Does metformin modify ovarian responsiveness during exogenous FSH ovulation induction in normogonadotrophic anovulation? A placebo-controlled double-blind assessment

Evert J P van Santbrink, Femke P Hohmann, Marinus J C Eijkemans, Joop S E Laven and Bart C J M Fauser

Division of Reproductive Medicine, Department of Obstetrics and Gynaecology and Centre for Clinical Decision Sciences, Department of Public Health, Erasmus Medical Centre, Rotterdam, The Netherlands and Department of Reproductive Medicine, University Medical Centre, Utrecht, The Netherlands

(Correspondence should be addressed to E J P van Santbrink, Department of Obstetrics and Gynaecology, Erasmus Medical Centre, Dr Molewaterplein 40, 3015 GD, Rotterdam, The Netherlands; Email: e.vansantbrink@erasmusmc.nl)

Abstract

**Objective**: To assess whether the addition of metformin to gonadotrophin ovulation induction in insulin-resistant, normogonadotrophic, anovulatory women alters ovarian responsiveness to exogenous FSH.

**Design**: Placebo-controlled double-blind assessment in an academic hospital.

**Results**: After a progestagen withdrawal bleeding, patients were randomised for either metformin (n = 11) or placebo (n = 9) treatment. In cases of absent ovulation, exogenous FSH was subsequently administered to induce ovulation. Only during metformin treatment did body mass index and androgen (androstenedione and testosterone) levels decrease, whereas FSH and LH levels increased significantly. In the metformin group, a single patient ovulated before the initiation of exogenous FSH. Significantly more monofollicular cycles and lower preovulatory oestradiol concentrations were observed in women receiving FSH with metformin compared with FSH alone.

**Conclusions**: Metformin co-treatment in a group of insulin-resistant, normogonadotrophic, anovulatory patients resulted in normalization of the endocrine profile and facilitated monofollicular development during the FSH induction of ovulation.

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Introduction

One of the major problems associated with gonadotrophin induction of ovulation in patients with normogonadotrophic anovulation is multifollicular development, resulting in multiple pregnancy and ovarian hyperstimulation syndrome (1, 2). Surpassing the follicle-stimulating hormone (FSH) threshold for inducing follicular growth for too long may result in multifollicular development (3, 4). In order to reduce the above-mentioned complications and improve the effectiveness of treatment, new approaches have been suggested, such as patient selection on the basis of initial screening characteristics (5, 6) or alternative dose regimens for FSH ovulation induction (7). Also new compounds such as aromatase inhibitors (8) or insulin sensitisers (9) are clinically applied in an attempt to improve treatment outcome.

In normogonadotrophic anovulation (World Health Organisation (WHO) classification group II) including polycystic ovary syndrome (PCOS), hyperandrogenism and insulin resistance are prominent features (10, 11). Although the role of hyperinsulinaemia in PCOS has not yet been entirely elucidated, it is suggested that high insulin concentrations at the ovarian level increase androgen production (12, 13), increase oestradiol (E2) synthesis by granulosa cells (14, 15) and may cause arrested early antral follicle development (16). Hyperandrogenism may be induced by increased luteinizing hormone (LH) serum concentrations stimulating theca cell androgen biosynthesis (13), by inducing increased sensitivity of theca cells for stimulation by LH (17) or by dysregulating the FSH-dependent aromatase activity in granulosa cells (18, 19). Development of clinical hyperandrogenism may also be enhanced by hyperinsulinaemia by lowering sex hormone-binding globulin (SHBG) production in the liver (20).

It is postulated that the use of insulin sensitisers, such as metformin, in normogonadotrophic anovulation may improve the endocrine milieu by decreasing both insulin resistance and hyperandrogenism (21). These changes may restore menstrual cyclicity and spontaneous ovulation (22–24), or may render these women more responsive to ovulation-inducing drugs such as clomiphene citrate (21) or exogenous FSH (25, 26). Ovulatory response to clomiphene citrate is...
enhanced by the addition of metformin in clomiphene-resistant and obese PCOS patients (21, 27). In ovulation induction, the degree of hyperinsulinaemia is proposed to be the best estimation of requirement for (exogenous) FSH (28). Furthermore, it is suggested that the addition of metformin to FSH ovulation induction modulates ovarian response resulting in more monofollicular cycles (25).

These findings suggest that follicular sensitivity for FSH is enhanced by metformin. The aim of the current study is to explore the potential additional effect of metformin on ovarian responsiveness during gonadotrophin induction of ovulation.

Materials and methods

Subjects and study protocol

Subjects The local Ethics Review Committee approved this study. Between July 1999 and June 2001, all patients attending the outpatient clinic of the Erasmus Medical Centre with an amenorrhea or an oligomenorrhea of at least 56 days fulfilling the inclusion criteria as outlined below were approached to volunteer for participation in this study. After screening, 20 participants were selected. Written informed consent was obtained from each participant.

Inclusion criteria were women seeking pregnancy with: (i) age at time of screening between 18 and 37 years; (ii) normal serum E2 and FSH concentrations (as published previously (7)); (iii) severe oligomenorrhea (interval between bleeding > 56 days) or amenorrhea (interval between bleeding > 6 months); (iv) insulin resistance (defined as a fasting glucose–insulin ratio < 4.5 mg/100 ml U (29)); (v) normal serum prolactin and thyroxine levels; (vi) no signs of liver or kidney insufficiency and heart or vascular disease; (vii) failure to ovulate (clomiphene-resistant anovulation) or conceive (clomiphene failure) during clomiphene citrate treatment. Patients with overt diabetes mellitus were excluded.

Study design This was a randomised, double-blind, placebo-controlled study comparing FSH gonadotrophin induction of ovulation with or without metformin. All participants were randomised for metformin or placebo using sealed and numbered envelopes by the pharmacy of the Erasmus Medical Centre. Initial screening was performed within 2 months before inclusion and included a full history (age, menstrual cycle length), laboratory (FSH, LH, E2, androstenedione (AD), testosterone, SHBG, insulin, glucose, prolactin, thyroid-stimulating hormone, liver and kidney function) and ultrasound assessment. Ultrasound examinations were performed by a single observer (E P H) using a 6.5 MHz transvaginal transducer (as published before (7)).

First phase

At study day 1, patients visited the clinic after 10–12 h of fasting, at 0800 h for a glucose tolerance test (GTT), blood sampling (i.e. FSH, LH, E2, AD, testosterone, SHBG), length and weight measuring, blood pressure test, a urine pregnancy test and a transvaginal ultrasound (TVS). In cases of a negative pregnancy test, patients started the same day with progestins (Duprost Solvay Pharma, Weesp, The Netherlands; 10 mg, 2 × 1 tablet) for 7 days. Patients returned at day 8 for blood sampling and a TVS (Fig. 1). On this day patients were randomised for the two different therapies (metformin (Merck Nederland bv, Amsterdam, The Netherlands) or placebo). On the same day patients started with either metformin (2 × 1 tablet of 850 mg daily) or placebo (2 × 1 tablet daily).

Patients returned on day 11–12 and were subsequently monitored (TVS and blood sampling) every 3–4 days until ovarian response (Fig. 1). All visits were in the morning between 0900 and 1100 h. Ovarian response was defined as one or more follicles with a mean diameter of at least 10 mm. In cases of ovarian response and follicular growth, patients were subsequently monitored every 1–2 days until ovulation. One or two days after the day of ovarian response patients returned for a GTT at 0800 h, and for weight measuring and blood pressure testing. No FSH was administered to patients who were ovulatory with metformin or placebo. One week after ovulation patients returned for a TVS and blood sampling for progesterone. In cases where menstruation did not occur 14 days after ovulation, a urine pregnancy test was performed. In cases of a positive pregnancy test, a TVS was performed after 7 to 8 weeks (to determine the viability of the pregnancy) and after 12 weeks amenorrhea (to determine whether the pregnancy was ongoing). In cases of an absent ovarian response, patients continued their daily medication and monitoring every 3–4 days until day 36.

Second phase

In all patients who did not show an ovarian response within 35 days, FSH ovulation induction was started. At day 36, patients returned at 0800 h, after 10–12 h of fasting, for a GTT, weight measuring, blood pressure test, blood sampling and a TVS. Patients were instructed by the clinical research nurse for subcutaneous self-injections. Patients continued to use metformin or placebo therapy. From day 36 onwards, patients were monitored (TVS and blood sampling) every 3–4 days until an ovarian response. On the same day patients started with recombinant FSH (recFSH) stimulation according to a low-dose step-up regimen (30). The medication used was recFSH (Gonal-F; Serono Benelux BV, The Hague, The Netherlands), applying a starting dose of 50 IU recFSH s.c. daily in the morning between 0900 and 1200 h (fixed time for each patient). If no response was observed within 7 days of stimulation, the first dose of
recFSH was increased by 25 IU/day. A further dose increase of 37.5 IU/day was performed weekly if no response was detected. The maximum dosage was 225 IU recFSH/day. In the case of no response at maximum dosage the study was ended for this particular subject.

As soon as an ovarian response was documented, the patient was monitored every 1–2 days and continued on the same dosage of recFSH until the largest follicle reached a mean diameter of at least 18 mm. One or 2 days after response, the patient returned at 0800 h, after 10–12 h of fasting, for a GTT, weight measuring and blood pressure check. As the largest follicle reached this diameter of 18 mm or more, a dose of 5000 IU human chorionic gonadotrophin (hCG; Profasi; Serono Benelux BV) s.c. was administered at 1100 h to induce ovulation. Patients were advised to have intercourse approximately 36 h after the injection. In cases of more than three follicles with a diameter of ≥15 mm, no hCG was administered and the use of contraceptives was advised. Two days after the administration of hCG, patients returned for TVS to be examined for indirect signs of ovulation on ultrasound (free fluid, disappearance or decrease of size or changed shape of the dominant follicle) and blood sampling. The day after ovulation, the study medication (metformin/placebo) was stopped. The date of the next menstruation was recorded. If menstruation had not yet started 14 days after ovulation, a urine pregnancy test was performed.

**Assay methods**

The GTT was performed after 8–12 h of fasting. It started at 0800 h with a fasting blood withdrawal (glucose and insulin) followed by an oral intake of 75 g glucose. Blood withdrawal followed at 30, 60, 90 and 120 min thereafter (29). The method of blood withdrawal, the assays used and the intra- and inter-assay coefficients of variation valid for this study have all been described previously (31).

**Data analysis**

Before the initiation of the study, power calculations were performed to determine the required number of patients for the detection of significant differences in the primary study endpoints, i.e. duration of the stimulation phase and total amount of recFSH used to reach ovulation. Randomisation was performed by the pharmacy of the Erasmus Medical Center. The patient and the doctor were unaware of the randomisation and medication during the entire study. In cases of response and ovulation before starting FSH ovulation induction, the duration of stimulation and ampoules of FSH needed are defined as zero. Based on a median duration of FSH stimulation of 17 days in cases of co-treatment with placebo (7) and 14 days in cases of metformin (27), the difference was calculated to be apparent with at least 22 patients. Values given are means ± S.D. unless stated otherwise. Analysis of the data was performed using the SPSS software package SPSS version 10.1 (SPSS Inc., Chicago, IL, USA). The P values given are two-sided, and 0.05 was considered the limit of statistical significance. Differences between patient groups were tested using the Student’s t-test or Chi-square test for paired data on an interval and nominal scale respectively. A biochemical pregnancy is defined as a positive urine pregnancy test; an ongoing
pregnancy is defined as positive heart action on ultrasound after 12 weeks of pregnancy.

Results

Twenty WHO II anovulatory patients were included in the study of which nine patients were randomised for placebo and eleven for metformin treatment. Initial patient characteristics are shown in Table 1. At randomisation, patient characteristics did not significantly differ between groups. All patients were randomised after a progestagen-induced withdrawal bleeding. Patient characteristics after metformin/placebo treatment compared with initial characteristics are shown in Table 2. In both groups the insulin–glucose ratio did not change significantly, but androgen (both AD and testosterone) serum concentrations decreased significantly \( (*P = 0.03\) for AD and \( *P = 0.01\) for testosterone) in the metformin group only. Also body mass index (BMI = weight/height\(^2\) (kg/m\(^2\))) \( (*P = 0.00\) for AD and \( *P = 0.01\) for testosterone) and gonadotrophins \( (*P = 0.02\) for FSH and \( *P = 0.04\) for LH) decreased significantly in the metformin group but not in the placebo group. Two patients (both in the placebo group) presented with an ovarian cyst on the day on which FSH treatment should have started and were excluded from further evaluation. One patient was ovulatory on metformin alone (before FSH treatment). Seventeen patients (seven patients in the placebo and ten patients in the metformin group) received exogenous FSH. Ovulation was confirmed in all patients receiving hCG. Two patients in the metformin group presented with a mild ovarian hyper-response (without hospitalisation) and hCG was withheld. Differences between placebo and metformin treatment characteristics (Fig. 2) were assessed in terms of duration of stimulation (33 vs 15 days), total amount of FSH needed (3238 vs 950IU), preovulatory E\(_2\) serum levels (150 vs 75IU), preovulatory E\(_2\) serum levels (1203 vs 615 pmol/l) and monofollicular cycles defined as one follicle > 12 mm on the day of hCG (14 vs 75%). Only E\(_2\) serum concentration and monofollicular growth on the day of hCG were statistically significantly different (Fig. 3). In the metformin group, three pregnancies (one early miscarriage and two ongoing singleton pregnancies) occurred while in the placebo group none

Table 1 Initial characteristics of 20 normogonadotrophic, insulin-resistant anovulatory patients randomised for receiving either metformin or placebo. Median and range and statistically significant differences between groups are given.

<table>
<thead>
<tr>
<th>Placebo</th>
<th>Metformin</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>Age (years)</td>
<td>28 (24–34)</td>
<td>28 (22–32)</td>
</tr>
<tr>
<td>Primary infertility (%)</td>
<td>78</td>
<td>36</td>
</tr>
<tr>
<td>Body mass index (kg/m(^2))</td>
<td>34 (27–44)</td>
<td>38 (28–51)</td>
</tr>
<tr>
<td>Amenorrhoea (%)</td>
<td>44</td>
<td>36</td>
</tr>
<tr>
<td>Laboratory tests</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>2.7 (2.0–5.7)</td>
<td>4.0 (1.4–12.2)</td>
</tr>
<tr>
<td>E(_2) (pmol/l)</td>
<td>205 (112–278)</td>
<td>220 (102–366)</td>
</tr>
<tr>
<td>Glucose/insulin ratio</td>
<td>0.19 (0.09–0.24)</td>
<td>0.16 (0.07–0.23)</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>2.0 (0.4–3.7)</td>
<td>1.6 (0.8–4.3)</td>
</tr>
<tr>
<td>AD (nmol/l)</td>
<td>14.3 (2.4–16.3)</td>
<td>10.0 (5.8–23.1)</td>
</tr>
<tr>
<td>FAI (testosterone (\times) 100/SHBG)</td>
<td>13.4 (2.3–33.6)</td>
<td>12.3 (3.8–35.6)</td>
</tr>
</tbody>
</table>

n.s., not significant. *Chi-square test. FAI, free androgen index.

Table 2 Patient characteristic compared before and after 5 weeks of treatment with either metformin or placebo in 20 normogonadotrophic insulin-resistant anovulatory patients. Means±s.d. are given.

<table>
<thead>
<tr>
<th>Placebo</th>
<th>Metformin</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>Body mass index (kg/m(^2))</td>
<td>34±5</td>
<td>34±5</td>
</tr>
<tr>
<td>Laboratory tests</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>2.4±1.5</td>
<td>4.2±1.5</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>5.8±4.4</td>
<td>5.3±3.2</td>
</tr>
<tr>
<td>E(_2) (pmol/l)</td>
<td>344±313</td>
<td>256±170</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>1.9±0.9</td>
<td>2.0±1.2</td>
</tr>
<tr>
<td>AD (nmol/l)</td>
<td>12.5±6.0</td>
<td>14.6±6.9</td>
</tr>
<tr>
<td>FAI (testosterone (\times) 100/SHBG)</td>
<td>11.7±10.3</td>
<td>15.3±10.4</td>
</tr>
<tr>
<td>Glucose/insulin ratio</td>
<td>0.2±0.1</td>
<td>0.2±0.1</td>
</tr>
</tbody>
</table>

* t-test.
occurred. Serious gastro-intestinal problems during the use of the study medication were not reported.

Discussion

The FSH threshold for initiation of gonadotrophin-independent follicle growth has been recognised as an important entity for many years (3, 4). The period of time during which the FSH threshold is surpassed (the FSH window) determines whether one or more follicles are selected for ongoing growth and ovulation. It is generally thought that in PCOS patients the FSH threshold is higher than in regularly menstruating women. More FSH is required to start follicle growth and this can be managed by enhancing the endogenous FSH production by clomiphene citrate or by giving exogenous FSH.

A crucial question is why the endogenous hormone feedback system is not responding to this relative shortage of FSH in PCOS patients. One reason might be that PCOS ovaries produce more E₂ and androgens that inhibit FSH release. It is suggested that hyperinsulinaemia plays a key role in this by stimulating cytokine P450c17 in theca cells (9, 20) and aromatase in granulosa cells (15, 18, 19) directly, independent of FSH. This results in enhanced production of testosterone, AD and E₂. The change in endocrine milieu may produce an over-reaction of negative feedback during early follicular growth resulting in an early FSH drop followed by follicle growth arrest and the well-known polycystic image of the ovaries on sonography.

Clomiphene citrate is the first choice of treatment for WHO II women. About 75% of all treated patients will respond with ovulation and about 45% conceive (31). BMI, hyperandrogenism (free androgen index), leptin and hyperinsulinaemia (fasting insulin and insulin-like growth factor binding protein-I) were determined to

Figure 2 Results of FSH ovulation induction using a low-dose step-up protocol in 17 normogonadotropic insulin-resistant anovulatory subfertile patients after randomization and co-treatment with metformin or placebo (n = 7). Differences are not statistically significant (NS).

Figure 3 Results of FSH ovulation induction using a low-dose step-up protocol in 17 normogonadotropic insulin-resistant anovulatory subfertile patients after randomization and co-treatment with metformin or placebo (n = 7).
be the strongest predictors for remaining anovulatory during clomiphene citrate treatment (32, 33, 34). Although hyperinsulinaemia seems more profound in obese women (35), lean PCOS women (mean BMI 22 kg/m²) are also shown to have a significant reduction in insulin response to glucose load and androgen serum concentrations (20, 36). The addition of metformin to clomiphene in clomiphene-resistant anovulation is recognised as a valuable treatment option before starting with exogenous gonadotrophins (21, 27).

Less is known about the addition of metformin during gonadotrophin induction of ovulation. Two limited studies have described co-administration of metformin with gonadotrophin induction of ovulation in normogonadotrophic anovulatory patients (25, 26). The first study (25) included PCOS patients with clomiphene-resistant anovulation or failure to conceive and they were randomized for treatment A or B. The insulin resistance status of the included patients is unknown. Treatment A consisted of two conventional urinary FSH ovulation induction step-up cycles followed by one cycle with the addition of metformin (n = 10), treatment B offered metformin pretreatment followed by a conventional FSH ovulation induction step-up cycle combined with metformin (n = 10). It was concluded (25) that androgens and E₂ serum concentrations were significantly lower in cycles with metformin co-treatment and significantly more cycles with monofollicular growth occurred. The second study (26) selected 32 PCOS patients with normal glucose tolerance and clomiphene-resistant anovulation. These patients were randomised for metformin or placebo and a 6-week pretreatment period followed. All anovulatory patients were treated with FSH using a low-dose step-up protocol (30). No significant differences were determined in ovarian response after metformin or placebo treatment between these groups although significantly lower serum androgens (free testosterone) were described after metformin treatment.

In our study population we included insulin-resistant patients only, because this subgroup was expected to react favourably to metformin treatment. Although metformin treatment did not result in a (significant) decrease of insulin resistance, it did cause a significant decrease in androgen serum concentrations and improved the endogenous gonadotrophin–oestrogen balance, as was demonstrated in the former studies (25, 26). This endocrine shift might explain the following benefits during the FSH treatment phase: although not significant, considerably less FSH and a shorter treatment period were required to grow a dominant follicle. The dosage of FSH on the day on which a dominant follicle could be recognised by TVS was lower, but not significantly so, in the metformin group. In addition, on the day on which hCG was given significantly lower E₂ serum levels and significantly more cycles with monofollicular (not more than one follicle > 12 mm) growth were established. These results are comparable with former published studies (25).

In conclusion, these results suggest that metformin may improve the endocrine profile (by decreasing hyperandrogenaemia) and, in that way, facilitates monofollicular development during gonadotrophin ovulation induction in WHO II patients. This effect may be more pronounced in patients with insulin resistance. As this study was made with only a small population, larger studies are required to further evaluate the effects of metformin co-treatment in normogonadotropic FSH ovulation induction, especially on conception and birth rates.

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