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**N-Acetylcysteine improves oocyte and embryo quality in polycystic ovary syndrome patients undergoing intracytoplasmic sperm injection: an alternative to metformin**

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**Abstract.** Polycystic ovary syndrome (PCOS) is associated with low-quality oocytes. The aim of the present study was to investigate the effects of metformin (MET), N-acetylcysteine (NAC) and their combination on follicular fluid parameters, oocytes and embryo quality in PCOS patients. A prospective randomised placebo-controlled pilot study on 60 Iranian women with PCOS (aged 25–35 years) undergoing intracytoplasmic sperm injection (ICSI) was designed. Women were divided into four groups (\(n = 15\) in each): (1) an MET, administered 1500 mg day\(^{-1}\); (2) an NAC group, administered 1800 mg day\(^{-1}\); (3) an NAC + MET group; and (4) a placebo group. Drugs were administered from the 3rd day of previous cycle until the day of oocyte aspiration (6 weeks treatment in total). Data were analysed by one-way ANOVA, with significance set at \(P < 0.05\). The number of immature and abnormal oocytes decreased significantly in the NAC compared with placebo group, with a concomitant increase in the number of good-quality embryos in the NAC group (\(P < 0.05\)). Malondialdehyde levels decreased significantly in the NAC and NAC + MET groups compared with the placebo-treated group (\(P < 0.02\)). In addition, there were significant decreases in leptin levels in the NAC, MET and NAC + MET groups compared with the placebo group (\(P < 0.001\)). Insulin and LH levels were significantly lower in the MET and NAC groups compared with the placebo-treated group (\(P < 0.02\)). We concluded that NAC improves oocyte and embryo quality and could be administered as an alternative to MET.

**Additional keywords:** fertilisation rate, follicular fluid, ovulation induction.

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**Introduction**

Polycystic ovary syndrome (PCOS) is the most prevalent endocrine disorder resulting in anovulation, affecting up to 10% of women of childbearing age (Franks 1995). Oocyte quality is one of the most important factors determining the rate of success in assisted reproductive technologies (ART). PCOS patients are commonly distinguished by an increased number of oocytes during the course of ovulation stimulation; nevertheless, these oocytes are often of low quality, leading to low fertilisation, cleavage and implantation rates (Urman \textit{et al.} 2004). Typically, more than half the oocytes (\(\sim 60\%–70\%\)) retrieved from stimulated cycles exhibit at least one abnormal morphological characteristic (Balaban and Urman 2006). Research in recent years has revealed that insulin resistance and hyperinsulinaemia play a major role in the pathogenesis of PCOS (Tsilchorozidou \textit{et al.} 2004). Therefore, there is presently much interest in the use of insulin-sensitising drugs in PCOS patients. In most cases, treatment with such drugs overcomes anovulation associated with PCOS, leading to reductions in hyperandrogenism and cardiovascular risk factors, and improves the response to ovulation induction. Furthermore, it has been suggested that lowering insulin levels with these agents may be useful in PCOS women, undergoing IVF (Tso \textit{et al.} 2009).

Metformin (MET), a water-soluble insulin-lowering agent, has been investigated extensively for the management of PCOS. Many studies have demonstrated conclusively that MET can
induce regular menstrual cycles and increase the ovulation rate by approximately 75% in patients with PCOS (Palomba et al. 2009). However, the efficacy of MET treatment is still debated, and its administration is associated with a high incidence of side effects (Lord et al. 2003). Evidence obtained by some studies indicates that the administration of MET to PCOS women undergoing IVF or intracytoplasmic sperm injection (ICSI) treatment does not improve the clinical outcomes of these procedures (Tang et al. 2006). In contrast, two independent studies by Stadtmueller et al. (2001) and Fedorcsek et al. (2003) have shown that MET increases the number of oocytes collected and pregnancy rates.

N-acetylcysteine (NAC), as a mucolytic agent, is able to improve insulin secretion and is considered as a safe and well-tolerated drug (Fulghesu et al. 2002). NAC may provide a novel approach for increasing or inducing ovulation in patients with chronic anovulation, including PCOS. NAC is also commonly used to treat several other diseases related to oxidative stress and/or reduced glutathione (GSH) deficiency, such as human immunodeficiency virus (HIV) infection and lung and heart diseases (De Rosa et al. 2000). The pharmacological properties of NAC include antioxidant, anti-inflammatory and anti-apoptotic actions. NAC also preserves vascular integrity and has an immunological effect (De Flora et al. 2001). Furthermore, the potential benefits of NAC therapy in patients with chronic anovulation have been demonstrated (Fulghesu et al. 2002; Rizk et al. 2005); this emphasises its use in human fertility.

Thus, we speculated that the combined administration of MET and NAC should improve insulin function and enhance ovulation. Consequently, we designed a randomised placebo-controlled pilot trial to investigate the effects of NAC versus MET and their combination in improving the quality of oocytes and embryos, as well as the biochemical factors in the follicular fluid of PCOS women undergoing ovulation induction for ICSI.

Materials and methods

Study design and objective

In all, 80 infertile Iranian women with PCOS (aged 25–35 years) who were candidates for ICSI were included in the present prospective randomised placebo-controlled pilot trial between July 2012 and February 2013. This study was conducted in the IVF Unit of the Infertility Research Center of the Academic Center for Education, Culture and Research (ACECR) (Qom, Iran).

Patients needed to fulfil the diagnostic criteria for PCOS based on the Rotterdam Consensus Workshop in 2003 (The Rotterdam ESHRE/ASRM-Sponsored PCOS Work Group 2004), including having at least of the two following: chronic oligo- or anovulation; clinical or biochemical hyperandrogenism; or polycystic ovaries on ultrasound examination. Exclusion criteria included hypersensitivity to either MET or NAC, infertility factors other than anovulation, male infertility, pelvic organic pathologies, congenital adrenal hyperplasia, thyroid dysfunction, Cushing’s syndrome, hyperprolactinaemia, androgen-secreting neoplasia, diabetes mellitus, consumption of medications affecting carbohydrate metabolism and hormonal analogues other than progesterone 2 months prior to enrolment in the study and severe hepatic or kidney disease. Semen samples from male partners were assessed according to World Health Organization (WHO) guidelines (Cooper et al. 2010), and women with partners presenting abnormal semen parameters were excluded from the study.

The study was approved by the Research Ethics Committee of Royan Institute (Tehran, Iran; EC/91/1041) and informed consent was obtained from all participants. All subjects were asked to avoid any changes in their normal physical activity and diet, and also not to undergo any new pharmacotherapy during the study.

Treatment design and ovulation induction

Because no randomized control trials have so far been performed to investigate the use of MET plus NAC during IVF treatment, the power calculation in the present study was based on data from retrospective studies on MET or NAC (Yarali et al. 2002; Tang et al. 2006; Elnashar et al. 2007; Elgindy et al. 2010; Kjøtrød et al. 2011). Common findings for patients with PCOS undergoing IVF are low fertilisation rates and impaired oocyte quality. Overall, considering a 95% confidence interval and 80% power, 80 patients were required as the sample population. These patients were divided into four groups. Medication was provided to the patients by a midwife. Therefore, both the patient and the physician were blinded to the type of treatment regimen provided.

The dose and duration of NAC were chosen based on previous studies (Fulghesu et al. 2002; Elnashar et al. 2007; Oner and Muderris 2011). A total of 80 patients was examined, with patients being randomly divided into four groups (n = 20 in each) as follows: (1) a placebo-treated group that was administered oral rehydration solution (Poursina, Tehran, Iran) three times daily; (2) an MET-treated group administered MET (Glucophage; Merck, West Drayton, UK; 500 mg) three times daily; (3) an NAC-treated group administered NAC (Batch no. 6N5483; Holzkirchen, Bavaria, Germany; 600 mg) three times daily; and (4) a group treated with a combination of the same doses of MET + NAC three times daily. All treatments were administered for a period of 6 weeks.

The PCOS patients undergoing ICSI treatment using a long gonadotrophin-releasing hormone agonist protocol received either placebo, MET, NAC or MET + NAC randomly from the 3rd day of their last menstrual period (LMP) in the previous cycle until the day of oocyte aspiration. All patients received oral contraceptive pills for 21 days starting simultaneously with placebo, MET, NAC or MET + NAC on Day 3 of menses of the cycle before the treatment cycle. Ovarian downregulation was initiated with daily buserelin acetate (1 mg; Suprefact; Aventis, Frankfurt, Germany) beginning on Day 19 of the preceding menstrual cycle (luteal phase) and after ovarian downregulation was achieved (the second day of the LMP of the previous cycle); then, the dose was reduced to 0.5 mg when the thickness of the endometrium was <4 mm. Ovarian stimulation began with daily injections of an average 2 ampoules of recombinant FSH (Gonal-f; Merck Serono, Geneva, Switzerland). Cycles were monitored using vaginal sonography (HS 4000; Holzkirchen, Bavaria, Germany).
Assessment of baseline hormonal status and clinical features

Body mass index (BMI), the waist : hip ratio (WHR) and blood pressure were recorded for each patient before initiation of treatment on the 3rd day of menstruation. Fasting blood samples were collected and stored at −70°C until analysis. Following follicular aspiration and OPU, follicular fluid (FF) was collected from the first aspirated follicle that did not contain any visible blood contamination. The FF samples were immediately centrifuged at 1500g for 10 min at room temperature and the supernatants were aspirated from 30 s exposure to 20 IU mL−1 hyaluronidase (ART-4007A; SAGE BioPharma, Pasadena, CA, USA) in HEPES-based medium followed by thorough washing with HEPES-buffered human tubal fluid (HTF) containing 5 mg mL−1 human albumin (ART-3001; SAGE BioPharma) and mechanical pipetting. Mature (MII) oocytes were identified in the FF using the thiobarbituric acid (TBA) colorimetric method and a thioarbituric acid-reactive substances (TBARS) assay kit (catalogue no. KA1381; Abnova Corp., Taipei, Taiwan) by sandwich enzyme immunoassay. Concentrations of malondialdehyde (MDA; in μM), a naturally occurring product of lipid peroxidation, were determined in the FF using the thiobarbituric acid (TBA) colorimetric method and a thioarbituric acid-reactive substances (TBARS) assay kit (catalogue no. KA1381; Abnova Corp.). Dilutions were performed using PSB buffer before measurement of AMH (1 : 20) in FF depending on the calibration range.

Oocyte retrieval, ICSI and embryo culture

Oocyte retrieval was performed by ultrasound-guided transvaginal aspiration using a single-lumen needle (Reproline Medical, Rheinbach, Germany). All oocytes had their cumulus cells removed by 30 s exposure to 20 IU mL−1 hyaluronidase (ART-4007A; SAGE BioPharma, Pasadena, CA, USA) in HEPES-based medium followed by thorough washing with HEPES-buffered human tubal fluid (HTF) containing 5 mg mL−1 human albumin (ART-3001; SAGE BioPharma) and mechanical pipetting. Mature (MII) oocytes were identified and matured overnight in HEPES-based medium containing 5 mg mL−1 human chorionic gonadotrophin (hCG, catalogue no. DE2935) and 0.3 mg mL−1 luteinizing hormone (hLH; catalogue no. DE1289). Embryos were further incubated until the 3rd day of development (24–36 h post-fertilization).

Quality control of the embryos.

Due to adverse drug (n = 3) Failure of ovulation induction (n = 0) Mono-follicular development (n = 0) Lack of suitable embryos (n = 0)

Due to adverse drug (n = 0) Failure of ovulation induction (n = 1) Mono-follicular development (n = 1) Lack of suitable embryos (n = 1) Due to protocol violation and voluntary drop out (n = 2)

Due to adverse drug (n = 2) Failure of ovulation induction (n = 1) Mono-follicular development (n = 0) Lack of suitable embryos (n = 2)

Due to adverse drug (n = 0) Failure of ovulation induction (n = 1) Mono-follicular development (n = 1) Lack of suitable embryos (n = 2) No oocytes retrieved (n = 1)

Fig. 1. Flow chart of the study participants. MET, metformin; NAC, N-acetylcysteine.
Table 1. Baseline and clinical characteristics of the four polycystic ovary syndrome patient groups (age range 25–35 years)

Data are the mean ± s.d. Statistical analyses were performed by ANOVA followed by Tukey’s test for multiple comparisons. NS, no significant differences were found between mean values in the treatment groups compared with the placebo group (P > 0.05). NAC, N-acetylcysteine; MET, metformin; WC, waist circumference; HC, hip circumference; WHR, waist : hip ratio; BMI, body mass index; TT, total testosterone; E2, oestradiol.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NAC</th>
<th>MET</th>
<th>NAC + MET</th>
<th>Placebo</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>29.67 ± 3.35</td>
<td>28.07 ± 3.41</td>
<td>28.67 ± 3.86</td>
<td>27.9 ± 2.8</td>
<td>0.491 (NS)</td>
</tr>
<tr>
<td>Duration of marriage (years)</td>
<td>8.07 ± 3.97</td>
<td>8.6 ± 3.2</td>
<td>8.2 ± 2.8</td>
<td>8.57 ± 3.05</td>
<td>0.960 (NS)</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>94 ± 11</td>
<td>91.1 ± 15.3</td>
<td>90.9 ± 13.2</td>
<td>91.7 ± 15.4</td>
<td>0.931 (NS)</td>
</tr>
<tr>
<td>HC (cm)</td>
<td>108.3 ± 11.3</td>
<td>107.4 ± 13.5</td>
<td>106.4 ± 13.1</td>
<td>108.4 ± 13.4</td>
<td>0.970 (NS)</td>
</tr>
<tr>
<td>WHR</td>
<td>0.86 ± 0.04</td>
<td>0.84 ± 0.04</td>
<td>0.84 ± 0.04</td>
<td>0.83 ± 0.05</td>
<td>0.366 (NS)</td>
</tr>
<tr>
<td>BMI (kg m⁻²)</td>
<td>27.7 ± 4.5</td>
<td>27.9 ± 3.1</td>
<td>27.8 ± 3.6</td>
<td>26.9 ± 2.3</td>
<td>0.853 (NS)</td>
</tr>
<tr>
<td>Insulin (mU L⁻¹)</td>
<td>17.5 ± 1.6</td>
<td>17.45 ± 1.97</td>
<td>17.88 ± 1.91</td>
<td>18.04 ± 2.25</td>
<td>0.810 (NS)</td>
</tr>
<tr>
<td>LH (mIU mL⁻¹)</td>
<td>11.51 ± 2.47</td>
<td>10.68 ± 1.88</td>
<td>10.47 ± 1.53</td>
<td>10.77 ± 2.41</td>
<td>0.566 (NS)</td>
</tr>
<tr>
<td>FSH (mIU mL⁻¹)</td>
<td>5.3 ± 1.7</td>
<td>4.9 ± 1.6</td>
<td>5.25 ± 0.97</td>
<td>5.46 ± 0.96</td>
<td>0.709 (NS)</td>
</tr>
<tr>
<td>TT (ng mL⁻¹)</td>
<td>1.11 ± 0.54</td>
<td>1.1 ± 0.5</td>
<td>1.0 ± 0.4</td>
<td>1.19 ± 0.48</td>
<td>0.799 (NS)</td>
</tr>
<tr>
<td>E2 (pg mL⁻¹)</td>
<td>69.1 ± 14.6</td>
<td>69.2 ± 13.6</td>
<td>64.6 ± 14.4</td>
<td>70.2 ± 16.6</td>
<td>0.734 (NS)</td>
</tr>
</tbody>
</table>

by the presence of the first polar body under a stereomicroscope (Olympus, Tokyo, Japan). Only those oocytes that had extruded the first polar body (MII oocytes) were used for ICSI. Immediately before injection, the processed sperm suspension was added to a 50-μL droplet of polyvinylpolypyrrolidone (PVP; ART-4006-A; SAGE BioPharma). Four hours after oocyte retrieval, a single motile spermatozoon with apparently normal morphology was immobilised and used to inseminate the oocyte. Inseminated oocytes were transferred to the fertilisation medium (ART-1520; SAGE BioPharma), covered with mineral oil (Reproline Medical). Fertilisation was assessed the next day and if two pronuclei (2PN) were present, fertilised oocytes were transferred to the fertilisation medium (CRi PolScope Technology, London, UK). Assessment of oocyte morphology, fertilisation and embryo quality

Assessment of oocyte morphology, fertilisation and embryo quality

Nuclear maturation of oocytes was determined by the identification of the first polar body just before the ICSI procedure under an Olympus inverted microscope (IX71) with a Hoffmann modulation contrast system at ×400 magnification. The morphology of the oocytes was evaluated based on the MII oocyte morphological scoring system (MOMS) and the grading system described by Rienzi et al. (2012) on the basis of colouration, granularity (large or small granules; homogeneous distribution or clustering of granules; and in the centre or periphery of the oocyte), size of the perivitelline space and the distribution of organelles (vacuoles and endoplasmic reticulum). According to these morphological criteria, oocytes were classified as: (1) normal oocytes; (2) oocytes with extracytoplasmic abnormalities (dark zona pellucida, large perivitelline space and fragmented polar body); or (3) oocytes with intracytoplasmic abnormalities (dark or granular cytoplasm, vacuolated, structural deformities and cytoplasmic fragments). The meiotic spindle was identified by the help of polarised light (CRi PolScope Technology, London, UK).

The fertilisation results were assessed 12–16 h after ICSI for the appearance of two distinct pronuclei. Cleavage was evaluated 24–36 h after fertilisation. Embryo quality was assessed on the 3rd day of insemination and graded as follows: Grade I, symmetric blastomeres and no fragmentation; Grade II, unequal blastomeres and <30% fragmentation; and Grade III, unequal blastomeres and >30% fragmentation (Brezinova et al. 2009).

Statistical analysis

The normality of continuous variables was confirmed using the Kolmogrov–Smirnov test, and data are reported as the mean ± s.d. Data were analysed by one-way ANOVA. Tukey’s and Dunnett’s T3 tests for post hoc analysis were used. A Chi-squared test was used for statistical analysis where appropriate. Mean values were considered significantly different at P < 0.05 (two-tailed). Pearson’s correlation test and multivariate linear regression analysis were performed to define the correlation between variables. All data were analysed using SPSS (version 16.0) for Windows (SPSS Inc., Chicago, IL, USA).

Results

Clinical and demographic characteristics

There were no significant differences in age, duration of marriage, duration of marriage infertility, BMI, waist circumference, hip circumference, WHR, oligomenorrhoea, amenorrhoea, hirsutism or concentrations of insulin, LH, FSH, TT and E2 among the four groups prior to treatment (Table 1). Analysis of the male partner’s sperm parameters revealed no significant differences among the different groups (Table 2).
Table 2. Characteristics of semen parameters from fertile men whose partners had polycystic ovary syndrome

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NAC</th>
<th>MET</th>
<th>NAC + MET</th>
<th>Placebo</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm count (×10^6/mL⁻¹)</td>
<td>14 ± 5</td>
<td>15 ± 5.7</td>
<td>12.5 ± 3.7</td>
<td>13.6 ± 4.7</td>
<td>0.516 (NS)</td>
</tr>
<tr>
<td>Total motility (%)</td>
<td>42.3 ± 6.5</td>
<td>44.3 ± 6.2</td>
<td>45.3 ± 6.4</td>
<td>43.7 ± 5.5</td>
<td>0.603 (NS)</td>
</tr>
<tr>
<td>Normal forms (%)</td>
<td>5.2 ± 2.7</td>
<td>5.6 ± 2.7</td>
<td>5.1 ± 2.4</td>
<td>6 ± 3</td>
<td>0.771 (NS)</td>
</tr>
</tbody>
</table>

Table 3. Comparison of biochemical and hormonal parameters of the follicular fluid from polycystic ovary syndrome patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NAC</th>
<th>MET</th>
<th>NAC + MET</th>
<th>Placebo</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (mU L⁻¹)</td>
<td>6.5 ± 2.2*</td>
<td>6.4 ± 2.1*</td>
<td>6.8 ± 2.5 (NS)</td>
<td>8.9 ± 2.6</td>
<td>0.013</td>
</tr>
<tr>
<td>LH (mIU mL⁻¹)</td>
<td>1.0 ± 0.3*</td>
<td>1.0 ± 0.3*</td>
<td>1.03 ± 0.4 (NS)</td>
<td>1.3 ± 0.4</td>
<td>0.018</td>
</tr>
<tr>
<td>TT (ng mL⁻¹)</td>
<td>7.8 ± 3.4</td>
<td>7.9 ± 3.9</td>
<td>8.6 ± 3.7</td>
<td>8.68 ± 3.1</td>
<td>0.858</td>
</tr>
<tr>
<td>E2 (pg mL⁻¹)</td>
<td>466.6 ± 135.1</td>
<td>496.6 ± 176.1</td>
<td>470.2 ± 187.5</td>
<td>436 ± 155.5</td>
<td>0.796</td>
</tr>
<tr>
<td>Leptin (ng mL⁻¹)</td>
<td>19.82 ± 4.73</td>
<td>19.67 ± 4.19</td>
<td>20.42 ± 4.01</td>
<td>25.3 ± 4.04</td>
<td>0.001</td>
</tr>
<tr>
<td>AMH (ng mL⁻¹)</td>
<td>503.9 ± 160.3</td>
<td>426.2 ± 117.4</td>
<td>491.6 ± 173.9</td>
<td>548.3 ± 164.1</td>
<td>0.204</td>
</tr>
<tr>
<td>MDA (µM)</td>
<td>3.78 ± 0.39*</td>
<td>4.54 ± 0.37 (NS)</td>
<td>4.03 ± 0.30*</td>
<td>5.61 ± 0.52</td>
<td>0.012</td>
</tr>
</tbody>
</table>

\( ^*P\)-values for the significance of differences between mean values in the treatment groups compared with placebo. Significant values are bolded.

Evaluation of endocrine characteristics

There was a significant decrease in FF leptin concentrations in all three treatment groups compared with the placebo group \((P < 0.001)\). In addition, there were significant decreases in FF insulin and LH in the MET- and NAC-treated groups compared with the placebo group \((P < 0.02)\). However, there were no significant differences in FF concentrations of TT, E2 or AMH between the treatment groups and the placebo group \((P > 0.05)\). There was a significant decrease in FF MDA levels in the NAC- and MET + NAC-treated groups, but not in the MET-treated group, compared with the placebo group \((P < 0.02; \text{Table 3})\).

Evaluation of oocyte morphology and embryos

The total number of oocytes retrieved in the three treatment groups did not differ significantly from that retrieved in the placebo group \((P > 0.05)\). The number of immature oocytes (MI + germinal vesicle (GV) stage) and the number of abnormal mature oocytes decreased significantly in the NAC group \((P < 0.01)\), but no significant reductions were seen in the MET and MET + NAC groups \((P > 0.05)\). The number of MI oocytes in the MET- and NAC-treated groups and the number of meiotic spindles in mature (MII) oocytes in the vicinity of the second polar body increased significantly in all treatment groups compared with the placebo group \((P < 0.01)\). There were no significant differences in the fertilisation rate of MII oocytes and the number of cleaved embryos among any of the study groups \((P > 0.05)\). The number of good embryos (Grade I) formed on Day 3 increased significantly only in the NAC-treated group \((P < 0.05)\), and not in the MET and MET + NAC groups, compared with the placebo group \((P > 0.05)\). The number of embryos transferred (Grade I + II) and the clinical pregnancy rate did not differ significantly among the four groups \((P > 0.05)\). In addition, the rate of ovarian hyperstimulation syndrome (OHSS) and endometrial thickness did not differ significantly in the treatment groups compared with the placebo group \((P > 0.05; \text{Table 4})\). A significant negative correlation was detected between the number of mature oocytes with meiotic spindle and MDA levels in FF \((r = -0.294; P < 0.022)\), and between fertilisation rate and MDA levels in FF \((r = -0.284; P < 0.028)\).

Investigating intra- and extracytoplasmic abnormalities in oocytes revealed a significant reduction in the fragmentation rate of the polar body, a large perivitelline space and granular cytoplasm in the NAC-treated group \((P < 0.05)\), but no significant difference was observed in the MET and MET + NAC groups compared with the placebo group. Conversely, there was a significant decrease in the number of vacuolated oocytes in the NAC, MET and NAC + MET groups compared with the
fertilisation, cleavage and implantation rates, increased oocytes following ovulation induction, which leads to lower commonly distinguished by an increased number of low-quality associated with insulin resistance and hyperinsulinaemia. It is PCOS is an endocrine–metabolic disorder that is closely asso-

Discussion

PCOS is an endocrine–metabolic disorder that is closely asso-

Table 4. Distribution of oocytes retrieved, quality of oocytes and embryos and pregnancy outcome in polycystic ovary syndrome patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NAC</th>
<th>MET</th>
<th>NAC + MET</th>
<th>Placebo</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. oocytes retrieved</td>
<td>12.1 ± 6.1</td>
<td>15.7 ± 6.6</td>
<td>14.7 ± 7.5</td>
<td>10 ± 4</td>
<td>0.06</td>
</tr>
<tr>
<td>No. immature oocytes (GV + MI)</td>
<td>1.27 ± 0.88*†</td>
<td>3 ± 2 (NS)</td>
<td>2.27 ± 1.94 (NS)</td>
<td>3.33 ± 1.35</td>
<td>0.006</td>
</tr>
<tr>
<td>No. mature oocytes (MII)</td>
<td>10.8 ± 5.4*</td>
<td>12.2 ± 5.3*</td>
<td>12.07 ± 6.05*</td>
<td>6.3 ± 2.5</td>
<td>0.006</td>
</tr>
<tr>
<td>No. normal MII oocytes</td>
<td>8.7 ± 5.4*</td>
<td>8.5 ± 3.7*</td>
<td>7.6 ± 3.6*</td>
<td>3 ± 1</td>
<td>0.001</td>
</tr>
<tr>
<td>No. abnormal MII oocytes</td>
<td>1.9 ± 0.9*†</td>
<td>4.2 ± 1.5 (NS)</td>
<td>4.5 ± 2.6 (NS)</td>
<td>3.5 ± 1.5</td>
<td>0.001</td>
</tr>
<tr>
<td>No. MII oocytes with normal spindle visible</td>
<td>9.9 ± 5.8*</td>
<td>10.5 ± 4.5*</td>
<td>10.6 ± 5.3*</td>
<td>5.7 ± 2.3</td>
<td>0.01</td>
</tr>
<tr>
<td>No. fertilised oocytes (2PN)</td>
<td>7.7 ± 3.9</td>
<td>9.2 ± 4.5</td>
<td>8.3 ± 4.5</td>
<td>5.6 ± 2.2</td>
<td>0.09</td>
</tr>
<tr>
<td>No. cleaved embryos</td>
<td>7.3 ± 3.4</td>
<td>8.5 ± 4.1</td>
<td>7.53 ± 3.52</td>
<td>5.13 ± 2.13</td>
<td>0.06</td>
</tr>
<tr>
<td>No. Grade I embryos</td>
<td>2.87 ± 1.45*</td>
<td>2.5 ± 1.3 (NS)</td>
<td>2.3 ± 0.9 (NS)</td>
<td>1.53 ± 0.83</td>
<td>0.021</td>
</tr>
<tr>
<td>No. Grade II embryos</td>
<td>2.7 ± 1.7</td>
<td>2.7 ± 1.6</td>
<td>2.4 ± 1.2</td>
<td>1.9 ± 0.9</td>
<td>0.295</td>
</tr>
<tr>
<td>No. Grade III embryos</td>
<td>1.7 ± 1.3</td>
<td>3.4 ± 2.3</td>
<td>2.8 ± 2.1</td>
<td>1.7 ± 0.8</td>
<td>0.06</td>
</tr>
<tr>
<td>No. embryos transferred (Grades I + II)</td>
<td>2.07 ± 0.46</td>
<td>2.13 ± 0.35</td>
<td>2.2 ± 0.6</td>
<td>2.2 ± 0.4</td>
<td>0.826</td>
</tr>
<tr>
<td>No. OHSS (%)</td>
<td>2 (13.3%)</td>
<td>4 (26.7%)</td>
<td>4 (26.7%)</td>
<td>5 (33.3%)</td>
<td>0.656</td>
</tr>
<tr>
<td>Endometrial thickness (mm)</td>
<td>9.4 ± 0.9</td>
<td>9.9 ± 1.9</td>
<td>9.6 ± 1.2</td>
<td>8.9 ± 1.6</td>
<td>0.307</td>
</tr>
<tr>
<td>No. clinical pregnancy (%)</td>
<td>3 (20%)</td>
<td>4 (26.7%)</td>
<td>2 (13.3%)</td>
<td>2 (13.3%)</td>
<td>0.761</td>
</tr>
</tbody>
</table>

AStatistical analyses performed by Dunnett’s T3 test for multiple comparisons.
BStatistical analyses performed by the Chi-squared test for multiple comparisons.
P-values for the significance of differences between mean of values in the treatment groups compared with placebo. Significant values are bolded.

Placebo group (P < 0.001). Although a tendency for decreased rates of dark zona, subzonal fragmentation and morphological deformities was observed in the treatment groups compared with the placebo group, the differences did not reach statistical significance (Table 5; Fig. S1 available as Supplementary Material to this paper).

Discussion

PCOS is an endocrine–metabolic disorder that is closely asso-

Table 5. Distribution of oocyte abnormalities in polycystic ovary syndrome patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NAC</th>
<th>MET</th>
<th>NAC + MET</th>
<th>Placebo</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. extracytoplasmic abnormalities (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dark zona</td>
<td>2 (13.3%)</td>
<td>5 (33.3%)</td>
<td>6 (40%)</td>
<td>6 (40%)</td>
<td>0.346</td>
</tr>
<tr>
<td>Large PVS</td>
<td>6 (40%)*</td>
<td>13 (80%) (NS)</td>
<td>13 (80%) (NS)</td>
<td>13 (80%)</td>
<td>0.015</td>
</tr>
<tr>
<td>Fragmented polar body</td>
<td>2 (13.3%)*</td>
<td>7 (46.7%) (NS)</td>
<td>8 (53.3%) (NS)</td>
<td>10 (66.7%)</td>
<td>0.025</td>
</tr>
<tr>
<td>No. intracytoplasmic abnormalities (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Granulated</td>
<td>3 (20%)*</td>
<td>6 (40%) (NS)</td>
<td>6 (40%) (NS)</td>
<td>10 (66.7%)</td>
<td>0.07</td>
</tr>
<tr>
<td>Vacuolated</td>
<td>1 (6.7%)*</td>
<td>3 (20%)*</td>
<td>4 (26.7%)*</td>
<td>11 (73.3%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Subzonal fragmentation</td>
<td>3 (30%)</td>
<td>9 (60%)</td>
<td>7 (46.7%)</td>
<td>8 (53.3%)</td>
<td>0.137</td>
</tr>
<tr>
<td>Morphological abnormalities</td>
<td>2 (13.3%)</td>
<td>5 (33.3%)</td>
<td>4 (26.7%)</td>
<td>5 (33.3%)</td>
<td>0.581</td>
</tr>
</tbody>
</table>

No. fertilised oocytes (2PN) 7.7/C6
No. MII oocytes 10.8/C6
No. mature oocytes (MII) 10.8/C6
No. normal MII oocytes 8.7/C6
No. abnormal MII oocytes 1.9/C6
No. MII oocytes with normal spindle visible 9.9/C6
No. fertilised oocytes (2PN) 7.7/C6
No. cleaved embryos 7.3/C6
No. Grade I embryos 2.87/C6
No. Grade II embryos 2.7/C6
No. Grade III embryos 1.7/C6
No. embryos transferred (Grades I + II) 2.07/C6
No. OHSS (%) 2/C6
Endometrial thickness (mm) 9.4/C6
No. intracytoplasmic abnormalities (%) 2 (13.3%)
No. extracytoplasmic abnormalities (%) 2 (13.3%)
No. clinical pregnancy (%) 3 (20%)

Embryonic fragmentation and a higher miscarriage rate (Urman et al. 2004). In these patients, insulin may induce local androgen production by stimulating activity of 17α-hydroxylase, resulting in low-quality oocytes (Dumesic et al. 2008). Moreover, high LH levels (Urman et al. 2004), increases in androgen (Kurzawa et al. 2008) and leptin (Amfiandis et al. 2005) levels and increased ROS in FF and decreased total antioxidant capacity (TAC; Agarwal et al. 2012) have been associated with a significant decrease in oocyte quality, low maturation, low fertilisation rate, impaired embryo quality and consequently lower pregnancy and higher miscarriage rates. Elevated serum AMH concentrations are also clearly correlated with increased testosterone and/or LH levels in PCOS patients, impaired oocyte
maturity and therefore reduced embryo quality (Desforges-Bullet et al. 2010). Thus, improving metabolic–endocrine factors in these patients may increase the quality of oocytes and embryos. In the present study, the effects of MET and NAC alone and in combination on FF endocrine parameters and the quality of oocytes and embryos in PCOS patients undergoing ovulation induction for ICSI were investigated.

Previous studies have shown that MET administration in PCOS women lowers serum fasting insulin, TT and free testosterone, improves E2 levels on the day of oocyte retrieval, enhances clinical pregnancy and live birth rates and severely diminishes the risk of OHSS (Tang et al. 2006). It is believed that MET directly inhibits androgen production in human ovarian thecal cells by decreasing the activity of cytochrome P450-C17a and/or insulin concentrations, thus increasing the response to ovulation induction (Attia et al. 2001). The present study revealed that MET is effective in improving insulin, LH and leptin levels in FF, and hence increases mature and normal oocytes compared with the placebo group. These findings are consistent with those of other studies (Stadtmueller et al. 2001; Fedorcsek et al. 2003). In addition, the results of the present study showed that MET treatment did not increase the number of oocytes retrieved, the rate of fertilisation and cleavage and the quality of embryos; these findings are in agreement with those of previous studies (Tang et al. 2006; Kjøtrød et al. 2011). In contrast, others have reported that MET increases the number of oocytes retrieved (Fedorcsék et al. 2003) and the rate of fertilisation (Stadtmueller et al. 2001). Because the present study was a pilot study and the P-values obtained for these results were close to being significant, further studies are required to clarify the effects of MET. In addition, the different results reported by other studies are likely due to differences in treatment duration and the dose of metformin, as well as genetic differences in the populations studied.

In the present study, similar to other studies (Fedorcsék et al. 2003; Tang et al. 2006), we administered MET for a period of 6 weeks starting from ovulation induction on the 3rd day of the last menstrual cycle until oocyte aspiration. Other studies have concluded that treatment with a higher dosage and/or duration of MET before and during the long protocol IVF–ICSI cycle in women with PCOS does not improve the out come of ART (Kjøtrød et al. 2004, 2011; Önalan et al. 2005). Conversely, long-term treatment may not be well tolerated by patients; therefore, we may not be able to control the study as required. In some PCOS patients, due to the lack of tolerance during MET administration and to limit side effects, the dose of MET must be gradually increased during the first 2 weeks of treatment. However, this gradual dose increase can interfere with the results. Therefore, we preferred to treat patients with the selected dose for 6 weeks during ovulation induction without any gradual increase in dose.

It is well known that NAC has multiple biological effects, preserving the follicles in the ovary and improving the pregnancy rate through its anti-apoptotic action (Fulghesu et al. 2002; Hildebrandt et al. 2004; Rizk et al. 2005; Nasr 2010; Salehpour et al. 2012). However, Elgindy et al. (2010) reported that 1200 mg NAC supplementation in ICSI cycles, using the long agonist protocol, did not significantly increase the number of Grade I embryos or the rate of fertilisation and pregnancy. Elgindy et al. (2010) suggested that larger-scale studies, possibly with higher doses and/or longer duration of NAC administration, should be performed to identify any significant effects of NAC. In this regard, Liu et al. (2012) recently reported that the mice treated for 2 months with NAC exhibited an increased number and quality of oocytes and improved embryo development. It is likely that NAC has beneficial effects in reducing follicle atresia and ensuring oocyte quality, particularly by increasing telomerase activity and telomere length. The results of the present study show that NAC is effective in decreasing insulin, LH, leptin and MDA levels in FF through antioxidant and anti-apoptotic actions. This improves the maturation and quality of oocytes and embryo development, and decreases the number of immature oocytes. The results of the present study confirm those of previous studies (Badawy and Abdelgawad 2007; Oner and Muderris 2011; Liu et al. 2012). Conversely, similar to the results obtained by Elgindy et al. (2010), NAC did not significantly increase the number of oocytes retrieved or the rate of fertilisation and pregnancy compared with the placebo group. Modifications in the dose and/or duration of treatment, sampling size and ovulation induction protocols may improve the results.

Bonnefont-Rousselot et al. (2003) reported that MET reduces oxidative stress, whereas others have reported that it increases oxidative stress (Pavlovic et al. 2000; Yilmaz et al. 2005). In the present study, no significant decrease in MDA concentrations was observed following MET administration. Unlike MET, NAC seems to decrease elevated oxidative stress in PCOS patients more effectively, which is likely due to its antioxidant and anti-apoptotic properties. This is probably the reason for the increase in the quality and maturity of oocytes observed in the NAC-treated group. The fact that these results were not obtained in the NAC + MET-treated group may be due to the fact that MET cannot significantly decrease MDA levels, as a marker of lipid peroxidation. Conversely, it may be that there is no synergistic effect between the two drugs in the NAC + MET-treated group or that we used an insufficient dosage of NAC or MET in the combination treatment.

It is believed that oocyte morphological abnormalities can affect fertilisation rate and embryo development (Balaban and Urman 2006; Ebner et al. 2006) in such a way that cytoplasmic abnormalities that occur during oocyte maturation, especially those that interfere with the meiotic spindle and intricate cytoskeletal structure, can adversely affect the ability of oocytes to undergo normal fertilisation and embryo development. Higher ROS concentrations in FF and a lower proportion of oocytes with the meiotic spindle in PCOS patients may contribute to lower fertilisation and pregnancy rates and embryos quality, as well as higher abortion rates (Rajani et al. 2012). Our findings are similar to those of Rajani et al. (2012), who found that an increased number of oocytes without a normal meiotic spindle and with high MDA concentrations decreased both the fertilisation rate and embryo quality. Furthermore, our results showed a significant negative correlation between the number of mature oocytes with meiotic spindle and MDA levels in FF, and between fertilisation rate and MDA levels in FF.

The present study is the first pilot trial focusing on the effects of MET, NAC and their combination on the quality of oocytes
and embryos in PCOS patients undergoing ovulation induction. To summarise, our data revealed that NAC treatment reduces the number of immature oocytes and morphological abnormalities, increases the normality and maturity of oocytes and the number of good embryo (Grade I) formed, and lowers concentrations of endocrine parameters such as insulin, LH and MA in the FF. In addition, we found that MET treatment increases the normality and maturity of oocytes and lowers the level of endocrine parameters, such as insulin and LH in FF.

Although MET is believed to be useful for the treatment of many women with PCOS, NAC, with antioxidant, anti-apoptotic and lipid peroxidation-lowering properties, may be considered as an alternative supplement (Fulghesu et al. 2002; Rizk et al. 2005; Elgindy et al. 2010). Considering the fact that NAC improves oocyte maturation and embryo quality, and decreases the rate of immature oocytes in women with PCOS while being a safe and well-tolerated agent, we also suggest the administration of NAC as an alternative to other insulin-sensitising agents like MET.

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References


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