Use of metformin before and during assisted reproductive technology in non-obese young infertile women with polycystic ovary syndrome: a prospective, randomized, double-blind, multi-centre study

S.B. Kjøtrød1,*, S.M. Carlsen2,3, P.E. Rasmussen4, T. Holst-Larsen5, J. Mellembakken6, A. Thurin-Kjellberg7, K. HaapaniemiKouru8, L. Morin-Papunen9, P. Humaidan10, A. Sunde1, and V. von Düring1

1Department of Gynaecology and Obstetrics, Fertility Clinic, Trondheim University Hospital, 7030 Trondheim, Norway 2Unit for Applied Clinical Research, Department of Cancer Research and Molecular Medicine, Norwegian University for Science and Technology, Olav Kyres gate 9, 7489 Trondheim, Norway 3Department of Endocrinology, St Olavs Hospital, Trondheim University Hospital, Olav Kyres gate 17, 7030 Trondheim, Norway 4The Fertility Clinic, Odense University Hospital, DK 5000 Odense, Denmark 5Haugesund Fertility Centre, 5504 Haugesund, Norway 6Department of Gynaecology, Section of Reproductive Medicine, Oslo University Hospital, 0424 Oslo, Norway 7Sahlgrenska Academy, Sahlgrenska University Hospital, Reproductive Medicine, 41345 Gothenburg, Sweden 8Fertility Unit, Karolinska University Hospital, 141 86 Stockholm, Sweden 9Department of Obstetrics and Gynaecology, University Hospital of Oulu, University of Oulu, 90029 OYS Oulu, Finland 10The Fertility Clinic, Skive Regional Hospital, 7800 Skive, Denmark

*Correspondence address. Tel: +47-72573812; E-mail: sigrun.kjotrod@stolav.no

Submitted on October 6, 2010; resubmitted on March 4, 2011; accepted on March 21, 2011

BACKGROUND: To study the effect of metformin before and during assisted reproductive technology (ART) on the clinical pregnancy rate (CPR) in non-obese women with polycystic ovary syndrome (PCOS).

METHODS: A multi-centre, prospective, randomized, double-blind study was conducted in eight IVF clinics in four Nordic countries. We enrolled 150 PCOS women with a body mass index <28 kg/m², and treated them with 2000 mg/day metformin or identical placebo tablets for ≥12 weeks prior to and during long protocol IVF or ICSI and until the day of pregnancy testing. The primary outcome measure was CPR. Secondary outcome measures included spontaneous pregnancy rates during the pretreatment period, and the live birth rate (LBR).

RESULTS: Among IVF treated women (n = 112), biochemical pregnancy rates were identical in both groups (42.9%), and there were no significant differences in the metformin versus the placebo group in CPR [39.3 versus 30.4%; 95% confidence interval (CI): −8.6 to 26.5]. The LBR was 37.5 versus 28.6% (95% CI: −8.4 to 26.3). However, prior to IVF there were 15 (20.3%) spontaneous pregnancies in the metformin group and eight (10.7%) in the placebo group (95% CI: −1.9 to 21.1; P = 0.1047). According to intention to treat analyses (n = 149); significantly higher overall CPR were observed in the metformin versus placebo group (50.0 versus 33.3%; 95% CI: −1.1 to 32.3; P = 0.0391). LBR was also significantly higher with use of metformin versus placebo (48.6 versus 32.0; 95% CI: 1.1 to 32.2; P = 0.0383). No major unexpected safety issues or multiple births were reported. More gastrointestinal side effects occurred in the metformin group (41 versus 12%; 95% CI: 0.15 to 0.42; P < 0.001).

CONCLUSIONS: Metformin treatment for 12 weeks before and during IVF or ICSI in non-obese women with PCOS significantly increases pregnancy and LBRs compared with placebo. However, there was no effect on the outcome of ART per se.

Trial registration: ClinicalTrials.gov Identifier: NCT00159575.

Key words: polycystic ovarian syndrome / metformin / insulin sensitizers / clinical pregnancy rate / live birth rate
Introduction

International estimates indicate that ~9% of couples are infertile (Boivin et al., 2007). Around one-third of women who attend infertility clinics have anovulatory infertility, and polycystic ovary syndrome (PCOS) is the cause of 90% of such cases (Balen and Michelmore, 2002; Balen and Rutherford, 2007). PCOS is characterized by hyperandrogenism, oligo- or anovulation and polycystic ovaries on ultrasound (The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). Insulin resistance and compensatory hyperinsulinemia are also common features of PCOS, and appear to play a pathophysiological role in hyperandrogenism (Dunaif et al., 1989; Tsilchorozidou et al., 2004). However, patients with PCOS comprise a heterogeneous population (Balen et al., 1995). This has caused difficulty in establishing the aetiological causes (Diamanti-Kandarakis et al., 2006; Franks et al., 2006, diagnostic criteria (Azizz, 2005, 2006; Franks et al., 2006) and treatment algorithms for PCOS (Balen and Rutherford, 2007). The severity of PCOS is influenced by environmental factors, such as nutrition and lifestyle (Holte et al., 1995; Norman et al., 2004; Franks et al., 2006). Obesity has an additive negative impact on hyperandrogenism and anovulation, and substantially increases insulin resistance (Ciampelli et al., 1999; Legro et al., 2004). Dietary and lifestyle modifications should be considered mandatory for all patients with PCOS prior to specific fertility treatment; indeed, weight loss of only a few kilograms may induce ovulation (Norman et al., 2004; Balen and Rutherford, 2007).

Since the first paper (Velazquez et al., 1994), numerous studies on metformin treatment and PCOS have been performed, and yet very few clear conclusions can be drawn. However, according to a recent Cochrane analysis (Tang et al., 2010) metformin is still of benefit in improving clinical pregnancy and ovulation rates although no clear evidence exists that metformin improves live birth rates (LBRS) whether it is used alone, in combination with clomiphene or compared with clomiphene.

In addition, suppression of insulin levels in women with PCOS who are undergoing IVF or ICSI may potentially ameliorate the adverse effects of ovarian stimulation and improve treatment outcomes (Kjotrod et al., 2004; Tang et al., 2006). In our previous pilot study (Kjotrod et al., 2004), randomization was stratified according to whether the woman was obese, defined as a BMI ≥ 28 kg/m² (n = 40), or non-obese, defined as BMI <28 kg/m² (n = 33). Pretreatment with metformin did not improve stimulation or clinical outcome. However, among the non-obese women metformin tended to improve clinical pregnancy rates (CPRs) as there were eight clinical pregnancies following IVF among 14 metformin-treated women, versus three clinical pregnancies among 13 women in the placebo group. A recent systematic review of adjunctive metformin treatment before and during assisted reproductive technology (ART) among women with PCOS showed that the risk of ovarian hyperstimulation syndrome (OHSS) was reduced with metformin (Tso et al., 2009). However, there was a lack of evidence to conclude that metformin before and/or during ART increased pregnancy or LBRS (Tso et al., 2009). Further large studies are needed to definitely answer whether the use of metformin in PCOS women undergoing ART improves live birth and pregnancy rates and based on the results from our previous pilot study we decided to focus on the non-obese group.

Materials and Methods

Stud y design and objective

A prospective, randomized, double-blind, study was performed in four Nordic countries to evaluate whether 12 weeks of metformin before and during IVF/ICSI increases the CPR compared with placebo in non-obese women with PCOS scheduled for IVF/ICSI. The study was planned as a multi-centre study (EUDRACTnr-2004-001 124-20). Originally a centre in Leeds, UK was planned to be included; but we did not get the approval for study medications by the medical authorities in UK. One private IVF clinic in Helsinki, one in Oslo and one in Copenhagen were also supposed to participate, but dropped out very early due to recruitment problems. To compensate for this, The Oslo University Hospital was recruited into the study during the last 1.5 years of the inclusion period.

The study was conducted in full accordance with the Edinburgh (2000) amendment of the Declaration of Helsinki 1964, the International Conference on Harmonisation guidelines for Good Clinical Practice, and with local laws and regulations for clinical research. Written informed consent was obtained from all patients prior to enrolment.

Participants

Women diagnosed with PCOS, aged <38 years and with a BMI of <28 kg/m², who were scheduled to undergo a first or second cycle of IVF or ICSI were recruited from eight infertility treatment centres in Denmark, Finland, Norway and Sweden. This limit for BMI was chosen to have the same BMI cut-off as we used in our previous study. Moreover, prior studies indicate increased insulin resistance at a BMI as low as 27 kg/m² in PCOS patients (Gennarelli et al., 2000). The included patients had been trying, unsuccessfully, to conceive for at least 1 year and have a diagnosis of PCOS based on fulfilling at least two of the following three criteria: oligomenorrhoea/amenorrhoea, clinical or biochemical hyperandrogenism and/or polycystic ovaries on ultrasound (The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004).

Patients were excluded if they were contraindicated for a starting dose of 112.5 IU recombinant human follicle-stimulating hormone (r-hFSH), or had a basal serum FSH level of >10 IU/l. Patients with liver or kidney disease, diabetes mellitus (or fasting plasma glucose ≥ 7.0 mmol/l), alcoholism or drug abuse were excluded. Patients with hyperprolactinaemia (serum prolactin >700 mIE/l), abnormal thyroid function tests, congenital adrenal hyperplasia, androgen-secreting tumours or Cushing’s syndrome were also excluded. Finally, patients who had received oral steroid hormones, cinetidine, anticoagulants, erythromycin or other macrolides were also excluded. A 1-month washout period was required for women who had previously received metformin.

Interventions

 Patients underwent a screening visit to establish suitability for inclusion in the study. Advice (both oral and written) on diet and lifestyle was given and folic acid supplementation (≥ 1.2 mg per day) was initiated. Following a negative urinary pregnancy test, patients were randomized 1:1 to receive metformin (metformin hydrochloride, immediate release formulae provided by Weifa AS) or matching placebo tablets for ≥12 weeks prior to controlled ovarian stimulation (COS), throughout IVF/ICSI, and until the day of pregnancy testing. The dose of metformin was gradually increased from 500 to 2000 mg per day during the first 2 weeks of treatment. To limit the incidence of gastrointestinal side effects, doses were taken with meals, and dose reductions were permitted if troublesome gastrointestinal side effects occurred.
Following a spontaneous menstrual period or a gestagen-induced shedding of the endometrium, pituitary down-regulation (nafarelin, 400 µg administered twice daily intranasally) was initiated (on cycle Day 20) and continued for at least 14 days. After a further negative urinary pregnancy test, COS using r-hFSH (GONAL-f® at 112.5 IU/day) was administered. Ultrasound monitoring was performed on cycle Day 8 and the dose of r-hFSH was tailored accordingly. Patients were monitored using regular ultrasound scans and coating was permitted if considered necessary to prevent OHSS. A single dose of human chorionic gonadotrophin (hCG, either Ovitrelle®, 250 µg/0.5 ml, or Pregnyl®, 5000 or 10 000 IU) was injected to induce final follicular maturation when at least one follicle reached >17 mm in diameter. The formulation and dose of hCG was selected by the treating physician.

IVF, ICSI or both procedures were performed in accordance with the routine practice at the individual treatment centre. Preferably one, or a maximum of two, Day-2 or Day-3 embryos were transferred in utero. All patients received 14 days of luteal phase support; the type and dosage were selected by the treating physician. A serum pregnancy test was performed on Days 13–15 after embryo transfer; women with a positive test underwent an ultrasound in Week 7 of pregnancy.

The patient height and weight were measured at the screening visit. Weight was also recorded on the day of oocyte collection. Blood samples were drawn at the screening visit, at cycle Day 20 or within 5 days prior to initiation of pituitary down-regulation, immediately prior to hCG injection, on the day of oocyte collection, on Day 14 after embryo transfer and within 1 week after a spontaneous positive pregnancy test. Basal levels of the following analytes were quantified by a central laboratory: fasting plasma glucose, insulin, androstenedione, dehydroepiandrosterone, testosterone, sex-hormone-binding globulin, serum hCG, estradiol, FSH, prolactin, free thyroxine and thyroid-stimulating hormone.

Treatment adherence was evaluated by counting unused study medication and calculating the number of tablets taken as a proportion of the number of tablets prescribed according to protocol.

Randomization
The trial clinician and a study nurse at each site enrolled the patients. Randomization was performed in blocks of four by the hospital pharmacy using a computer-generated list. Identical blister packs containing metformin or placebo tablets (of the same appearance, smell and taste) were made, and each centre was assigned 20 identical packs. The study medicine was delivered to the patients either by the hospital pharmacy or by a third, independent person who was not involved in the study. The patient screened and randomized as number one in the centre received package number one, randomization patient number two received package number two, etc. Randomization codes remained blinded until the database lock had taken place. All study site personnel, the sponsor and the monitor operationally involved in the monitoring or conduct of the study were blinded to the study drug codes.

Study populations
The following populations were defined: the intention-to-treat (ITT) population included all women randomized to treatment; the spontaneous pregnancy (SP) population included all women with a positive urinary pregnancy test prior to COS; the IVF/ICSI (ART) population included all women who started gonadotrophin stimulation; and the safety population included all women who received at least one dose of metformin or placebo.

Outcomes
The primary efficacy end-point was the CPR, defined by ultrasound evidence of an intrauterine gestational sac (with a beating heart) at Week 7 in the ITT population.

Secondary efficacy end-points included the SP rate during the pretreatment period (SP population); biochemical pregnancy (defined by a positive serum hCG test on Day 14 after embryo transfer) and CPRs following IVF/ICSI in the ART population and LBR (in the ITT, ART and SP populations).

Safety variables included the incidence of adverse events (AEs), OHSS and coating. According to prespecified criteria, OHSS and coating were not considered AEs.

Sample size and statistical methods
In our previous pilot study (Kjotrod et al., 2004) an increased pregnancy rate of almost 100% was observed among metformin-relative to placebo-treated non-obese women with PCOS. A CPR of ~0.35 was expected in the placebo group. With a study power of 0.80 and a significance level of 0.05, it was estimated that 120 patients were needed in each group to demonstrate a 50% increase in the CPR in the metformin group. To allow for dropouts, the planned sample size was 300 (150 in each group). The primary end-point was to be analysed according to the ITT principle; ART analyses were performed when suitable.

Clinical and biochemical pregnancy rates, LBRs and the number of transferred embryos were analysed using Pearson’s χ² test. The number of oocytes collected, and the days and dose of gonadotrophin treatment required were analysed using a t-distribution. The fertilization rate, number of cleaved embryos at Day 2, frozen embryos and good quality embryos were analysed using a generalized linear model.

The following rules were applied when data were missing: in the analyses of pregnancy and fertilization outcomes, missing outcomes were imputed with a negative result or an answer of zero; if no oocytes were collected, the fertilization rate was regarded as missing.

Subgroup analyses of the clinical pregnancy and LBRs were undertaken in patients with a BMI categorized as either above or below 25 kg/m² using logistic regression analysis. SAS software (version 9.2) was used for all statistical analyses.

Changes in the conduct of the study or planned analyses
Recruitment was stopped after the randomization of 150 women, owing to slow patient recruitment and expiry of study medication. Clinical laboratory evaluations were performed only on the screening samples; monitoring changes in levels of serum analytes to evaluate safety was therefore not possible. Unblinding was performed prior to serum analyses; at that time, there were only eight pregnancies with an undetermined outcome, all were beyond 30 weeks of gestation.

Results
Study population
The total study period was ~5 years: the first patient was recruited in February 2005 and the last birth occurred in March 2010.

The flow of participants throughout the study is presented in Fig. 1. A total of 150 women were randomized to treatment (metformin group, n = 74; placebo group, n = 76). One patient in the placebo group withdrew her consent just after randomization, with the explicit instruction not to include any data obtained during the trial. The ITT...
population therefore comprised 149 women (metformin group, \(n = 74\); placebo group, \(n = 75\); Table I). Of these, 23 women achieved a pregnancy prior to gonadotrophin treatment (SP population) and 112 women proceeded to start gonadotrophin stimulation (ART population). In total, 71 patients underwent IVF (metformin, \(n = 40\); placebo, \(n = 31\)); 31 patients underwent ICSI (metformin, \(n = 11\); placebo, \(n = 20\)); 5 patients underwent both procedures (metformin, \(n = 3\); placebo, \(n = 2\)).

A total of 53 patients were withdrawn during the study period (Fig. 1). The most common reason for withdrawal was SP (\(n = 23\)). Baseline demographic and disease characteristics, including age, weight and BMI, were similar in the two treatment groups (Table II). The mean (standard deviation, SD) age of patients was 29.5 (3.6) years. The majority of patients had previously received unsuccessful clomiphene citrate (CC) treatment (\(n = 59\) in each treatment group). The mean (SD) number of previous cycles of CC was 3.7 (1.8) in the metformin group and 3.5 (1.9) in the placebo group.

The metformin group experienced significant reductions in mean weight and BMI between screening and the day of oocyte collection compared with the placebo group. The mean treatment difference in weight (kg) was \(-1.6\) [95% confidence interval (CI): \(-2.6\) to \(-0.68\); \(P = 0.001\)] and in BMI (kg/m\(^2\)) was \(-0.60\) (95% CI: \(-0.95\) to \(-0.25\); \(P = 0.0009\)). In the metformin group, the weight

![Figure 1](image-url) Study design showing patient numbers. IVF, in vitro fertilization; OHSS, ovarian hyperstimulation syndrome; PCOS, polycystic ovary syndrome.

<table>
<thead>
<tr>
<th>Table I Study populations.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
</tr>
<tr>
<td>Intention-to-treat (ITT)</td>
</tr>
<tr>
<td>SP</td>
</tr>
<tr>
<td>ART population</td>
</tr>
</tbody>
</table>

*One patient was found not to meet the required criteria for a diagnosis of PCOS.*
reduction (kg) was $-0.6$ (95% CI: $-1.3$ to $+0.13$), and in placebo group there was a weight increase $+1.1$ (95% CI: $+0.38$ to $1.79$).

**Primary efficacy end-point**

A total of 62 clinical pregnancies were reported in the ITT population: $37/74$ (50.0%) in the metformin group and $25/75$ (33.3%) in the placebo group. The CPR was significantly higher in the metformin versus the placebo group, with a difference of 16.7% (95% CI: 1.1 to 32.3; $P = 0.0391$; Table IVa).

**Secondary efficacy end-points**

**Fertilization outcomes**

There were no significant differences between the treatments groups for any of the fertilization outcomes assessed (including the number of oocytes collected, fertilization rate and number of embryos transferred; Table III).

**Pregnancy outcomes**

A total of 60 live births were reported in the ITT population: $36/74$ (48.6%) in the metformin group and $24/75$ (32.0%) in the placebo group; the LBR was significantly higher in the metformin group versus placebo: 16.7% (95% CI: 1.1 to 32.2; $P = 0.0383$; Table IVa).

In the metformin and placebo groups spontaneous pregnancies were reported in 15 of 74 (20.3%) and 8 of 75 (10.7%); the difference in the SP rate was 9.6% (95% CI: $-1.9$ to $21.1$; $P = 0.1047$; Table IVb). Similar biochemical pregnancy, clinical pregnancy and LBRs were observed in each treatment group following IVF or ICSI (the ART population; Table IVc).

In total, 10 miscarriages occurred in the ART population (metformin, $n = 3$; placebo, $n = 7$). Five were biochemical pregnancies (metformin, $n = 2$; placebo, $n = 3$) and the remaining five were miscarriages that occurred prior to 12 weeks of pregnancy (metformin, $n = 1$; placebo, $n = 4$). There were no miscarriages between 12 and 22 weeks of pregnancy. No miscarriages occurred in the SP population. One ectopic pregnancy and one vanishing twin pregnancy occurred in the ITT population (both in the placebo group). No multiple births were reported during the study.

**Gonadotrophin treatment**

There were no significant differences between treatments groups in the mean duration or total dose of r-hFSH required. The mean (SD) number of days of r-hFSH stimulation was 12.4 (2.9) days for the metformin group and 12.1 (3.3) days for the placebo group ($P = 0.6262$). The mean (SD) total dose (IU) of r-hFSH was 1553 (579) IU for the metformin group and 1532 (650) IU for the placebo group ($P = 0.8528$).

**Subgroup analyses**

A subgroup analysis of women with BMI $<25$ kg/m$^2$ ($n = 95$) versus 25–28 kg/m$^2$ ($n = 54$) showed no significant difference in treatment effect on the CPR [estimated odds ratio (95% CI); $1.92$ (0.827–4.454) versus $1.98$ (0.662–5.944); $P = 0.9631$] or LBR [1.92 (0.827–4.454) versus 2.05 (0.680–6.186); $P = 0.9254$].

---

**Table II** Baseline demographic and clinical characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Metformin ($n = 74$)</th>
<th>Placebo ($n = 75$)</th>
<th>Total ($N = 149$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD) age, years</td>
<td>29.6 (3.4)</td>
<td>29.5 (3.8)</td>
<td>29.5 (3.6)</td>
</tr>
<tr>
<td>Mean (SD) body weight, kg</td>
<td>67.7 (8.6)</td>
<td>65.9 (8.9)</td>
<td>66.8 (8.8)</td>
</tr>
<tr>
<td>Mean (SD) BMI, kg/m$^2$</td>
<td>24.0 (7.2)</td>
<td>23.6 (2.8)</td>
<td>23.8 (2.7)</td>
</tr>
<tr>
<td>Mean (SD) duration of infertility, years</td>
<td>2.6 (1.8)</td>
<td>2.8 (1.8)</td>
<td>2.7 (1.8)</td>
</tr>
<tr>
<td>Cause of infertility, n</td>
<td>PCOS only</td>
<td>45</td>
<td>44</td>
</tr>
<tr>
<td>Additional male factor</td>
<td>21</td>
<td>26</td>
<td>47</td>
</tr>
<tr>
<td>Additional tubal disease</td>
<td>6$^a$</td>
<td>6$^b$</td>
<td>12</td>
</tr>
<tr>
<td>Additional endometriosis</td>
<td>4$^d$</td>
<td>2$^d$</td>
<td>6$^d$</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>PCOS diagnosis, n</td>
<td>Oligomenorrhoea/amenorrhoea</td>
<td>72</td>
<td>71</td>
</tr>
<tr>
<td>Hyperandrogenism</td>
<td>39</td>
<td>46</td>
<td>85</td>
</tr>
<tr>
<td>Polycystic ovaries</td>
<td>71</td>
<td>75</td>
<td>146</td>
</tr>
</tbody>
</table>

BMI, body mass index; PCOS, polycystic ovary syndrome; SD, standard deviation.

$^a$ n = 38; $^b$ n = 40; $^c$ n = 78; $^d$ n = 36; $^e$ n = 48.

**Table III** Fertilization outcomes.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Metformin ($n = 56$) Mean (SD)</th>
<th>Placebo ($n = 56$) Mean (SD)</th>
<th>Effect size (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of oocytes collected</td>
<td>11.6 (6.1)</td>
<td>13.2 (7.2)</td>
<td>$-1.5$ ($-4.0$ to $0.97$)</td>
<td>0.2267</td>
</tr>
<tr>
<td>Fertilization rate$^a$</td>
<td>0.53 (0.28)</td>
<td>0.54 (0.25)</td>
<td>$0.01$ ($-0.01$ to $0.09$)</td>
<td>0.8186</td>
</tr>
<tr>
<td>Number of cleaved embryos at Day 2$^b$</td>
<td>6.0 (4.5)</td>
<td>7.6 (5.6)</td>
<td>0.79 (0.58 to 1.1)</td>
<td>0.1313</td>
</tr>
<tr>
<td>Number of transferred embryos$^c$</td>
<td>1.2 (0.39)</td>
<td>1.1 (0.28)</td>
<td>0.10 ($-0.03$ to $0.24$)</td>
<td>0.1359</td>
</tr>
<tr>
<td>Number of frozen embryos$^b$</td>
<td>2.5 (3.0)</td>
<td>2.7 (3.0)</td>
<td>0.93 (0.56 to 1.5)</td>
<td>0.7667</td>
</tr>
<tr>
<td>Number of good quality embryos$^b$</td>
<td>3.5 (3.2)</td>
<td>3.6 (3.0)</td>
<td>0.97 (0.70 to 1.3)</td>
<td>0.8553</td>
</tr>
</tbody>
</table>

CI, confidence interval; SD, standard deviation.

$^a$ Metformin, $n = 54$; placebo, $n = 53$.

$^b$ Effect size: Relative difference in mean (due to skewed data).

$^c$ Metformin, $n = 48$; placebo, $n = 46$. 

---
Safety outcomes
A total of 196 AEs were reported during the study. Women in the metformin group experienced more AEs than those receiving placebo [overall AEs (135 versus 61), moderate/severe AEs (49 versus 13) and study drug-related AEs (76 versus 16)]. The commonly reported study drug-related AEs were nausea (metformin group, 21 patients; placebo group, 4 patients; Table V) and mild headache (metformin group, 15 patients; placebo group, 9 patients).
A total of five women withdrew from the study because of an adverse drug reaction (metformin group, n = 2; placebo group, n = 3). Two patients (both in the metformin group) reported serious AEs: one had a kidney stone (considered unrelated to the study drug); the other achieved an SP but received an IVF treatment cycle following a false-negative urinary pregnancy test (considered possibly related to the study drug).

There were a total of 10 reported cases of OHSS (5 in each treatment group): of these, 5 required hospitalization (metformin, n = 2; placebo, n = 3; Table VI). A total of 20 patients were managed using coasting (metformin, n = 8; placebo, n = 12). Threatened OHSS led to cycle cancellation for four women: in order to avoid development of OHSS, one patient in the placebo group was not given an injection of hCG and three patients (metformin, n = 1; placebo, n = 2) did not undergo embryo transfer.

Treatment adherence
A high level of adherence to treatment was observed. The mean (SD) adherence for the combined ART and SP populations was 94.3% (13.6%) for the metformin group and 92.3% (16.8%) for the placebo group.

Discussion
The present prospective, multi-centre, placebo-controlled, double-blind study explored the effect on pregnancy outcomes of metformin for ≥12 weeks prior to, and during, IVF/ICSI (or until SP) in non-obese women with PCOS. In the ITT population, significantly higher overall clinical pregnancy and LBRs were observed (both 50% higher in the metformin versus placebo group). Similar CPRs were found among women who underwent IVF/ICSI (ART population) in both treatment groups, but there was a trend towards a higher SP rate in those pretreated with metformin versus placebo. Therefore, the combined effect of pretreatment, and treatment during IVF/ICSI and through the luteal phase gave significantly increased clinical pregnancy and LBRs. No significant differences in the duration of r-hFSH

### Table IV Pregnancy outcomes.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Metformin n (%)</th>
<th>Placebo n (%)</th>
<th>Difference (95% CI) %</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) ITT population</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical pregnancy</td>
<td>37 / 74 (50.0)</td>
<td>25 / 75 (33.3)</td>
<td>16.7 (1.1 to 32.3)</td>
<td>0.0391</td>
</tr>
<tr>
<td>Live birth</td>
<td>36 / 74 (48.6)</td>
<td>24 / 75 (32.0)</td>
<td>16.7 (1.1 to 32.2)</td>
<td>0.0383</td>
</tr>
<tr>
<td>(b) Spontaneous pregnancy population</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spontaneous clinical pregnancy</td>
<td>15 / 74 (20.3)</td>
<td>8 / 75 (10.7)</td>
<td>9.6 (−1.9 to 21.1)</td>
<td>0.1047</td>
</tr>
<tr>
<td>(c) ART population</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biochemical pregnancy</td>
<td>24 / 56 (42.9)*</td>
<td>24 / 56 (42.9)*</td>
<td>0.0 (−18.3 to 18.3)</td>
<td>1.000</td>
</tr>
<tr>
<td>Clinical pregnancy</td>
<td>22 / 56 (39.3)</td>
<td>17 / 56 (30.4)</td>
<td>8.9 (−8.6 to 26.5)</td>
<td>0.3212</td>
</tr>
<tr>
<td>Live birth</td>
<td>21 / 56 (37.5)</td>
<td>16 / 56 (28.6)</td>
<td>8.9 (−8.4 to 26.3)</td>
<td>0.3151</td>
</tr>
</tbody>
</table>

CI, confidence interval.
*Two biochemical pregnancies; one clinical miscarriage.
*Three biochemical pregnancies; three cases of non-viable intrauterine gestational sac at Week 7; one ectopic pregnancy; one miscarriage at Week 10.
*Defined by ultrasound evidence of a viable intrauterine gestational sac at Week 7 following embryo transfer.

### Table V Gastrointestinal side effects (ITT population).

<table>
<thead>
<tr>
<th></th>
<th>Metformin n (%)</th>
<th>Placebo n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. patients with some gastrointestinal side effects</td>
<td>30 (41)</td>
<td>9 (12)</td>
</tr>
<tr>
<td>Nausea</td>
<td>21 (28)</td>
<td>4 (5)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>4 (5)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Diarrhoea/loose stools</td>
<td>9 (12)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Abdominal pain/discomfort</td>
<td>10 (14)</td>
<td>6 (8)</td>
</tr>
<tr>
<td>Dropout due to side effects</td>
<td>2 (3)</td>
<td>3 (4)</td>
</tr>
</tbody>
</table>

### Table VI OHSS (ART population).

<table>
<thead>
<tr>
<th></th>
<th>Metformin n = 12</th>
<th>Placebo n = 18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coasting performed</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Cancelled IVF/ICSI</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Outpatient follow-up</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Hospitalization</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

Coasting performed or IVF/ICSI cancelled to avoid development of OHSS. Established OHSS for a total of 10 patients (4 of them were coasted to try to avoid OHSS). ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; OHSS, ovarian hyperstimulation syndrome.
stimulation, number of oocytes retrieved, fertilization rates or embryo quality were found between the treatment groups. Our results suggest that metformin pretreatment improves the SP rate in non-obese women with PCOS, but does not affect IVF/ICSI outcomes per se. However, it is possible that the non-significant effect during pretreatment and the lack of effect on ART outcomes may be due to the fact that we did not reach the target sample size. The study may have been underpowered to detect a difference in ART outcomes, which could lead to false-negative assumptions. Ideally, larger SP and ART populations would have been enrolled to reliably distinguish the effects of metformin on these outcomes.

The slow enrolment and the fact that we had to stop the study before achieving the estimated 300 patients due to expiration of study medication is certainly a limitation. Furthermore, this was an investigator-initiated study without economic incentives, which may have had a negative impact on inclusion. Motivation in participating centres in long-term studies, such as this one that ran for 5 years, remains a problem in randomized controlled trials. Furthermore, it is more difficult to recruit non-obese PCOS than PCOS with BMI \( \geq 28 \) kg/m\(^2\). A good proportion of non-obese PCOS become pregnant before they have to be enrolled in an IVF programme. Finally, during the study period metformin became more or less established as a ‘proven’ drug in the treatment of PCOS, making it progressively more difficult to include women with PCOS into a study with a 50% chance of getting placebo (Vanky et al., 2010).

The use of a homogeneous population (BMI \(<28 \) kg/m\(^2\)) is a key strength of the current study. Further studies on homogeneous patient populations are needed. Previous studies on the effect of metformin pretreatment prior to IVF/ICSI enrolled heterogeneous patient populations (Tso et al., 2009). However, one randomized controlled trial enrolled a population with a mean BMI of \( \sim 27 \) kg/m\(^2\); significantly higher pregnancy and LBRs were reported with metformin versus placebo in that study (Tang et al., 2006). Moreover, a subgroup analysis (n = 33) of our previous prospective, randomized, double-blind study suggested that metformin pretreatment for 16 weeks before IVF/ICSI may improve biochemical pregnancy rates in women with PCOS and a BMI of \(<28 \) kg/m\(^2\) (Kjotrod et al., 2004). The significant improvement in the CPR in non-obese women with PCOS treated with metformin demonstrated in the current study concurs with these earlier observations. We acknowledge that no difference in treatment effect was detected between women with a BMI of either \(<25\) or \(\geq 25\) kg/m\(^2\). Although this finding does not fully support earlier observations, it may be related to the small sample size in the current study.

The birth of a healthy baby is the only meaningful outcome for a couple with infertility. For women with PCOS, diet and lifestyle advice, and weight reduction, are essential to increase pregnancy rates and minimize the incidence of complications during pregnancy and birth (Boomsma et al., 2006). Furthermore, obesity is negatively affecting ovulation, oocyte maturation, endometrial development, uterine reception, implantation and miscarriage (Robker, 2008; Brewer and Balen, 2010). A small but significant reduction in weight and BMI was observed in the metformin-treated group during the current study. It is possible that this weight reduction may have contributed to the observed increased pregnancy and LBRs in the metformin group. However, it is difficult to speculate on mechanisms as no central assays or biochemistry was performed during the study. Any study repeating these findings would be strengthened by including such investigations.

While it is not conventional practice to delay IVF for 12 weeks, in this study, we nevertheless, had 23 babies born (15 versus 8 in metformin and placebo groups). We think this highlights the importance of optimizing prepregnancy diet and lifestyle in non-obese PCOS women. Furthermore, it has been shown previously that a substantial proportion of infertile women will become spontaneously pregnant while on the waiting list for IVF (Eijkemans et al., 2008, Pinborg et al., 2009).

Safety is a key consideration for women undergoing ART. Few serious AEs and no unexpected major safety issues were reported in this study. In line with previous knowledge (Legro et al., 2007), a substantial number of patients do suffer gastrointestinal side effects, and this may limit the clinical acceptance of metformin. It has been suggested that the incidence of early miscarriage is increased in women with PCOS (Homburg, 2006). The current study was the first, to our knowledge, to continue metformin through the luteal phase and no deleterious effects (no increase in miscarriage rate) were observed in treated patients [overall miscarriage rate: 3/24 (12.5%) in the metformin group versus 7/24 (29%) in the placebo group; no miscarriages in the 23 spontaneous pregnancies]. However, more data are needed to determine whether metformin during the first trimester might actually reduce miscarriage. Importantly, no multiple births occurred in the current study; the mean number of embryos transferred was 1.2 in the metformin versus 1.1 in the placebo group. Apparently, metformin is not associated with multiple pregnancies (Legro et al., 2007).

In conclusion, we demonstrated a considerable increase in both clinical pregnancy and LBRs with an easy, safe and low-cost drug. Our data suggest that treatment with metformin, for at least 12 weeks prior to, and during, IVF/ICSI is worth considering as a management approach for non-obese women with PCOS. However, more studies are needed to clarify the effect in IVF per se. Further studies on the effect of metformin in homogeneous patient populations are required, and the LBR is recommended as the primary end-point.

### Authors’ roles

The study was planned and designed by S.B.K., S.M.C., A.S. and V.D. S.B.K. ran the study, developed and managed the main database, interpreted the analyses and drafted the paper. S.M.C., V.D., A.S., L.M.-P. and A.T.-K. contributed extensively in interpretation of data, main conclusions and carefully revising the manuscript. P.E.R., T.H.-L., J.M., K.H.K., P.H., A.T.-K., L.M.-P. and SBK ran the study and collected data in one centre each. All authors participated in the finalization of the manuscript and approved the final draft.

### Acknowledgements

We would like to acknowledge Mrs Louise Reinertsen (employed by St Olavs University Hospital) for carefully monitoring the study in all eight participating centres.
Conflict of interest

All authors have completed the Unified Competing Interest form and declare that (1) S.B.K., S.M.C., A.S. and V.D. have support from Merck Serono Norway (an affiliate of E. Merck AB) for the submitted work; (2) S.B.K. has received honoraria from Schering Plough/MSD for lectures and meetings/conferences; P.H. has received an unrestricted research grant and honoraria for lectures from Serono and Schering Plough; L.M.-P. has received expenses from Serono-Merck for travel/accommodation for a workshop; A.S. has received consultancy fees, honoraria for lectures at international conferences and expenses for travel/accommodation from Merck Serono, Geneva, Schering Plough and MSD, USA and Ferring, Denmark; V.D. has received expenses for travel/accommodation from Merck Serono, Schering Plough, MSD and Ferring; A.T.-K. has received advisory board consultancy fees from Merck Serono, Sweden and has acted as a moderator at a scientific meeting sponsored by Ferring; (3) the spouses, partners or children of all authors have no financial relationships that may be relevant to the submitted work and (4) all authors have no non-financial interests that may be relevant to the submitted work.

Funding

This was an investigator-initiated study. Merck Serono Norway (an affiliate of E. Merck AB) provided a grant to help fund the study. The statistical analyses were performed by an independent company (Inge Christoffer Olsen, PhD, Statistician, Smerud Medical Research International AS Drammensveien 41, N-0271 Oslo, Norway) and these expenses were covered by Merck. Smerud also provided the Clinical Study Report (CSR), which are strictly required by the medical authorities in Norway. A professional company (Hannah Wills and MailLee Wong in Caudex Medical in Oxford) was used primarily to speed up the publication process. Merck has had no access to the raw data and no influence on the statistical evaluations or writing of the manuscript at any time point.

The manuscript was reviewed by Merck Serono S.A—Geneva, Switzerland (an affiliate of Merck KGaA, Darmstadt, Germany), but all decisions regarding the final content lay solely with the authors. The views expressed by the authors therefore do not necessarily reflect the views, ideas or policy of the company. Merck Serono makes no guarantee of the completeness, accuracy or reliability of the information provided. Thus, Merck Serono does not accept any liability for reliance placed on the information. Metformin and placebo tablets were provided free of charge, by Weifa A/S, Oslo, Norway. Weifa had no involvement in the study. S.B.K. is a paid employee of Trondheim University Hospital/St Olavs Hospital HF and would like to thank her employers for permitting time to be spent on this study during working hours. S.B.K. would also like to thank UNIMED research fund for providing a scholarship, which supported her during the writing of this manuscript.

References


Pinborg A, Hougaard CO, Nyboe Andersen A, Molbo D, Schmidt L. Prospective longitudinal cohort study on cumulative 5-year delivery


Tang T, Lord JM, Norman RJ, Yasmin E, Balen AH. Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligoamenorrhoea and subfertility. *Cochrane Database Syst Rev* 2010;1:CD003053. (Review)

