.1)</td>
<td></td>
</tr>
<tr>
<td>Ovulation rate (no. of ovulating cycles/no. of cancelled cycles, %)</td>
<td>74/78 (94.9)</td>
<td>71/73 (97.3)</td>
<td>0.682</td>
</tr>
<tr>
<td>Mono-ovulatory cycle rate (no. of mono-ovulatory cycles/no. of non-cancelled cycles, %)</td>
<td>67/78 (85.9)</td>
<td>47/73 (64.4)</td>
<td>0.002</td>
</tr>
<tr>
<td>Duration of stimulation of non-cancelled cycles (days)</td>
<td>15.6 ±4.1</td>
<td>12.6 ±2.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gonadotrophin vials used in non-cancelled cycles/no. of non-cancelled cycles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dominant follicles on day of HCG administration (n)</td>
<td>1.2 ±0.6</td>
<td>1.5 ±0.7</td>
<td>0.019</td>
</tr>
<tr>
<td>Peak estradiol levels on day of HCG administration/dominant follicles (pg/ml)</td>
<td>256.4 ±37.6</td>
<td>278.7 ±44.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Pregnancy rate 'a' (no. of pregnancies/no. of cycles, %)</td>
<td>8/85 (21.2)</td>
<td>14/87 (16.1)</td>
<td>0.392</td>
</tr>
<tr>
<td>Pregnancy rate 'b' (no. of pregnancies/no. of non-cancelled cycles, %)</td>
<td>8/78 (10.3)</td>
<td>14/73 (19.1)</td>
<td>0.558</td>
</tr>
<tr>
<td>Multiple pregnancy rate 'a' (no. of multiple pregnancies/no. of non-cancelled cycles, %)</td>
<td>2/78 (2.6)</td>
<td>5/73 (6.9)</td>
<td>0.264</td>
</tr>
<tr>
<td>Multiple pregnancy rate 'b' (no. of multiple pregnancies/no. of pregnancies, %)</td>
<td>2/18 (11.1)</td>
<td>5/14 (35.7)</td>
<td>0.195</td>
</tr>
<tr>
<td>Cumulative pregnancy rate (no. of pregnancies/no. of patients, %)</td>
<td>18/35 (51.4)</td>
<td>14/35 (40.0)</td>
<td>0.337</td>
</tr>
<tr>
<td>Abortion rate (no. of abortions/no. of pregnancies, %)</td>
<td>1/18 (5.6)</td>
<td>2/14 (14.3)</td>
<td></td>
</tr>
<tr>
<td>Live-birth rate (no. of babies/no. of pregnancies, %)</td>
<td>17/18 (94.4)</td>
<td>12/14 (85.7)</td>
<td></td>
</tr>
<tr>
<td>OHSS rate (no. of OHSS/no. of cycles, %)</td>
<td>0/85 (0.0)</td>
<td>1/87 (1.1)</td>
<td>1.000</td>
</tr>
</tbody>
</table>

OHSS = ovarian hyperstimulation syndrome.
cyclicity and reproductive outcomes. On the contrary, few data are available regarding the use of metformin in PCOS patients treated with exogenous gonadotrophins (De Leo et al., 1999; Stadtmauer et al., 2001, 2002; Yarali et al., 2002; Fedorcsak et al., 2003; Kjotrod et al., 2004).

Based on these considerations, our study protocol was designed to evaluate the effects of metformin administration in a subgroup of PCOS women with insulin resistance and thus at high risk of multifollicular development during gonadotrophin treatment.

In the present study, the low-dose step-up protocol was a useful regimen for inducing a high rate of mono-ovulatory cycles in this population at significant risk for ovarian hyper-stimulation, and its association with the subsequent IUI/II was an effective treatment for infertile PCOS patients.

Our data demonstrate that the pre-treatment and co-administration of metformin in insulin-resistant PCOS patients treated with hpFSH induces a more physiological growth of follicles and a reduction in multifollicular development. In fact, a lower number of dominant follicles and E2 levels per dominant follicle on the day of HCG administration was observed.

An increased duration of the COS and the need for more gonadotrophin vials in PCOS patients who received metformin was detected in the current study. This result could be considered surprising in relation to previous data (De Leo et al., 1999; Yarali et al., 2002); however, it may also be further evidence of a normalization of ovarian response to exogenous gonadotrophins (Rice et al., 2005). Specifically, we suggest that in the placebo-treated PCOS subjects there is an insulin-promoted LH activity and that this promotes FSH sensitivity. Therefore, metformin treatment could reduce the degree of LH-promoted follicular activity and this should result in both fewer follicles per cycle and longer stimulation. Unfortunately, no assay of serum LH and androgen levels was performed after treatment in order to test this hypothesis.

De Leo et al. (1999) first studied the efficacy of metformin co-administration in 20 PCOS women undergoing ovarian stimulation with uFSH. In this study (De Leo et al., 1999) the gonadotrophin treatment consisted of a personalized protocol, the metformin was administered as pre-treatment and as co-administration with gonadotrophin, but was stopped on the day of HCG administration. An important confounding factor makes the data of the De Leo’s study difficult to interpret: data for 50% of cycles performed with metformin plus gonadotrophin co-administration were obtained from the same patients of the control group previously treated with gonadotrophin for the previous two cycles.

More recently, Yarali et al. (2002) in a prospective placebo-controlled randomized trial showed that 6 weeks of 1700 mg daily metformin administration had no significant effects on insulin resistance and ovarian response in obese clomiphene citrate-resistant PCOS patients treated with rFSH in a low-dose step-up protocol. A trend was observed towards lower duration of stimulation, lower E2 levels on the day of HCG, and smaller total number of vials used after metformin treatment, but the sample size was too small to detect significant differences between groups.

The discrepancies between our data and those obtained by Yarali et al. (2002) can be explained by differences in the sample population and in treatments used. First, in our series we studied non-obese insulin-resistant PCOS women, whereas in Yarali’s study obese PCOS patients and PCOS patients not selected for insulin resistance were enrolled. Obesity (and weight loss) is an important metabolic and confounding clinical factor in reproductive biology. In addition, there are contrasting data regarding the effectiveness of metformin in obese patients with PCOS (Maciel et al., 2004). For these reasons, we selected only non-obese (i.e. normal-weight and overweight) women who did not intend to follow a diet and/or a physical activity programme. Second, our PCOS patients were treated with metformin for a period of 3 months before gonadotrophin administration, whereas in Yarali’s study the metformin pre-treatment was for only 6 weeks, probably too short to exert a significant action on insulin resistance in obese patients. Finally, different gonadotrophins were used in the two studies (rFSH versus hpFSH), probably with different ovarian responses.

In a retrospective study on infertile PCOS women undergoing IVF, Stadtmauer et al. (2001) observed that 1500 mg daily metformin treatment prior to and during leuprolide acetate administration followed by COS with rFSH decreased the total number of follicles without changing the number of dominant follicles, increased the mean number of oocytes retrieved and the embryos cleaved, and the fertilization and pregnancy rates. In addition, metformin administration was shown to reduce the duration of the ‘coasting’ and the E2 concentrations, improving the clinical pregnancy rate (Stadtmauer et al., 2002). Fedorcsak et al. (2003) conducted a prospective, randomized, open-label study on the effects of metformin administration in a small sample of insulin-resistant obese PCOS women undergoing IVF/ICSI. Co-administration of metformin, at doses of 1500 mg daily for 3 weeks before treatment, increased the number of oocytes collected, but did not alter gonadotrophin requirements (Fedorcsak et al., 2003). More recently, in a prospective, randomized, double-blind, controlled trial, Kjotrod et al. (2004) have shown that pre-treatment with 1000 mg daily metformin did not facilitate ovarian stimulation and did not improve any reproductive outcomes in infertile PCOS patients. Unfortunately, the down-regulation caused by the GnRH analogue and the use of rFSH can be considered confounding factors in all these IVF studies (Stadtmauer et al., 2002; Fedorcsak et al., 2003; Kjotrod et al., 2004).

Although the aim of the present study was not to elucidate the mechanism by which metformin exerts its beneficial action, we can hypothesize that metformin acts on the regulation of ovarian response to exogenous gonadotrophins, improving insulin-resistance. An improvement in serum testosterone and insulin levels in follicular fluid has been observed after metformin treatment (Stadtmauer et al., 2002). According to this view, the decrease in the androgen and insulin levels locally in the ovary could be important factors leading to more normal folliculogenesis, homogeneous development of follicles and atresia of the small cohort of follicles. This is consistent with a direct effect of metformin on the androgen-producing thecal cells. Specifically, it is possible that metformin, decreasing
insulin levels, reduces the activity of ovarian cytochrome P450c-17α, an enzyme hyperexpressed and hyperstimulated by insulin in PCOS women, and responsible for intraovarian and plasma production of androgens (Nestler and Jakubowicz, 1996). Experimental data (la Marca et al., 1999; Koivunen et al., 2001) have shown that in PCOS women metformin administration leads to a reduction in serum 17OH-progesterone levels, a marker of cytochrome P450c-17α hyperactivity, after an HCG challenge test. In addition, several other mechanisms have been described to explain the beneficial actions of metformin on the ovary, such as an inhibition of aromatase activity in granulosa cells or an increase in the insulin-like growth factor binding protein-1 levels (la Marca et al., 2000; Vrbikova et al., 2001; Mansfield et al., 2003; Pawelczyk et al., 2004).

In conclusion, our results demonstrate that metformin pre-treatment and co-administration with hFSH in a low-dose step-up protocol can be useful because it increases mono-ovulation in appropriately selected normal- and overweight insulin-resistant patients with PCOS. Well-designed trials on a wider sample of infertile PCOS patients are necessary to detect an effect, if any, of metformin administration on other reproductive end-points.

References


Metformin and gonadotrophins in PCOS women


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