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ARTICLE

# Effect of *N*-acetyl-cysteine after ovarian drilling in clomiphene citrate-resistant PCOS women: a pilot study

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**Abstract** The aim of this randomized double-blind placebo-controlled pilot study was to evaluate *N*-acetyl-cysteine (NAC) as an adjunctive therapy following unilateral laparoscopic ovarian drilling (LOD) for clomiphene citrate-resistant women with polycystic ovary syndrome (PCOS). A total of 60 patients with clomiphene citrate-resistant PCOS who underwent unilateral LOD were assigned randomly to receive either NAC 1.2 g/d (group A = 30) or placebo (group B = 30) for 5 days starting at day 3 of the cycle for 12 consecutive cycles. The primary outcome was pregnancy rate; secondary outcomes were ovulation rates, endometrial thickness and pregnancy outcome. Baseline clinical, endocrine, and sonographic characteristics were similar in the two groups. A significant increase in both ovulation and pregnancy rates was observed in the NAC group, compared with placebo [87% versus 67% (RR 1.3; 95% CI 1.2–2.7) and 77% versus 57% (RR 1.4; 95% CI 1.1–2.7), respectively, $P < 0.01$]. Moreover, miscarriage rates were significantly lower and live birth rates were significantly higher in the NAC group [8.7% versus 23.5% (RR 0.4; 95% CI 0.1–3.7) and 67% versus 40% (RR 1.7; 95% CI 0.3–3.5), respectively, $P < 0.01$]. In conclusion, NAC is a novel adjuvant therapy after unilateral LOD which might help improve overall reproductive outcome.



**KEYWORDS:** clomiphene citrate resistance, laparoscopic ovarian drilling (LOD), *N*-acetyl-cysteine, polycystic ovary syndrome (PCOS), RCT

## Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies affecting 5–10% of reproductive-age women (Yavasoglu et al., 2009). It is the most frequent cause of ovarian hyperandrogenism and chronic anovulation (Homburg, 2008). Clomiphene citrate is still the first-line medication for the induction of ovulation in PCOS women.

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A collection of published results of treatment with clomiphene citrate indicates a pregnancy rate of 36% and a miscarriage rate of 20.4% (Homburg, 2008).

Resistance to clomiphene citrate is a challenging problem occurring in up to 40% of PCOS patients (Homburg, 2008; Pritts, 2002; Wolf, 2000). Clomiphene citrate resistance is commonly defined as failure to ovulate with a maximal dose of clomiphene citrate (150 mg/d for 5 days) and requires a change of treatment mode (Homburg, 2008; van Wely et al., 2005).

Several adjuvants have been used to improve treatment outcomes for women with clomiphene citrate-resistant PCOS, including metformin (Siebert et al., 2006), tamoxifen (Homburg, 2008) and dexamethazone (Elnashar et al., 2006). *N*-acetyl-cysteine (NAC) is a mucolytic drug with insulin-sensitizing properties that has been used successfully as an adjuvant therapy in subjects with clomiphene citrate-resistant PCOS (Rizk et al., 2005). NAC alone, however, was not effective in inducing ovulation (Elnashar et al., 2007). Gonadotrophin therapy has classically been the next step for clomiphene citrate-resistant patients; treatment is associated with risk of ovarian hyperstimulation syndrome (OHSS), multiple gestation and recurrent abortions (Beck et al., 2005; Farquhar et al., 2002).

Laparoscopic ovarian drilling (LOD) is currently accepted as a successful second-line treatment for ovulation induction in clomiphene citrate-resistant women with PCOS (Sastre et al., 2009). LOD can avoid or reduce the need for gonadotrophins. In a recent Cochrane review, no evidence of a difference in the live birth or miscarriage rates in women with clomiphene citrate-resistant PCOS undergoing LOD compared with gonadotrophin treatment was reported (Farquhar et al., 2007). Despite the fact that reduction in OHSS and multiple pregnancy rates in women undergoing LOD makes this option attractive, there are ongoing concerns about its long-term effects on ovarian function (Farquhar et al., 2007) and adhesion formation (Mercorio et al., 2008). Unilateral LOD was found to be equally efficacious as bilateral drilling in inducing ovulation and achieving pregnancy in clomiphene citrate-resistant PCOS women, which makes it a more suitable option because of the reduced potential for adhesion formation (Al-Mizyen and Grudzinskas, 2007; Roy et al., 2009).

The aim of this randomized double-blind placebo-controlled pilot study was to evaluate the role of NAC as an adjuvant therapy after unilateral LOD for clomiphene citrate-resistant PCOS women.

## Materials and methods

### Diagnosis of PCOS

The diagnosis of PCOS was made according to the revised 2003 European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM) Rotterdam criteria with the presence of at least two of the following three features after exclusion of other aetiologies through history, clinical examination and laboratory investigations if needed: (i) oligo- or anovulation, (ii) clinical and/or biochemical hyperandrogenism and (iii) ultrasound findings of polycystic ovaries [presence of $\geq$12 follicles in each ovary measuring 2–9 mm in diameter, and/or increased ovarian volume ($>$10 ml)] (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). Women were considered to have clomiphene citrate-resistant PCOS when they failed to ovulate with clomiphene citrate at a dose of 150 mg/d for 5 days (Homburg, 2008; van Wely et al., 2005).

### Laparoscopic ovarian drilling technique

Triple puncture laparoscopy was performed under general endotracheal anaesthesia using Storz Laparoscopy Equipment (Tuttlingen, Germany). After confirming the diagnosis of polycystic ovaries (enlarged ovaries with smooth pearly white capsule) and careful inspection of all pelvic organs, chromopertubation using methylene blue was carried out to check tubal patency. The equipment for monopolar diathermy consisted of the electrosurgical unit (Erbotom, Erbe, Hamburg, Germany) and pointed needle electrode (Corson combined suction irrigation cannula with a built-in channel for the needle electrode, 1.4 mm, Storz, Germany). Four punctures with a depth of 4 mm were made in the cortex of only the right ovary. The electric current used was set at 40 W and applied for 4 s for each puncture. The power was activated just before touching the ovary, and then the needle electrode was held against the antimesenteric surface of the ovary for 4 s until penetration of the ovarian capsule. The ovary was cooled in the pool of saline after each cauterization both to minimize adhesion formation and to prevent thermal injury to adjacent viscera. Complete haemostasis was ensured. At the end of the procedure, the ovary was copiously rinsed with saline or Ringer's lactate (Aqua-purator, Storz, Germany). About 200 ml of heparinized Ringer's lactate (500 IU/l) was left in the pelvis to avoid postoperative adhesions.

### Setting

This randomized double-blind placebo-controlled pilot study was performed at the Department of Obstetrics and Gynecology, Women's Health Centre, Assuit University, Egypt from January 2005 to June 2007. Institutional review board approval was attained before the beginning of the study. In addition, all randomized women gave their written consent for participation after receiving a full explanation of the study including the potential benefits and side effects.

### Eligibility, inclusion and randomization

Women undergoing unilateral LOD for clomiphene citrate-resistant PCOS were approached. An age range of 18–38 years, with at least 2 years of primary or secondary infertility due to anovulation, patent Fallopian tubes (as shown by hysterosalpingography or diagnostic laparoscopy) and a normal semen analysis (WHO criteria, 1999) served as inclusion criteria. Women should not have received hormonal therapy for at least 3 months before enrolment in the study. Other exclusion criteria included contraindications to laparoscopy or general anaesthesia. Study admission assessment included the following: a thorough history and clinical examination, transvaginal ultrasonographic examination (TVS) and

basal hormonal assays. TVS was carried out using the Siemens Sonoline Sienna Ultrasound Imaging System (Siemens, Germany) using a transvaginal probe (5 MHz) at cycle day 3—7 in oligomenorrhoeic patients and at random in amenorrhoeic patients. On cycle day 3 or at random in amenorrhoeic patients, the following hormones were assayed: luteinizing hormone (LH), follicle stimulating hormone (FSH), LH/FSH ratio and serum prolactin. Hormones were assayed by time-resolved fluoroimmunoassay (dissociation-enhanced lanthanide fluorescent immunoassay; DELFIA; Pharmacia, Wallace OY, and Turku, Finland). All sera were assayed on the same day to avoid interassay variation. Patients had the right to refuse to participate and/or withdraw from the study at any time without being denied their regular full clinical care. Personal information and medical data collected were strictly confidential and were not made available to a third party.

## Experimental protocol

**Figure 1** shows a flow diagram of patients' enrolment, allocation, follow up and analysis. A total number of 74 women with clomiphene citrate-resistant PCOS who underwent unilateral LOD were screened for eligibility. Six patients did not meet the inclusion criteria while eight refused to participate in the study. The remaining 60 patients were randomized to receive either a daily dose of 1.2 g of NAC in oral effervescent form (group A, $n = 30$; acetylcysteine, sachet form; Sedico, Egypt) or placebo (group B, $n = 30$) for 5 days starting at day 3 of the cycle for 12 consecutive cycles. Treatment was started immediately after LOD. The placebo sachets were specially manufactured to look identical to the NAC sachets. The sachets were placed in sacs and then stored in envelopes numbered from 1 to 60. The envelopes were numbered and randomized according to a computer-generated randomization table to ensure an equal number of patients in each arm. Throughout the study, access to the randomization code was available only to the pharmacist who manufactured the placebo and packed the envelopes and was not available to the treating gynaecologist or patients. In all women, cycles were monitored by TVS for the mean follicular diameter and endometrial thickness on days 10, 12, and 14 of the cycle. When one lead follicle attained a diameter of $\geq$18 mm,

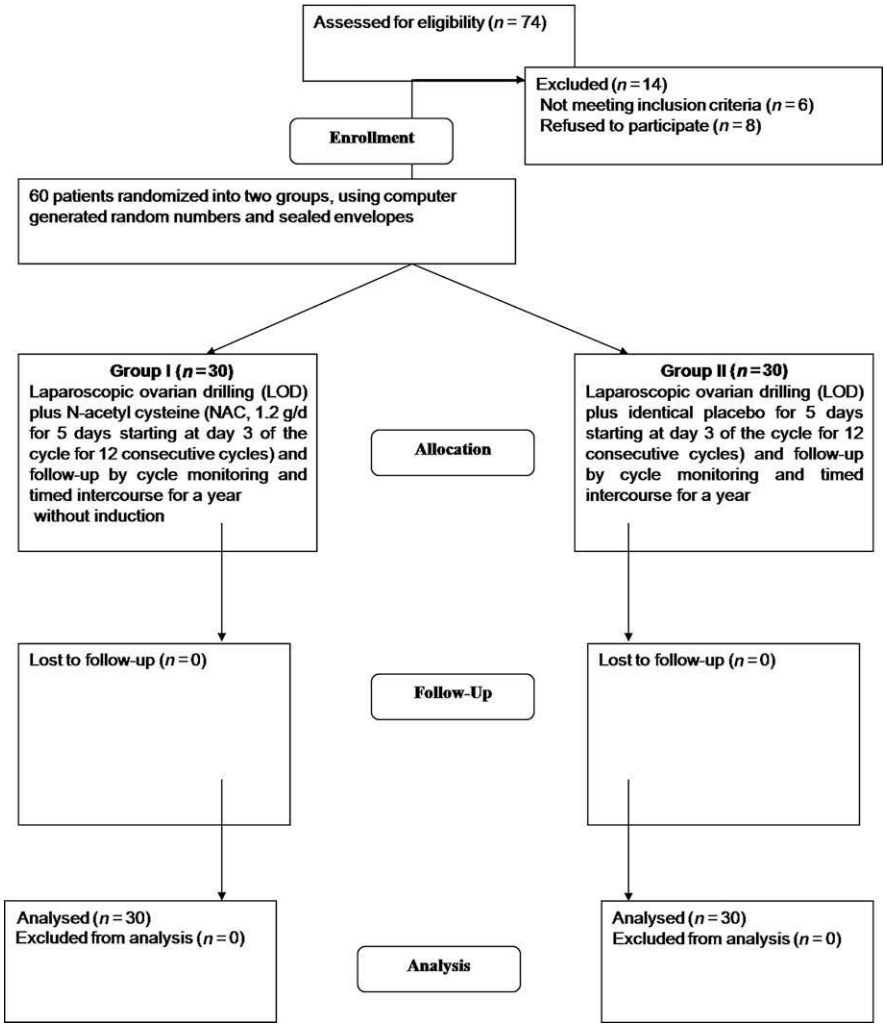


**Figure 1** Flow diagram of patient enrolment, allocation, follow up and analysis of clomiphene citrate-resistant polycystic ovary syndrome patients.

human chorionic gonadotrophin (HCG) injection (Pregnyl, 10,000 IU i.m.; Organon, Holland) was given. Patients were advised to have intercourse 24—36 h after the HCG injection. Criteria of ovulation included the characteristic transvaginal ultrasonography observations with follicle collapse and elevated serum progesterone measured between days 21 and 23 of the cycle by RIA using the antibody coated-tube method (Coat-A-Count; Diagnostic Product Corporation, Los Angeles, CA, USA). Treatment was repeated in patients failing to achieve pregnancy for 12 consecutive cycles. A serum βHCG concentration was determined 14 days after HCG injection. Pregnancy was defined as an increase in the serum βHCG concentration more than 25 mIU/ml or appearance of an intrauterine gestational sac by TVS. Miscarriages were defined as cases with positive βHCG testing who aborted spontaneously until the end of week 28 of gestation.

## Power calculations

The primary outcome was pregnancy rate; secondary outcomes were ovulation rates, endometrial thickness and pregnancy outcome. Sample size estimation was calculated based on the primary outcome. Pregnancy rates after LOD in clomiphene citrate-resistant PCOS patients were reported to be approximately 54% (Kaaijk et al., 1995). Assuming an ongoing pregnancy rate of 54% 12 months after LOD, with an $\alpha$ of 5% and a $\beta$ of 20%, and assuming a 20% difference between the study and control groups, it was calculated that 27 women were required in each arm of the study to detect a true difference at the 95% confidence level with 80% power. Taking dropouts into account, it was estimated that 30 women would be needed in each arm of the study to observe the difference described.

## Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) software, version 15.0 (SPSS, Chicago, IL, USA). Continuous data were described as mean ± standard deviation (SD). Categorical data were summarized as ratios. The independent sample *t*-test was used to assess the significance of the difference between continuous variables in the two groups. The chi-squared test or the Fisher's exact test was used to assess the statistical significance of categorical variables. A *P*-value $<0.05$ was considered statistically significant.

## Results

A total number of 60 women with clomiphene citrate-resistant PCOS who underwent unilateral LOD and were randomized to receive either a daily dose of 1.2 g of NAC (group A = 30) or placebo (group B = 30) for 5 days starting at day 3 of the cycle for 12 consecutive cycles were analysed. **Table 1** shows the clinical, ultrasonographic and hormonal data in both groups of PCOS patients. The two groups had similar baseline characteristics. **Table 2** summarizes the clinical and reproductive outcomes in the two study groups. A significant increase in both ovulation and pregnancy rates was observed in the NAC group, compared with placebo [87% versus 67% (relative risk, RR, 1.3; 95% confidence interval, CI, 1.2—2.7 and 77% versus 57% (RR 1.4; 95% CI 1.1—2.7), respectively, $P < 0.01$]. Likewise, the number of mature ovarian follicles ($\geq$18 mm in diameter) was significantly more in the NAC than the control group (2.9 versus 1.8, $P < 0.05$). Interestingly, the endometrial thickness measured on the day of HCG injection was significantly higher in group A than group B (8.7 versus 6.2 mm, $P < 0.01$). The ongoing pregnancy rate was also significantly higher in the NAC group than controls [70% versus 43% (RR 1.6; 95% CI 1.1—2.8), $P < 0.01$]. Moreover, miscarriage rates were significantly less and live birth rates were significantly higher in the NAC group [8.7% versus 23.5% (RR 0.4; 95% CI 0.1—3.7) and 67% versus 40% (RR 1.7; 95% CI 0.3—3.5), respectively, $P < 0.01$]. All miscarriages were in the first trimester.

**Table 1** Baseline characteristics of the two study groups.

| | *Group A (LOD + NAC) (n = 30)* | *Group B (LOD + placebo) (n = 30)* |
|---|---|---|
| Age (years) | 28.4 ± 4.2 | 29.2 ± 3.7 |
| Couples with primary infertility | 26/30 (87) | 27/30 (90) |
| Duration of infertility (years) | 5.3 ± 1.9 | 4.9 ± 2.1 |
| Women with amenorrhoea | 5/30 (17) | 6/30 (20) |
| Women with oligomenorrhoea | 25/30 (83) | 24/30 (80) |
| Women with hirsutism | 16/30 (53) | 18/30 (60) |
| Body mass index (kg/m$^2$) | 28.6 ± 3.7 | 29.1 ± 4.2 |
| Women with increased ovarian stromal echogenicity | 16/30 (53) | 16/30 (53) |
| Ovarian volume (ml) | 15.4 ± 2.7 | 16.1 ± 2.2 |
| Cycle day-3 serum LH (mIU/ml) | 17.7 ± 4.6 | 18.3 ± 5.5 |
| Cycle day-3 serum FSH (mIU/ml) | 2.7 ± 1.2 | 2.9 ± 1.7 |
| LH/FSH ratio | 5.8 ± 2.6 | 6.0 ± 1.9 |
| Cycle day-3 serum prolactin (ng/ml) | 8.2 ± 3.4 | 7.5 ± 2.8 |

Values are mean ± SD or number/total (%).
LOD = laparoscopic ovarian drilling; NAC = *N*-acetyl-cysteine. There were no statistically significant differences between the two groups.

**Table 2** Clinical and reproductive outcomes of the two study groups (12-month follow-up).

| | *Group A (LOD + NAC) (n = 30)* | *Group B (LOD + placebo) (n = 30)* | *RR (95% CI)* | *P-value* |
|---|---|---|---|---|
| Women resuming ovulation | 26/30 (87) | 20/30 (67) | 1.3 (1.2–2.7) | <0.01 |
| Number of follicles ≥18 mm | 2.9 ± 0.7 | 1.8 ± 0.8 | – | <0.05 |
| Endometrial thickness (mm)[a] | 8.7 ± 1.3 | 6.2 ± 1.4 | – | <0.01 |
| Women achieving pregnancy | 23/30 (77) | 17/30 (57) | 1.4 (1.1–2.7) | <0.01 |
| Number of miscarriages | 2/23 (9) | 4/17 (24) | 0.4 (0.1–3.7) | <0.01 |
| Number of multiple pregnancies | 0 | 0 | – | – |
| Ongoing pregnancy | 21/30 (70) | 13/30 (43) | 1.6 (1.1–2.8) | <0.01 |
| Number of preterm deliveries | 1/30 (3) | 1/30 (3) | – | – |
| Live births | 20/30 (67) | 12/30 (40) | 1.7 (0.3–3.5) | <0.01 |

Values are mean ± SD or number/total (%); LOD = laparoscopic ovarian drilling; NAC = *N*-acetyl-cysteine.
[a]Measured on the day of HCG injection.

## Discussion

Long known as a major cause of chronic anovulation, PCOS and PCOS-related infertility have always been an area of active research. Between the 1930s and the early 1960s surgical treatment of PCOS was the only treatment available. The original procedure was bilateral wedge resection, which required a laparotomy and removal of up to 75% of each ovary, and often resulted in extensive pelvic adhesions. Then clomiphene citrate became the first-line treatment for the management of anovulatory women with PCOS; however, resistance to clomiphene citrate is common. Gonadotrophin therapy has been effective in restoring ovulation and achieving pregnancy in clomiphene citrate-resistant patients. The modern-day minimal-access alternative to gonadotrophin therapy for clomiphene citrate-resistant PCOS is LOD. LOD has therefore, replaced ovarian wedge resection as the surgical treatment of choice for clomiphene citrate-resistant PCOS. Besides being as effective as gonadotrophin treatment with similar live birth and miscarriage rates, the technique has a number of other merits: ease, convenience, reduced risks of gonadotrophins, lack of intensive ultrasound monitoring, increased responsiveness of the ovary to oral ovulation induction agents, sustainability of ovarian activity as shown by consecutive spontaneous ovulations resulting years after LOD and the added value of making a laparoscopic assessment of the pelvis (Balen, 2006; Farquhar et al., 2002; Farquhar et al., 2007).

Various techniques were described for laparoscopic ovarian drilling of PCOS (Gordts et al., 2009). Ovarian drilling by transvaginal fertiloscopy with bipolar electrosurgery appeared to be an effective minimally invasive procedure in patients with PCOS resistant to clomiphene citrate, where 91% of women recovered ovulatory cycles and 60% achieved pregnancy during an 18-month follow-up period (Fernandez et al., 2004).

In the present study, unilateral LOD was preferred to bilateral LOD. Besides being equally effective, in terms of ovulation and pregnancy rates, unilateral LOD reduces the operative time as well as the potential for adhesion formation (Al-Mizyen and Grudzinskas, 2007; Roy et al., in press; Youssef and Atallah, 2007). Minimization of the technique is a welcome addition. Moreover, four punctures with a depth of 4 mm were made in the cortex of only the right ovary, thereby potentially reducing the chances of adhesion formation and premature ovarian failure. Whereas some authors recommended performing 10 holes in each ovary so as not to risk having lower than expected pregnancy rates (Farquhar et al., 2002), others, on the other hand, demonstrated that only four diathermy holes were needed in one ovary to induce ovulation (Balen and Jacobs, 1994).

In the present study, monopolar diathermy was used. Despite claims that monopolar energy can potentially increase the risk of adhesions, a recent experimental study found that LOD using bipolar electrocoagulation causes extensive destruction of the ovary resulting in significantly more destruction per burn than monopolar electrocoagulation (287.6 versus 70 $mm^3$). Again, bipolar electrocoagulation results in significantly more tissue damage than monopolar coagulation (2,876 versus 700 $mm^3$) and the authors concluded that the first choice for LOD would be monopolar electrocoagulation or $CO_2$ laser, given the same clinical effectiveness of the various procedures (Hendriks et al., in press).

In a meta-analysis of LOD, pregnancy rates have been reported to be 56% (Donesky and Adashi, 1995); however, individual case series have reported pregnancy rates as high as 88% (Tiitinen et al., 1993). This discrepancy is expected for new treatment strategies and emphasizes the continued and urgent need for careful evaluation with RCT (Farquhar et al., 2002).

In the present study, a mean pregnancy rate of 57% was reported 12 months after unilateral LOD, without using NAC, which is similar to that reported in a recent Cochrane review (Farquhar et al., 2007).

The present randomized double-blind placebo-controlled pilot study has demonstrated that cyclic use of NAC as an adjuvant treatment after unilateral LOD for 12 consecutive cycles resulted in a significantly higher ovulation (87%), pregnancy (77%) and live birth rates (67%) and a significantly lower miscarriage rate (8.7%). Increased body mass index in PCOS is associated with poorer reproductive outcomes, mostly due to insulin resistance (Farquhar et al., 2002). In the present study, women were overweight, which might have augmented the value of NAC as an insulin sensitizer.

As far as is known, this is the first published RCT to evaluate the role of NAC in women undergoing unilateral LOD for clomiphene citrate-resistant PCOS. NAC is a simple, safe mucolytic drug that was shown to exert insulin-sensitizing effects in clomiphene citrate-resistant PCOS women (Fulghesu et al., 2002; Kilic-Okman and Kucuk, 2004). It was used successfully as an adjunctive therapy to clomiphene citrate (Badawy et al., 2007; Rizk et al., 2005). However, NAC alone, unlike metformin, was not effective in inducing ovulation in women with clomiphene citrate-resistant PCOS (Elnashar et al., 2007). In the present study, a similar positive impact of NAC on reproductive outcomes was witnessed after unilateral LOD, which suggests that NAC functions as an adjuvant second player with LOD, akin to clomiphene citrate, in women with clomiphene citrate-resistant PCOS. The improved endometrial thickness with NAC use could be explained by its positive impact on follicular dynamics and ovarian oestradiol production in the follicular phase. In fact, the synergy in action of NAC and FSH was found to play an important role in follicle growth of ovarian tissue cultures, where a well-preserved preantral follicle was found, for the first time, in a culture of frozen–thawed human ovarian tissue (Fabbri et al., 2007). The reduced miscarriage rate, improved ongoing pregnancy and live birth rates seen in the current study has also been reported by other investigators. Due to the potent antioxidant properties of NAC, it was found to significantly increase the rate of continuation of a living pregnancy up to and beyond 20 weeks and the take-home baby rate in women with unexplained recurrent pregnancy loss (Amin et al., 2008). Moreover, NAC exerted a salient protective effect against fetal death and preterm labour induced by maternal inflammation in mice (Buhimschi et al., 2003). Although NAC resulted in a significant increase in the number of follicles $\geq$18 mm in diameter, there were no cases with multiple pregnancy in either group.

In the present study, all eligible women were approached and women rarely declined to enter the study. All recruited women were available for analysis. All operations have been carried out by the same surgeon (AN) using the very same technique on the same side to ensure consistency. The conclusions drawn from this study are limited by the small number of women recruited and the relatively short follow-up period (12 months). Undoubtedly, the need for larger RCT addressing this issue is overwhelming and can't be overemphasized.

In conclusion, this study has demonstrated that NAC may help improve the reproductive outcome in women undergoing unilateral LOD for clomiphene citrate–resistant PCOS. It is a safe well-tolerated drug that could be a potentially effective adjuvant treatment for such women, as has been previously witnessed with clomiphene citrate. The results of a larger RCT are, however, necessary before generalization of such a conclusion becomes plausible.

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