ARTICLE

Combined coenzyme Q10 and clomiphene citrate for ovulation induction in clomiphene-citrate-resistant polycystic ovary syndrome

Abdelaziz El Refaeey a, Amal Selem b, Ahmed Badawy a, *

Abstract This prospective randomized controlled trial evaluated the effect of combined oral coenzyme Q10 (CoQ10) and clomiphene citrate for ovulation induction in clomiphene-citrate-resistant polycystic ovary syndrome (PCOS). A total of 101 infertile women with PCOS resistant to clomiphene citrate were randomized either to combined CoQ10 and clomiphene citrate (51 patients, 82 cycles) or to clomiphene citrate alone (50 patients, 71 cycles). The outcome measures were number of follicles, serum oestradiol, serum progesterone, endometrial thickness and ovulation, clinical pregnancy and miscarriage rates. Numbers of follicles >14 mm and ≥18 mm were significantly higher in the CoQ10 group. Endometrial thickness on the day of human chorionic gonadotrophin was significantly greater in the CoQ10 group (8.82 ± 0.27 mm versus 7.03 ± 0.74 mm). Ovulation occurred in 54/82 cycles (65.9%) in the CoQ10 group and 11/71 cycles (15.5%) in the control group. Clinical pregnancy rate was significantly higher in the CoQ10 group (19/51, 37.3%) versus the control group (3/50, 6.0%). Combination of CoQ10 and clomiphene citrate in the treatment of clomiphene-citrate-resistant PCOS patients improves ovulation and clinical pregnancy rates. It is an effective and safe option and can be considered before gonadotrophin therapy or laparoscopic ovarian drilling.

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KEYWORDS: clomiphene citrate, coenzyme Q10, PCOS

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Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in reproductive age women. It is now well evidenced that PCOS is associated with obesity, insulin resistance, hyperandrogenism, abnormal lipid profile and oxidation stress (Imani et al., 1998; Kuscu and Var, 2009). Recently, PCOS was reported to be associated with mitochondrial dysfunction which has a negative impact on oocyte quality, compromising melotic spindle configuration and chromosomal misalignment and eventually causing oocyte death (May-Panloup et al., 2005, 2007). About 20–25% of PCOS women fail to ovulate with incremental doses of clomiphene citrate (Hull, 1987). Ovulation induction with gonadotrophins is the standard treatment for clomiphene-citrate-resistant women. This approach, however, is associated with complications and has the added disadvantage of high cost and need for careful monitoring. Hence, there is a clear need for an alternative less expensive therapy.

Coenzyme Q10 (CoQ10) is a fat-soluble coenzyme, found in the inner mitochondrial membrane, that plays a crucial role in the production of cellular energy and acts as an antioxidant. It has been used as a dietary supplement in the treatment of many disorders, such as cardiovascular diseases, high cholesterol and diabetes mellitus, and to improve immune function in people with HIV or AIDS and to act as anticancer agent in breast cancers (Buyukkaya et al., 2013; Cooney et al., 2011; Digiesi et al., 1994; Folkers et al., 1988; Kolahdouz Mohammadi et al., 2013; Lee et al., 2012; Wang et al., 2009). It is also widely used as a component in cosmetic products as some shampoos and conditioners (Portakal et al., 2000; Turunen et al., 2004). Evidence has been accumulating for a role of CoQ10 in the treatment of mitochondrial disorders from animal studies, as CoQ10 can greatly revitalize eggs in aged mice (Bentov et al., 2010). It is unknown if this improvement in eggs demonstrated in mice occurs in humans. The present prospective randomized controlled study was conducted to evaluate the effect of combination of coenzyme Q10 with clomiphene citrate in clomiphene-citrate-resistant PCOS.

Materials and methods

The study comprised of 110 women (153 cycles) with clomiphene-citrate-resistant PCOS among those attending the Fertility Outpatient Clinic in Mansoura University Hospitals, Mansoura University, Egypt and a private practice setting in the period from January 2010 to January 2013. Diagnosis of PCOS based on the revised 2003 consensus on diagnostic criteria and long-term health risks related to PCOS (The Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group, 2004). All women were previously treated with 150 mg clomiphene citrate daily for 5 days per cycle, for two or three cycles with persistent anovulation or ovulate with very thin endometrium (<5 mm) on the day of HCG administration (Homburg, 2005). The exclusion criteria of this study included patients with hyperprolactinaemia, hypercorticism and thyroid dysfunction. Patients receiving medications such as cholesterol-lowering drugs, beta-blockers and tricyclic antidepressants were also excluded. All women had patent Fallopian tubes proved by hysterosalpingography and normal semen analysis for their partners according to the modified criteria of World Health Organization (2010). The study was approved by the departmental ethical committee and all participants gave informed consent before inclusion in the trial (committee reference no. 231, approved December, 12 2009).

Patients were randomly allocated using a computer-generated random table and sealed envelopes into two groups: CoQ10 and clomiphene citrate (55 patients enrolled, of whom four patients dropped out, 82 cycles included) and clomiphene citrate only (55 patients enrolled of whom five patients dropped out, 71 cycles included). Allocation process was done by a third party (nurse). Withdrawal bleeding was achieved using 10 mg dydrogesterone tablets (Duphaston; Alexandria, Egypt) for 10 days before stimulation. CoQ10/clomiphene citrate patients received clomiphene citrate (Global Napi, Cairo, Egypt) 150 mg/day from cycle days 2–6 and CoQ10 (Mepaco; Enshas Elraml, Sharkia, Egypt), in a dose of 60 mg capsules orally t.i.d. starting on cycle day 2 and until the day of human chorionic gonadotrophin (HCG) administration. Choice of dose and duration of administration of CoQ10 was chosen arbitrarily because of the lack of previous human experience. Patients in the control group received clomiphene citrate 150 mg/day from cycle day 2 for 5 days. No patients received ovulatory medication in the 3 months previous to the study. All participants were instructed not to take any medications during the trial except after consultation of the infertility physician.

All patients were monitored by transvaginal ultrasound for follicular diameter and endometrial thickness on cycle days 10, 12 and 14. The physicians monitoring the cycles were blinded to the protocol of each group. Serum oestradiol (pg/ml) on the day of HCG injection was measured by radioimmunoassay (RIA) using direct double-antibody kits (Pantex, Santa Monica, CA, USA) and serum progesterone (ng/ml) was measured on cycle days 21–23 by RIA using an antibody-coated-tube method (Coat-A-Count; Diagnostic Product Corporation, Los Angeles, CA, USA). HCG injection (5000–10,000 IU i.m.; Premyn; Organon, Oss, Holland) was given when at least one follicle measuring at least 18 mm was found. Patients were advised to have intercourse 24–36 h after HCG injection. Serum HCG was determined 2 weeks after HCG injection in the absence of menstruation for diagnosis of pregnancy. Growing follicles were defined as those <14 mm and mature follicles ≥18–22 mm. Ovulation was defined by transvaginal ultrasound as the disappearance of the leading follicle, presence of follicular fluid in the Douglas pouch and midluteal progesterone >5 pg/ml.

The primary outcome measures were the number of growing and mature follicles, serum oestradiol, serum progesterone, ovulation rate and endometrial thickness. Secondary outcome measures were the occurrence of clinical pregnancy (ultrasound visualization of gestational sac with pulsating fetal pole) and miscarriage (spontaneous termination of a clinical pregnancy before 20 weeks of gestation).

Statistical analysis

Before starting the study, sample size calculation was done. Fifty cases were needed in each arm to gain a significant
difference of 22% in pregnancy rate at a significant level of 5% and a study power of 80%. Data obtained were statistically analysed using the Statistical Package for Social Sciences (SPSS, Chicago, IL, USA). Proportions were analysed by chi-squared test while mean and standard deviation was analysed by Student’s t-test. Differences were considered significant when \( P < 0.05 \).

**Results**

The study comprised 110 patients (153 cycles) in total. There were no statistically significant differences between the two groups with regard to age, parity, duration of infertility, body mass index, the presenting symptoms and signs and hormonal profile (Table 1).

The number of follicles \( > 14 \) mm and \( > 18 \) mm were significantly higher in the CoQ10 group \( (P < 0.05 \) and \( P < 0.001 \) respectively; Table 2). The endometrial thickness on the day of HCG administration was significantly greater in the CoQ10 group \( (8.82 \pm 0.27 \) mm versus \( 7.03 \pm 0.74 \) mm, \( P < 0.001 \)). Ovulation occurred in 54/82 cycles \( (65.9%) \) in the CoQ10 group and 11/71 cycles \( (15.5%) \) in the control group, which was significantly different \( (P < 0.001 \). Serum oestradiol and progesterone were significantly higher in the CoQ10 group \( (P < 0.05 \) and \( P < 0.001 \) respectively. In the CoQ10 group, clinical pregnancy occurred in 19/51 women \( (37.3\%) \), of which two ended in miscarriage, and in the control group, clinical pregnancy occurred in 3/50 women \( (6\%) \), with no miscarriages \( (P < 0.001 \) (Table 2). One twin pregnancy occurred in the CoQ10 group and none in the control group. No higher-order pregnancies or ovarian hyperstimulation syndrome occurred in both groups.

In the CoQ10 group, in the first treatment cycle, out of 51 women, 15 women conceived \( (29.4\%) \) and in the second treatment cycle, out of 31 women, four patients conceived \( (12.5\%) \). In the control group, in the first treatment cycle, out of 50 women, two conceived \( (4\%) \) and in the second treatment cycle, out of 21 women, one conceived \( (4.8\%) \). The mean duration of CoQ10 treatment in the first cycle was 16.2 \pm 2.1 days and in the second cycle 17.1 \pm 2.9 days.

In the CoQ10 group, 21 women with lean PCOS underwent 33 cycles, and the ovulation rate was 63.6% and the clinical pregnancy rate was 38.1%. Of 30 women with obese PCOS, the ovulation rate was 67.3% and the clinical pregnancy rate was 36.7%. Endometrial thickness was significantly increased \( (P < 0.0001 \) in women with lean PCOS versus obese PCOS. There were no statistically significant differences regarding number of follicles and serum oestradiol and midluteal progesterone concentrations (Table 3). When the control group was stratified into lean (17 women, 34 cycles) and obese (31 women, 37 cycles) women, no statistically significant differences were found in number of follicles, endometrial thickness, serum oestradiol and midluteal progesterone concentrations, ovulation rate \( (20.6\% \) versus 10.8\%) or clinical pregnancy rates \( (5.9\% \) versus 2.7%) (Table 4).

**Discussion**

As far as is known, this is the first report of the potential reproductive effects of CoQ10 in human females. Animal studies have reported that CoQ10 increases the reproductive lifetime of female mice by about 30% and that animals that receive more CoQ10 produce more and healthier eggs and show improved ovarian response and various consistent hormonal changes (Bentov et al., 2010).

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**Table 1** Patient characteristics.

<table>
<thead>
<tr>
<th>CoQ10-clomiphene citrate ( (n = 51) )</th>
<th>Clomiphene citrate ( (n = 50) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cycles</td>
<td>82</td>
</tr>
<tr>
<td>Age (years)</td>
<td>27.54 \pm 6.31</td>
</tr>
<tr>
<td>Parity</td>
<td>0.4 \pm 0.31</td>
</tr>
<tr>
<td>Clinical presentation</td>
<td></td>
</tr>
<tr>
<td>Oligo/anovulation</td>
<td>43 ( (84.3%) )</td>
</tr>
<tr>
<td>Hyperandrogenism</td>
<td>24 ( (47.1%) )</td>
</tr>
<tr>
<td>Polycystic ovaries</td>
<td>39 ( (76.5%) )</td>
</tr>
<tr>
<td>Duration of infertility ( (\text{years}) )</td>
<td>4.31 \pm 1.86</td>
</tr>
<tr>
<td>BMI ( (\text{kg/m}^2) )</td>
<td>29.31 \pm 3.92</td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>8.21 \pm 4.19</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>8.55 \pm 4.25</td>
</tr>
</tbody>
</table>

Values are mean \( \pm \) SD or \( n \) \( (\%) \). There were no statistically significant differences between the groups. BMI = body mass index.

**Table 2** Treatment outcomes.

<table>
<thead>
<tr>
<th>CoQ10-clomiphene citrate ( (n = 51) )</th>
<th>Clomiphene citrate ( (n = 50) )</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of follicles ( &gt; 14 ) mm</td>
<td>1.94 \pm 0.25</td>
<td>0.13 \pm 0.29</td>
</tr>
<tr>
<td>No. of follicles ( &gt; 18 ) mm</td>
<td>1.85 \pm 0.27</td>
<td>1.30 \pm 0.32</td>
</tr>
<tr>
<td>Endometrial thickness on day of HCG ( (\text{mm}) )</td>
<td>8.82 \pm 1.49</td>
<td>7.03 \pm 0.74</td>
</tr>
<tr>
<td>Serum oestradiol on the day of HCG ( (\text{pg/ml}) )</td>
<td>168.93 \pm 75.01</td>
<td>138.32 \pm 70.24</td>
</tr>
<tr>
<td>Midluteal progesterone ( (\text{pg/ml}) )</td>
<td>10.2 \pm 1.03</td>
<td>8.9 \pm 0.91</td>
</tr>
<tr>
<td>Ovulation per cycle</td>
<td>54/82 ( (65.9%) )</td>
<td>11/71 ( (15.5%) )</td>
</tr>
<tr>
<td>Clinical pregnancy per patient</td>
<td>19 ( (37.3%) )</td>
<td>3 ( (6.0%) )</td>
</tr>
</tbody>
</table>

Values are mean \( \pm \) SD or \( n \) \( (\%) \).
The oocyte has the largest number of mitochondria of any cell, (approximately $2 \times 10^5$ copies). The functional status of the mitochondria contributes to the quality of oocytes and plays an important role in the process of fertilization and embryo development (Cummins, 2004). Failure of the oocyte to increase the number of mitochondria during maturation may lead to a poor embryo outcome (May-Panloup et al., 2007; Victor et al., 2009). Mitochondrial dysfunction may contribute to the onset of metabolic syndrome including obesity, insulin resistance, abnormal lipid profile and an increased risk of coronary heart diseases later in life (Luce et al., 2010).

The results of the present study demonstrate that combined therapy with CoQ10-clomiphene citrate significantly improves ovulation rates in clomiphene-citrate-resistant PCOS women. There are a number of potential mechanisms by which CoQ10 could improve ovarian functions in clomiphene-citrate-resistant PCOS. Firstly, CoQ10 acts directly on the mitochondria, probably related to electron transfer in the respiratory chain, and plays a crucial role in the production of cellular ATP (Turunen et al., 2004). Secondly, CoQ10 is the only lipid-soluble antioxidant that is synthesized in human bodies and it could reduce oxidation stress within the ovary and protect DNA from free radical induced oxidative damage. CoQ10-treated mice have significantly higher CoQ10 concentration within their ovaries and ovarian veins and their ovaries contain less reactive oxygen species (Luce et al., 2010). CoQ10 acts as antioxidant itself, and in its reduced form, ubiquinol, it inhibits lipid peroxidation in biological membranes, and in low-density lipoproteins it also protects membrane proteins against oxidative damage. Ubiquinol can regenerate vitamin E from its oxidized form; this interaction with vitamin E is thought to be particularly important for the protection of low-density and other lipoproteins against oxidative damage (Bentinger et al., 2007; Portakal et al., 2000). Fourthly, CoQ10 is a micronutrient, and the extent of tissue uptake of CoQ10 correlates with the degree of tissue deficiency. Finally, it acts as antiapoptotic, and it is well known that apoptosis is the main mechanism involved in follicular cohort atresia. Microinjection of small numbers of mitochondria into mouse oocytes prevents the onset of apoptosis (Ochiai et al., 2007).

In this study, endometrial thickness was significantly increased in the CoQ10 group versus the control group, probably related to higher oestradiol concentration as a result of better ovulatory response. The results demonstrate that combined therapy with CoQ10-clomiphene citrate significantly improves clinical pregnancy rates in clomiphene-citrate-resistant PCOS women. The discrepancy between ovulation rate and clinical pregnancy rate among CoQ10-clomiphene citrate-treated women may be due to adverse effects of clomiphene citrate on the endometrium as well as on the cervical mucus. The improved clinical pregnancy rate in the CoQ10 group compared with the control group is probably related to improved ovarian response.
in the CoQ10 group. The use of CoQ10 in in-vitro culture of bovine embryos results in superior rate of early embryo cleavage and blastocyst formation, higher percentage of expanding blastocysts and a larger inner cell mass (Gosden, 2002; Perez et al., 2000). Also, the potential for embryo implantation is correlated with the ATP content of the embryo (Lerkom et al., 1995; Van Blerkom et al., 2000).

CoQ10 was well tolerated by all the patients and no adverse effects were observed. The lack of any statistically significant differences in the ovulation and clinical pregnancy rates between lean and obese PCOS in the CoQ10 group suggest that response to CoQ10 is independent of body weight, contrary to other methods of ovulation induction such as gonadotrophins. However, further studies with a large number of patients are recommended to verify these findings. To avoid bias, patients in the control group were informed not to take any medication unless with the permission of the treating physician while receiving clomiphene citrate, particularly CoQ10, as well as not to take medications known to affect CoQ10 such as beta-blockers and tricyclic antidepressants.

The results of this study are encouraging; however, the appropriate dosage of CoQ10 and the optimal duration of treatment needs to be further investigated. Moreover, the effects of CoQ10 therapy on hormonal and metabolic profiles, the symptoms of hyperandrogenism and cardiovascular risk factors need further assessment as to whether it is possible to modify these risk factors, particularly in PCOS. Comparing CoQ10 with other medical methods of ovulation induction in cases of clomiphene-citrate-resistant PCOS, CoQ10 is not as time consuming as metformin, which requires 1–6 months, and it is not as expensive as gonadotrophins and does not need intensive monitoring during and after treatment. So CoQ10 may be a valuable alternative in developing countries and for women aged >35 years who require a more rapid treatment protocol.

In conclusion, CoQ10 seems to be a promising adjuvant to oral ovulatory agents such as clomiphene citrate. Combination of COQ10 and clomiphene citrate is proved to be effective, inexpensive and safe for stimulating follicular development in clomiphene-citrate-resistant PCOS and can be tried successfully before a more complicated treatment such as gonadotrophins and laparoscopic ovarian drilling.

**Trial registration**

This trial is registered at ClinicalTrials.gov (ID NCT01910766).

**References**


Declaration: The authors report no financial or commercial conflicts of interest.

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