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The effect of coenzyme Q_{10} supplementation on partner pregnancy rate in infertile men with idiopathic oligoasthenoteratozoospermia: an open-label prospective study

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Abstract
Objective It has been shown that coenzyme Q_{10} (CoQ_{10}) supplementation in men with idiopathic oligoasthenoteratozoospermia (OAT) results in improved semen parameters. In present study, we evaluated the effects of coenzyme CoQ_{10} supplementation on semen parameters and pregnancy rates in infertile men with idiopathic OAT.
Patients and methods Two hundred and eighty-seven infertile men with idiopathic OAT were recruited in this study. These patients were treated with CoQ_{10} 300 mg orally twice daily for 12 months. Two semen analyses and determination of resting levels of sex hormones were done in all participants. Patients were followed up for another 12 months after CoQ_{10} discontinuation.
Results Mean sperm concentration, sperm progressive motility, and sperm with normal morphology improved significantly after 12-month CoQ_{10} therapy by 113.7, 104.8, and 78.9%, respectively (all Ps $<$ 0.05). The overall pregnancy rate was 34.1% within a mean of 8.4 $\pm$ 4.7 months.
Conclusions CoQ_{10} supplementation improves semen quality with beneficial effect on pregnancy rate.

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Keywords CoQ_{10} · Supplementation · Pregnancy rate · Male factor infertility · Treatment · Semen parameters

Abbreviation

OAT	Oligoasthenoteratozoospermia
CoQ_{10}	Coenzyme Q_{10}
LH	Luteinizing hormone (LH)
FSH	Follicle-stimulating hormone
PRL	Prolactin
TSH	Thyroid-stimulating hormone
WHO	World Health Organization
SOD	Superoxide dismutase
NBT	Nitroblue tetrazolium
hCG	Human chorionic gonadotropin
TEAEs	Treatment emergent adverse events

Introduction

Infertility affects 10–15% of couples, nearly 80 million couples worldwide [1, 2]. Male factors contribute to approximately 50% of cases of infertility. Studies on the causative factors of male factor infertility demonstrate that at least 30% of the patients suffer from idiopathic (not diagnosed) infertility [3]. Idiopathic oligoasthenoteratozoospermia (OAT) remains an emotionally and financially distressing situation facing many couples looking for reproductive assistance. Infertility can cause significant

tensions on interpersonal relationships and has been considered as the most stressful incident in the lives of infertile couples [4].

Sperm cells are capable of generating reactive oxygen species (ROS). Inequity between the production of ROS and the antioxidative mechanisms can cause cellular damage [5]. Some antioxidants, such as vitamins E and C, glutathione, and N-acetyl cysteine, have been used in the treatment for idiopathic male factor infertility with various efficacies [6].

Coenzyme Q_{10} (2,3-dimethoxy-5-methyl-6-decaprenyl-1,4-benzoquinone, CoQ_{10}) as a dietary, nutraceutical supplement has increased noticeably in the last decade. CoQ_{10} is a crucial electron carrier in the mitochondrial respiratory chain. It acts as an essential antioxidant and protects membrane phospholipids from lipid peroxidation [7]. In addition, CoQ_{10} is able to recycling and regenerating other antioxidants such as tocopherol and ascorbate [8]. It has been shown that the exogenous administration of CoQ_{10} in patients with idiopathic asthenozoospermia is effective in improving sperm kinetic features [9]. In our previous double-blind placebo-controlled, and randomized study, CoQ_{10} administration 300 mg/day significantly improved all three semen parameters (density, motility, and morphology) [10]. Apparently study outcomes based only on improvement in semen values are not convenience. A more accurate outcome measure would probably be the pregnancy rate, since that is the ultimate goal of infertility treatment. Yet while using pregnancy rate as the principal goal of therapy end points, it is imperative to take into account the spontaneous pregnancy rate in infertile couples on no treatment [11]. The objectives of this study were twofold: (1) to compare semen parameters in men with OAT with baseline values and (2) to determine whether supplementation of coenzyme Q_{10} affects the spontaneous pregnancy rates in couples with idiopathic male factor infertility.

Materials and methods

Study participants

From January 2005 to July 2007, a total of 354 consecutive and unselected infertile men aged 26–43 years (mean 32 ± 8) with idiopathic OAT were recruited for screening. They were referred by physicians or addressed themselves to our clinic for treatment. All had been married for more than 3 years and had had a minimum of 2 years of unprotected intercourse. In order to confirm the diagnosis of OAT, at least two semen analyses were performed at 1-month interval to eliminate inadvertent and possible adverse effects of various issues on spermatogenesis. Male infertility was diagnosed using World Health Organization (WHO) criteria [12]. The normal WHO values included $\geq 20 \times 10^6$/ml concentration with grade A motility in 25% or grade A + B motility in 50% of spermatozoa and normal morphology in at least 30% of the spermatozoa. In all cases, the wives had undergone gynecologic workups and were found to be fertile.

These patients had received at least two failed previous medications for improvement of semen values, each for at least 6 months. None of the participants received any treatment for infertility for at least 6 months prior to enrollment. Because of ethical reasons and the obligation to provide the best available treatment, this study lacks control groups. This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by the local Medical Ethic Committee at the participating site. Verbal informed consent was obtained for each couple. Verbal consent was witnessed and formally recorded.

Inclusion and exclusion criteria

Only men with idiopathic OAT and without a known cause for their infertility were included. Other inclusion criteria consisted of a sperm count of greater than 5×10^6/ml, more than 2 years of failed attempts at conception and no female factors.

Exclusion criteria included wife aged more than 40 years, abnormal testes on physical examination, total testicular volume <12 ml on ultrasound; alcohol, drug, or substance abuse; severe general diseases and endocrinopathies; and a history of cancer chemotherapy, or testosterone or antiandrogen use. Other exclusion criteria were as follows: Y chromosome deletions, abnormal karyotypes, tobacco use, and concomitant medical problems known to be associated with diminished fertility. Azoospermic patients, patients with body mass index of >30 kg/m^2 or <18 kg/m^2, and men who reported having used nutritional supplements during the previous year were also excluded.

Evaluations

After providing a complete medical and reproductive history, all the participants underwent a thorough physical examination, urine analysis and serum chemicals, and hematological laboratory tests. The resting levels of the following hormones were determined: total testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), thyroid-stimulating hormone (TSH), and inhibin B. At baseline, blood and seminal plasma samples were also analyzed for total CoQ_{10} using high-performance liquid chromatography. Genetic testing included peripheral blood karyotype and Y chromosome analyses in patients with a sperm count of less than 10×10^6/ml.

All of the wives received a complete infertility workup to rule out female factors.

Semen analysis

At baseline, at least two semen analyses were performed for each man. When values differed by more than 20%, a third test was done. The analyses of semen were performed by two technicians. The inter-observer coefficient of variation was 8% ($3.2 \pm 12\%$) for sperm density, 10% ($3.4 \pm 14\%$) for sperm motility, and 7% ($2.5 \pm 11\%$) for sperm morphology. This laboratory maintains weekly means quality control charts and participates in an external Quality Control Program for laboratories.

Following an instructed abstinence period of 3 days, each subject provided a semen sample into a sterile wide-mouth and metal-free plastic container. An average of two for each subject and each sperm parameter was taken for study purpose. The interval of time between the collections was 1 month. Each sample was tested at least twice for sperm concentration, motility, and morphology. All semen analyses were performed in accordance with established 1999 WHO guidelines, besides sperm morphology, which was evaluated using the Kruger parameters [13]. The normal WHO values included a volume of more than 2.0 ml, sperm concentration of 20×10^6 spermatozoa/ml or more, and motility of 50% or more with forward progression. Using the Kruger strict criteria, men with greater than 14% normal forms were regarded normal.

Of 354 screened subjects, 326 met inclusion and exclusion criteria and consented to proceed with study protocol.

Study intervention

The study consisted of a 2-month screening phase, a 12-month treatment phase, and a 12-month treatment-free period (Fig. 1). Eligible patients received 600 mg CoQ_{10} (Nutri Q10, Nutri Century, Toronto, Canada) orally daily for a minimum of 12 months in an open-label fashion. It has been showed that CoQ_{10} is well tolerated and safe for healthy adults at intake of up to 900 mg/day [14]. Medication was taken after lunch. No specific dietary regimen recommendations were given, but participants were instructed not to change their dietary during the study period. Compliance was assessed in two ways: first, by counting the number of capsules in each follow-up visits, and second, by determining plasma levels of CoQ_{10}. Patients acted as their own controls since we compared the effect of CoQ_{10} supplementation on pregnancy rate with time. All patients had their medication until their wives were pregnant or for a maximum of 12 months. No other fertility therapy was allowed neither for patients nor their partners. Couples were advised to engage in natural intercourse but had sexual intercourse on the expected ovulation day.

Follow-up

Patients and their wives were seen on the first day of CoQ_{10} administration and returned for a follow-up visit every 28 days during whole study period. Serum and seminal plasma CoQ_{10} level were determined sporadically, four times in each participant during CoQ_{10} administration, and 12-month follow-up periods. The seminal plasma enzymatic antioxidant levels of catalase and superoxide dismutase (SOD) were also assessed at baseline and every 3 months during study period. Seminal plasma antioxidant status was measured as described elsewhere [15]. In brief, SOD-like activity was measured by the inhibition of nitroblue tetrazolium (NBT) reduction by the combination xanthine + xanthine oxidase [16]. Catalase-like activity was measured by the decrease in concentration of H_2O_2 after incubation with the test samples using the method described by Yeung et al. [17]. Semen analyses were done 30 days before therapy, at the beginning of therapy and every 3 months during study period.

Following beginning of the study, to assess fertility outcome, women were asked by regular visits (every 28 days) to complete a health questionnaire. This

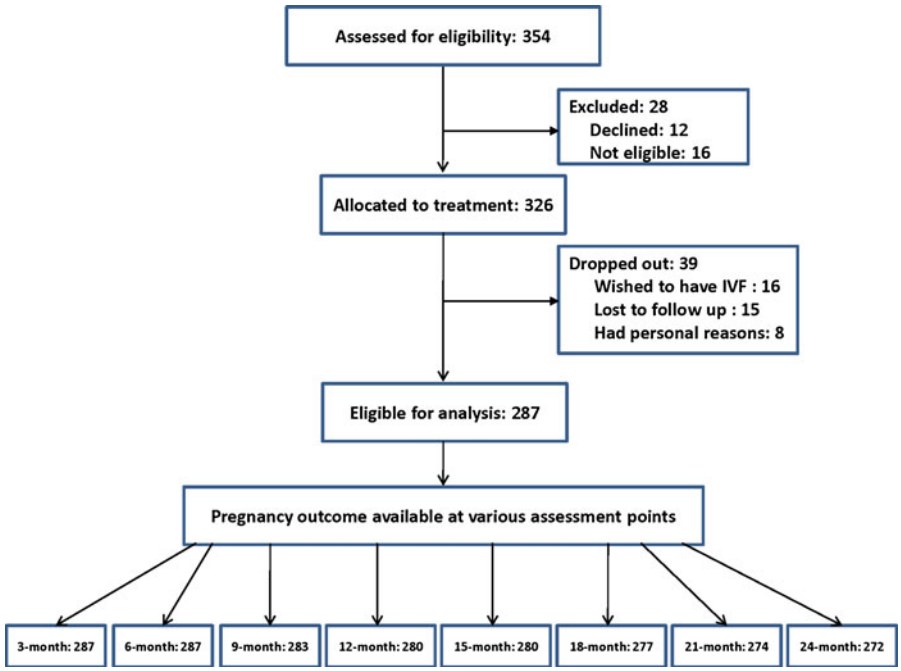

Fig. 1 Subject flow chart. *IVF* in vitro fertilization

questionnaire survey collected data regarding pregnancy, including date of last normal menstrual period, serum human chorionic gonadotropin (hCG) level, and ultrasound confirmation of clinical pregnancy. Pregnancy testing was performed by the quantitative measurement of serum hCG level in the absence of menstruation. A clinical pregnancy was defined as the presence of a gestational sac and fetal heart rate motion on transvaginal ultrasound scanning or histological confirmation of gestational product in miscarriages.

Outcomes

The primary endpoint with respect to the efficacy of CoQ_{10} administration was clinical pregnancy and live birth. The secondary outcome was to determine whether the different semen parameters influenced the pregnancy rate.

Safety assessment

Patients were asked to report all treatment emergent adverse events (TEAEs), which were assessed by the investigator using the medical dictionary for regulatory activities (version 5.0) for severity and relationship with the study drug. TEAEs were defined as any adverse event that first occurred or worsened after CoQ_{10} administration. Participants were asked at each visit to describe any adverse events that had occurred since the previous visit. Patients voluntarily reported adverse events throughout the study period.

Statistical analysis

For outcome analysis, only men with a minimum follow-up of 3 months were included in the present study. A priori power analysis was performed. For an expected pregnancy rate of 20%, a sample size of 270 patients was required for a statistical power of 90% ($\beta = 0.15$) at a P level of 0.05. Thus, assuming an overall 20% dropout rate, 324 men would be included. Univariate analyses were done using Student's t test for continuous variables and the χ^2 or Fischer's exact test for dichotomous variables when appropriate. Kolmogorov–Smirnov test was used for normality of all data, and the cube root transformation was applied to sperm density and total sperm count. Correlations were analyzed using Spearman's rank correlation coefficient. Odds ratios (ORs) and associated 95% confidence intervals (95% CIs) were calculated by logistic regression to estimate the chance of achieving pregnancy by measured variables. The Statistics Package for Social Science (SPSS 16.0; SPSS Inc.,

Chicago, IL) was used for statistical analyses. Statistical significance was set at $P < 0.05$.

Results

Baseline demographic and clinical characteristics of the participants are shown in Table 1. During the study period, 39 patients (12%) dropped out for various reasons (Fig. 1). Sixteen patients wished to have in vitro fertilization (IVF), 15 lost to follow-up, and eight had personal reasons for discontinuing. All of the remainder 287 patients completed 3-month follow-up and therefore were eligible for analysis. The age of men ranged from 26 to 43 years with mean of 32.8 ± 6.8 years; whereas the age of the female partner ranged from 22 to 37 years with mean of 29.4 ± 4.2 years. The number of coitus ranged from 2 to 5 times per week with mean of 4 ± 2 times. The mean value of testicular volume did not show any significant post-treatment difference.

Table 1 Patients' characteristics in the study at baseline

Variables	Mean ± SD
Male age (years)	32.8 ± 6.8
Female age (years)	29.4 ± 4.2
Body mass index (kg/m^2)	26.4 ± 2.5
Duration of marriage (years)	11 ± 6
Serum hormones	
Testosterone (nmol/l)	15.2 ± 4.8
LH (IU/l)	12.2 ± 2.4
FSH (IU/l)	16.8 ± 4.2
TSH (mIU/l)	2.8 ± 1.3
PRL (pmol/l)	368 ± 114
Inhibin B (ng/l)	84 ± 22
Semen parameters	
Ejaculate vol (ml)	2.6 ± 1.4
Total sperm/ejaculate ($\times 10^6$)	40.4 ± 8.5
Sperm density ($\times 10^6$/ml)	14.6 ± 4.8
Motility (%)	22.8 ± 2.5
Normal strict morphology (%)	7.1 ± 2.4
Plasma CoQ_{10}	
Blood (μg/ml)	1.1 ± 0.7
Seminal (ng/ml)	36.8 ± 10.3
Seminal plasma antioxidant status	
Catalase-like activity (U/ml)	305 ± 14
SOD-like activity (U/ml)	37.2 ± 1.4

LH luteinizing hormone, *FSH* follicle-stimulating hormone, *TSH* thyroid-stimulating hormone, *PRL* prolactin, *SOD* superoxide dismutase

CoQ_{10} concentrations and seminal plasma antioxidant activity

Without exception, all patients showed definite increases in blood and seminal plasma total CoQ_{10} levels during CoQ_{10} administration (Table 2). The mean levels of blood and seminal plasma total CoQ_{10} increased significantly by 142 and 146%, respectively, at 3-month treatment period (repeated measurement ANOVA, $P = 0.0001$). Blood and seminal plasma CoQ_{10} levels remained nearly unchanged after 3-month treatment with CoQ_{10} but then decreased after withdrawal. After 3 months of CoQ_{10} cessation, blood and seminal plasma CoQ_{10} concentrations were no longer significantly different from baseline values (Table 2). Seminal plasma SOD-like and catalase-like activities significantly increased during CoQ_{10} administration. The increases were 49 and 63%, respectively (repeated measurement ANOVA, $P = 0.001$). After CoQ_{10} withdrawal, the pattern of the decrease in seminal plasma SOD-like and catalase-like activity was somewhat different than the pattern observed in blood and seminal plasma CoQ_{10} concentrations. After CoQ_{10} cessation, seminal plasma SOD-like and catalase-like activity decreased gradually. By the end of follow-up at week 24, the seminal plasma antioxidant status was not significantly different from baseline (Table 2).

Semen variables

Table 3 summarizes the semen analyses results for the subjects. The baseline values in participants were as follows: semen volume 2.6 ± 1.4 ml, sperm concentration 14.6 ± 4.8 × 10^6/ml, total sperm count 40.4 ± 4.8 × 10^6 per ejaculate, motility 22.8% ± 2.5%, and strict morphology 7.1% ± 2.4%. Mean sperm concentration, motility percentage, total sperm count, and percentage of sperms with normal forms improved significantly after CoQ_{10} administration. At the end of 12-month CoQ_{10} supplementation, 48% had normal semen parameters using study limits, 20% were oligozoospermic, and 32% had normal sperm concentration (>20 × 10^6/ml) but reduced motility and/or morphology. When defining a positive response to

Table 2 Blood and seminal plasma CoQ_{10} concentrations and antioxidant activity of seminal plasma during study period

Variables	Baseline	3 months	6 months	9 months	12 months	15 months	18 months	21 months	24 months
Plasma CoQ_{10}									
Blood (μg/ml)	1.1 ± 0.7	2.66 ± 0.8[a]	2.68 ± 0.8[a]	2.64 ± 0.8[a]	2.66 ± 0.8[a]	1.2 ± 0.7[b]	1.1 ± 0.8[b]	1.1 ± 0.8[b]	1.1 ± 0.8[b]
Seminal (ng/ml)	36.8 ± 10.3	90.7 ± 12.4[a]	92.8 ± 12.5[a]	91.6 ± 12.4[a]	91.8 ± 12.3[a]	38.7 ± 12.3[b]	38.4 ± 10.2[b]	36.7 ± 10.4[b]	36.8 ± 10.2[b]
Seminal plasma antioxidant status									
Catalase-like activity (U/ml)	305 ± 14	484 ± 38[d]	497 ± 36[d]	495 ± 34[d]	498 ± 37[d]	357 ± 17[c]	340 ± 15[c]	326 ± 14[c]	316 ± 12[b]
SOD-like activity (U/ml)	37.2 ± 1.4	51.2 ± 2.7[d]	52.7 ± 2.8[d]	54.6 ± 2.6[d]	55.4 ± 2.8[d]	45.6 ± 2.4[c]	42.7 ± 2.4[c]	40.2 ± 2.7[c]	39.7 ± 2.6[b]

CoQ_{10} coenzyme Q10, *SOD* superoxide dismutase

All *P* values are versus baseline

[a] *P* value = 0.0001; [b] *P* value = not significant; [c] *P* value = 0.01–0.04; [d] *P* value = 0.001

CoQ_{10} administration as greater than a 50% increase in sperm concentration, 230 of 287 patients (80%) were responders. After 3 months of treatment, the mean total sperm count was significantly increased from baseline by 34.2% ($P = 0.005$; Fig. 2). After 6 months of treatment, the increases in total sperm counts from baseline were even greater. The subjects demonstrated an increase in total sperm count to greater than 100% of baseline at 12 months of therapy ($P = 0.001$). At 18-month follow-up on toward, sperm density begun to decrease; however, at the end of study (24 months), mean total sperm count per ejaculate and mean sperm density/ml were 83.7 and 96.6% higher than baseline values, respectively (both $P = 0.001$).

Sperm motility showed a significant increase, compared with baseline, after 3 months (increased by 17%; $P = 0.04$). Gradual and statistically significant increases in mean sperm motility from baseline were observed at all time points during treatment (by 72.6% at 9 months and by 113.7% at 12 months, both $P = 0.001$; Table 3 and Fig. 2). Sperm morphology was also significantly increased with CoQ_{10} supplementation (Table 3 and Fig. 2). In the overall population, mean percentage changes in strict sperm morphology from baseline at 3 and 6 months were 22.5% ($P = 0.03$) and 36.6% ($P = 0.01$), respectively. Mean changes from baseline in percent sperm with normal forms were remained significant at 24-month follow-up ($P = 0.01$; Fig. 2).

Serum hormones

Subjects showed little but increases in serum testosterone concentration at each follow-up visit up to month 6 (Fig. 3). The mean increases in serum testosterone levels from baseline at 3 and 6 months were 5.3 and 9.2%, respectively ($P = 0.07$ vs. baseline). During the follow-up period, serum testosterone concentrations remained nearly unchanged up to 12 months, thereafter gradually returned to baseline, although the recovery was not complete yet at 24-month follow-up visit. Mean baseline serum LH and FSH (gonadotropins) concentrations were in upper normal limits (Table 3). Significant decreases in serum LH levels of about 20% were observed 6 months after treatment was started (Fig. 3). Thereafter, serum LH levels were significantly and gradually decreased throughout the treatment period,

Table 3 Semen parameters, serum hormones, and spontaneous pregnancy data at various assessment points

Variables	Assessment points during CoQ_{10} administration				Assessment points during follow-up			
	3 months ($n = 287$)	6 months ($n = 287$)	9 months ($n = 283$)	12 -months ($n = 280$)	3 months ($n = 280$)	6 months ($n = 277$)	9 months ($n = 274$)	12 months ($n = 272$)
Semen parameters: (mean ± SD)								
Total sperm count ($\times 10^6$)	54.2 ± 10.4[c]	59.4 ± 12.5[b]	72.2 ± 12.1[c]	81.2 ± 14.8[c]	83.4 ± 14.4[c]	80.2 ± 14.1[c]	78.4 ± 15.1[b]	74.2 ± 12.7[b]
Sperm density ($\times 10^6$/ml)	17.2 ± 4.2[c]	21.2 ± 4.6[b]	25.2 ± 5.6[c]	31.2 ± 6.4[c]	32.3 ± 5.8[c]	30.2 ± 6.1[c]	29.6 ± 5.4[b]	28.7 ± 6.1[b]
Ejaculate vol (ml)	2.7 ± 1.4[d]	2.8 ± 1.4[d]	2.8 ± 1.4[d]	2.7 ± 1.5[d]	2.8 ± 1.5[d]	2.8 ± 1.4[d]	2.8 ± 1.3[d]	2.8 ± 1.4[d]
Sperm motility (%)	26.7 ± 2.4[a]	32.6 ± 2.5[c]	38.8 ± 4.2[c]	46.7 ± 6.6[c]	48.2 ± 5.4[c]	46.2 ± 6.1[c]	42.2 ± 4.6[b]	40.7 ± 5.2[b]
Normal strict morphology (%)	8.7 ± 2.4[a]	9.7 ± 2.4[b]	11.4 ± 3.2[b]	12.7 ± 4.4[c]	12.7 ± 4.4[c]	11.8 ± 4.4[b]	11.7 ± 3.7[b]	11.5 ± 3.4[b]
Serum hormones: (mean ± SD)								
Testosterone (nmol/l)	16.7 ± 5.4[d]	18.6 ± 5.4[d]	18.7 ± 5.2[d]	18.8 ± 5.1[d]	18.7 ± 5.4[d]	18.2 ± 4.6[d]	17.8 ± 5.1[d]	17.7 ± 5.3[d]
LH (IU/l)	10.7 ± 2.4[a]	10.1 ± 2.4[a]	8.7 ± 2.2[b]	8.1 ± 2.1[b]	8.1 ± 2.2[b]	8.7 ± 2.2[b]	9.1 ± 2.1[a]	9.3 ± 2.4[a]
FSH (IU/l)	14.4 ± 4.1[a]	10.4 ± 4.2[a]	9.7 ± 3.2[b]	9.4 ± 3.6[b]	9.2 ± 3.1[b]	9.4 ± 3.7[b]	10.0 ± 4.1[a]	10.4 ± 3.6[a]
TSH (mIU/l)	2.8 ± 1.4[d]	2.8 ± 1.3[d]	2.7 ± 1.4[d]	2.8 ± 1.3[d]	2.8 ± 1.2[d]	2.7 ± 1.4[d]	2.8 ± 1.2[d]	2.7 ± 1.2[d]
PRL (pmol/l)	356 ± 112[d]	346 ± 114[d]	332 ± 124[d]	326 ± 107[d]	324 ± 103[d]	328 ± 115[d]	330 ± 105[d]	336 ± 110[d]
Inhibin B (ng/l)	96 ± 27[a]	116 ± 24[a]	122 ± 28[b]	132 ± 26[c]	136 ± 22[c]	130 ± 21[c]	126 ± 27[b]	121 ± 20[b]
Pregnancy data no. (%)*								
Total pregnancy rate	0	8 (2.8)	20 (7.1)	31 (11.1)	12 (4.3)	12 (4.3)	8 (2.9)	7 (2.6)
Clinical pregnancy rate	0	8 (2.8)	20 (7.1)	31 (11.1)	12 (4.3)	12 (4.3)	8 (2.9)	7 (2.6)
Miscarriage rate	0	0 (0)	2 (10)	3 (9.7)	1 (8.3)	1 (8.3)	0 (0)	NA

CoQ_{10} coenzyme Q10, *LH* luteinizing hormone, *FSH* follicle-stimulating hormone, *TSH* thyroid-stimulating hormone, *PRL* prolactin, *NA* not applicable

All *P* values are versus baseline. * New cases between previous assessment point and current assessment point

[a] *P* value = 0.02–0.05; [b] *P* value = 0.01; [c] *P* value = 0.001–0.005; [d] *P* value not significant

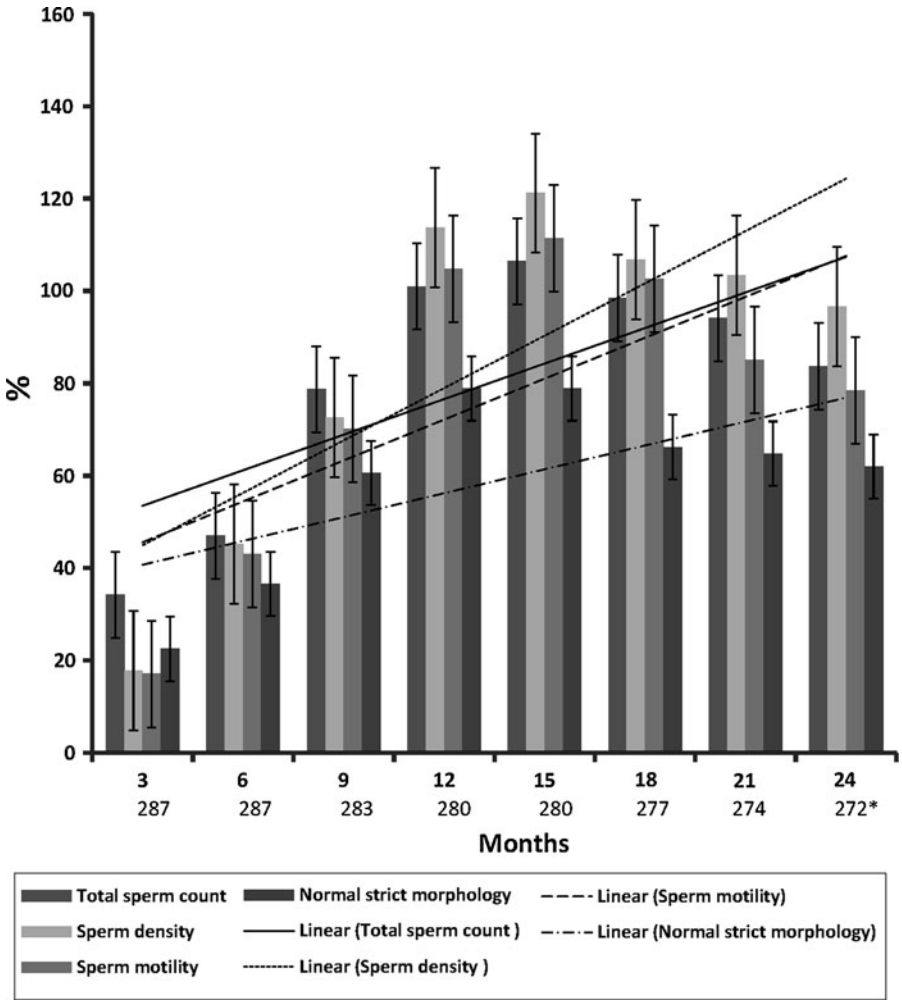

Fig. 2 Percent change from baseline in total sperm count, and sperm density, motility and morphology during whole study period. *Single asterisk* indicates number of patients analyzed

compared with baseline, whereas there was little elevation in the serum LH levels after discontinuation of CoQ_{10} administration. At the end of 24-month follow-up (12 months after withholding the CoQ_{10}), serum LH level was about 25% lower than baseline ($P = 0.02$ vs. baseline). Decrease in serum FSH level was more prominent. Serum FSH level began to decrease after 3-month treatment with CoQ_{10} (by 14%). This decrease continued throughout treatment period. At the end of 12-month therapy with CoQ_{10}, mean serum level of FSH was 44% lower than baseline ($P = 0.01$ vs. baseline). The decrease in serum FSH level continued during 3 months after medication discontinuation. Thereafter, serum FSH level begun to increase, but it never returned to baseline value completely. Serum FSH level was below 60% of baseline throughout the treatment period, and thereafter, it remained significantly decreased, so that, 12 months after CoQ_{10} discontinuation, serum FSH level was 38% lower than baseline ($P = 0.01$ vs. baseline). The maximal extent of FSH suppression (45.2%) encountered in 15-month follow-up assessment point ($P = 0.001$).

Serum inhibin B levels (Fig. 3) altered at 3 months and showed a considerable increase to approximately 60% baseline at 12 months ($P = 0.001$). Despite its decrease after CoQ_{10} discontinuation, serum inhibin B levels remained significantly higher than baseline at the end of 24-month study period ($P = 0.01$ vs. baseline). There were no significant changes from baseline in serum prolactin levels (Fig. 3).

Pregnancy rate

Ninety-eight of 287 couples conceived spontaneously during study period; 39 of these pregnancies were after

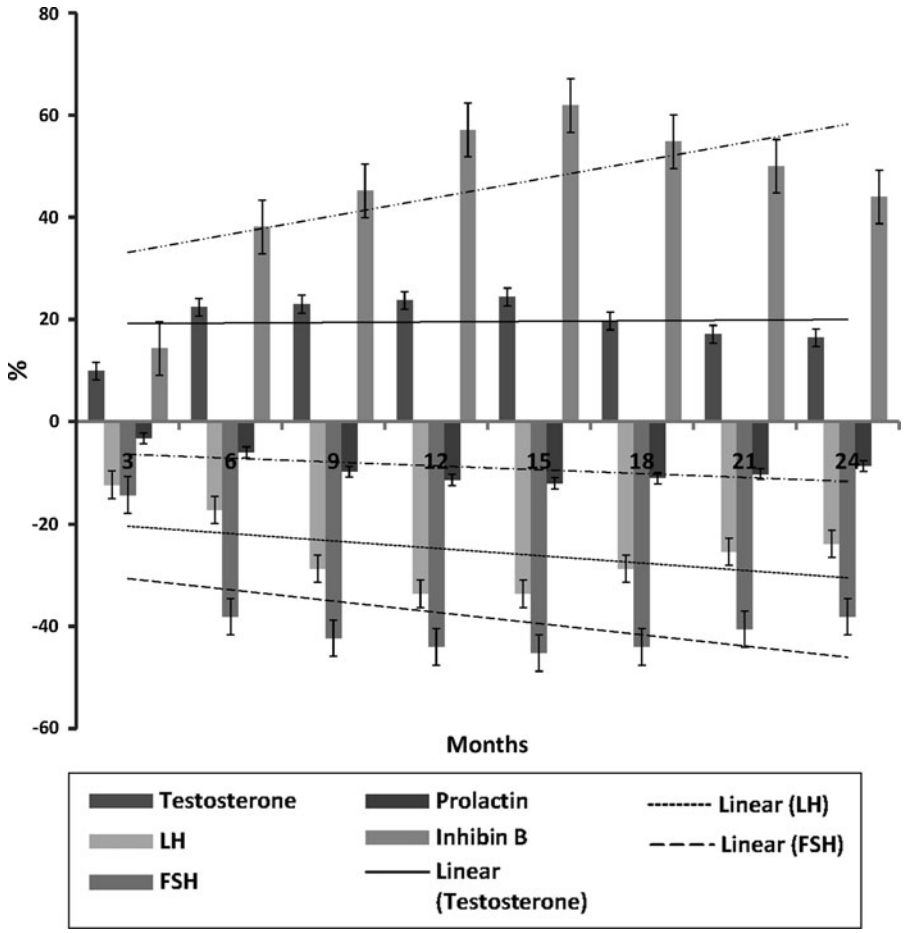

Fig. 3 Percent changes from baseline in serum testosterone, LH, FSH, PRL, and inhibin B during whole study period. *LH* luteinizing hormone, *FSH* follicle-stimulating hormone, *PRL* prolactin. *Single asterisk* indicates number of patients analyzed

the CoQ_{10} cessation. Total pregnancy rate (spontaneous clinical pregnancy) was 34.1%, within a mean of 8.4 ± 4.7 months. A pregnancy rate of 2.8, 9.9, and 21.1% was achieved after 6-, 9-, and 12-month treatment with CoQ_{10}, respectively (Table 3). There were 7 (7.1%) miscarriages up to 21-month follow-up visit, all of the remainder pregnancies ended with live birth. About one-third of pregnancies occurred between 9- and 12-month treatment periods. There were three multiple pregnancies (3.1%). All of them were twins. None of the participants, who their wives got pregnant, had post-treatment abnormal semen analysis according to study criteria.

Correlations

Seven variables were determined and incorporated into the multivariate analysis, namely male age; female age; duration of infertility (marriage); duration of CoQ_{10} administration; sperm density; sperm motility; and sperm morphology. The two factors most significantly related to outcome were the sperm density and the duration of CoQ_{10} administration, followed by female age (Table 4). All three variables showed a linear association with pregnancy rate. The chance of pregnancy increased with an increase in the number of sperms/million/ml [$r = 0.072$, odds ratio (OR) = 3.4, 95% confidence interval (CI) 2.7–5.6, $P = 0.001$] and decreased with rising age of the woman ($r = -0.062$, OR = 0.82, 95% CI 0.65–0.91, $P = 0.002$). The duration of CoQ_{10} administration was a continuous variable that was categorized into two different groups, namely <6 and >6 months. The pregnancy rate in the <6-month CoQ_{10} administration was 2.8% (8/287), and in the >6 months, CoQ_{10} therapy was 32.4% (90/287; $r = 0.064$, OR = 3.4, 95% CI 2.7–5.2, $P = 0.001$). Sperm motility was a significant predictor of pregnancy within the statistical

Table 4 Summary of multiple regression analysis of factors affecting spontaneous pregnancy rates in couples

Variables	Univariate			Multivariate[a]		
	r	*P*	Odds ratio (95% CI)	*r*	*P*	Odds ratio (95% CI)
Male age (years)	−0.026	0.03	0.92 (0.85–1.00)	−0.028	0.03	0.92 (0.85–1.00)
Female age (years)	−0.065	0.002	0.85 (0.65–0.90)	−0.062	0.002	0.82 (0.65–0.91)
Duration of marriage (years)	NS	0.172	NS	NS	0.654	
Duration of CoQ_{10} administration	0.066	0.001	3.7 (2.8–5.4)	0.064	0.001	3.4 (2.7–5.2)
Total sperm count ($\times 10^6$)	0.067	0.001	3.8 (2.4–5.2)	0.062	0.001	3.4 (2.2–4.7)
Sperm density ($\times 10^6$/ml)	0.071	0.001	3.9 (2.8–6.2)	0.072	0.001	3.4 (2.7–5.6)
Ejaculate vol (ml)	NS	0.154	NS	NS	0.157	
Sperm motility (%)	0.061	0.01	2.9 (1.8–5.2)	0.058	0.01	2.8 (1.7–5.1)
Normal strict morphology (%)	0.062	0.001	2.8 (1.6–4.3)	0.062	0.01	2.5 (1.7–4.6)

CoQ_{10} coenzyme Q10, *OR* odds ratio, *CI* confidence interval, *NS* not significant

model. With sperm motility of >40%, the chance of achieving pregnancy was almost three times the chance with motility <40% ($r = 0.058$, OR = 2.8, 95% CI 1.7–5.1, $P = 0.01$). Sperm morphology was also found to be a significant predictor of pregnancy rate within the multivariate analysis. When the normal sperm morphology (according to strict criteria) was >14%, the chance of achieving pregnancy was 2.5 times greater than when the percentage normal sperm morphology was <10% ($r = 0.062$, OR = 2.5, 95% CI 1.7–4.6, $P = 0.01$). Logistic regression analysis showed that duration of infertility (more than 2 years) was not a significant independent factor for the achievement of a spontaneous pregnancy ($r = -0.022$, OR = 1.82, 95% CI 0.95–2.1, $P = 0.07$).

Safety and adverse events

CoQ_{10} resulted in no clinically significant changes in vital signs, urinalyses, serum chemistry profiles, or hematological values. There were no serious adverse events and no withdrawals due to adverse events.

Discussion

In evaluating the effects of CoQ_{10} treatment on sperm parameters of infertile men with idiopathic OAT, we found a significant positive effect with favorable pregnancy rate. The detrimental effects of ROS on sperm cells have been demonstrated more than 60 years ago. Antioxidant enzymes, namely SOD, glutathione peroxidase, and catalase, operate to maintain oxidant–antioxidant balance in seminal plasma [18]. It has been previously shown that ubiquinone is present at considerable levels in human seminal fluid and has a direct correlation with the sperm count and motility [19]. Semen of infertile men contains considerable amount of ROS, whereas fertile men do not have detectable levels of ROS in their semen [20]. CoQ_{10} plays a key role in energy metabolism and has strong antioxidant properties for cellular membrane integrity [21]. It is to be noted that CoQ_{10} is identified to be the only endogenously synthesized fat-soluble antioxidant [22]. In addition, previous studies demonstrated that exogenous CoQ_{10} supplementation increases significantly CoQ_{10} levels in semen of oligoasthenozoospermic infertile men, with an improvement of semen values [10, 23]. There is a linear correlation between serum plasma and seminal plasma CoQ_{10} concentration. In our previous study on infertile men with idiopathic OAT, we found that, at the end of the 26-week treatment with 300 mg CoQ_{10}/day, percent increase in blood plasma and seminal plasma CoQ_{10} was 85.5 and 135.1%, respectively. Changes in blood plasma CoQ_{10} demonstrated significant positive correlations with the changes in seminal plasma CoQ_{10} [10].

We found a dramatic improvement in all three semen parameters (density, motility, and morphology) beyond the 6-month treatment period. At the end of 12-month CoQ_{10} supplementation, we noted more than 100% increase in sperm density and motility, and more than

70% increase in the percentages of sperms with normal morphology. Despite these paramount increases in semen values, they were well below from the usual values in normal fertile men. For instance, even with 113% increase in sperm density/ml, at the end of 12-month CoQ_{10} administration, overall mean sperm density was 31.2 ± 6.4 million/ml. Severe baseline OAT is probably the main reason for this finding.

In this study, significant improvement in semen quality translated into a biological significance reflected in favorable pregnancy rates. In the present study, there was a trend toward greater pregnancy rates with increasing the CoQ_{10} administration time beyond the 6th month.

Even while using pregnancy as the ultimate of infertility treatment outcomes, it is mandatory to consider the spontaneous pregnancy rate in reputedly infertile couples on no therapy. Evers et al. [24] studied treatment-independent pregnancy rate in patients with severe reproductive disorders. The crude 12-month cumulative spontaneous pregnancy rate on the waiting list for male subfertility patients was 6.4%. Our subjects had been undergone at least two previous failed infertility treatments. Therefore, 34.1% pregnancy rate in this study might not be occurred if these patients left untreated. SOD and catalase are important antioxidant enzymes that can scavenger excess free radicals such as superoxide anion and hydrogen peroxide, respectively [25, 26]. Seminal plasma SOD-like and catalase-like activity increased with the time of CoQ_{10} administration. Sustained improved semen parameters after CoQ_{10} withdrawal may be due to elevated seminal plasma antioxidant activity after it is withdrawn.

Infertility has serious psychological and particularly social consequences in the developing world [27]. Couples usually consult somewhat large numbers of physicians. For example, in a hospital in Cape Town, infertile couples consulted on average seven physicians, and over 20% of couples had between 11 and 40 consultations [28]. Therefore, developing low-cost, non-invasive, and effective treatment for male factor infertility has outmost importance. According to the main ethical theory, i.e., utilitarianism, more well-being would be created or more unhappiness avoided by providing effective treatment for male factor infertility.

Our study has limitations that should be taken into account when interpreting results. The major one is lack of control group. Owing to ethical issue, we did not include a control arm. The second was that the subjects were not a classic infertility subgroup; they had been trying to father a child for more than 24 months.

Conclusion

In conclusion, we observed a significant improvement in semen values following CoQ_{10} therapy with significant beneficial effect on pregnancy rate. Our observations should be confirmed by additional prospective controlled studies involving different groups of subjects and should lead to exploration of exact role of CoQ_{10} on the quantitative and qualitative aspects of spermatogenesis.

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Conflict of interest I have no conflict of interest whether of a financial or other nature.

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