Original Article

Effects of Coenzyme Q10 on Sperm Motility of Infertile Men with Pyospermia Treated with Doxycycline: A Randomized Controlled Trial

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Background: Pyospermia is a multifactorial disease. There is no ideal treatment, but the most promising treatment modalities are antibiotics and antioxidants.

Objective: To evaluate the effects of coenzyme Q10 (CoQ10) on sperm motility of men with pyospermia treated with doxycycline.

Materials and Methods: The present study, a double-blinded, randomized, placebo-controlled trial, enrolled 84 men who attended Siriraj Infertility Clinic, Faculty of Medicine Siriraj Hospital, Mahidol University with pyospermia on the initial semen analysis (SA) (T0). The participants were randomly assigned into two groups, group A received oral doxycycline 100 mg twice daily for 14 days plus oral CoQ10 200 mg per day for 30 days and group B received the same dose of doxycycline plus placebo for 30 days. The SA was repeated at 30±7 days (T1) and 60±7 days (T2) after initiating treatment.

Results: Of 70 eligible participants, the age of the participants was around 36 years. None of them had symptomatic urethral infection or abnormal urological examination. There was no difference in body mass index, education level, and female infertility factors between groups (p>0.05 for all). Two-thirds of both groups became non-pyospermic after the treatment. There was no difference of total sperm motility between group A and group B (46%; 32% to 55% versus 43%; 33% to 50% at T1 and 46%; 32% to 57% versus 45%; 32% to 53% at T2, p>0.05 for all).

Conclusion: A 30-day course of daily oral intake of 200 mg CoQ10 shows no additive benefit on total sperm motility in Thai men with pyospermia treated with doxycycline.

Trial registration: Thai Clinical Trials Registry, TCTR20140402002

Keywords: CoQ10, Doxycycline, Pyospermia, Sperm motility, Thai

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Pyospermia (leucospermia, leukocytospermia) implicates poor sperm functions as one cause of male infertility[1-3]. Pyospermia is defined as white blood cell (WBC) greater than 1 million per 1 mL of semen according to World Health organization (WHO) criteria 2010[4]. Leukocytes in semen may represent the infection or inflammation process in genital tract, which is indicated as a cause of poor sperm quality[1-3]. Pyospermia has multifactorial causes, including bacterial or viral infection, inflammation such as varicocele, alcohol, smoking, drug abuse, abstinence, vasovasostomy, and autoimmunity[5-7]. *Chlamydia trachomatis* is the most common bacterium that causes sexually transmitted infection[6,8,9]. This pathogen can cause prostatitis, orchitis and urethritis in men resulting in the deterioration of semen quality and pyospermia[7]. Overall prevalence of *C. trachomatis* in infertile men's seminal fluid was 2.5% to 42%[5-9]. At the moment, antibiotics and antioxidants have been the mainstay options of treatment[2,10]. Bezold

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et al demonstrated that men with pyospermia had lower total sperm count and normal morphology[5]. A previous study shows a significant recovery of sperm parameter from 10 days after initiating antibiotics to men with infection-caused pyospermia[3]. Cai et al have demonstrated that adding antioxidant with prulifloxin further enhance the return of sperm concentration and motility in patient with prostatitis from chlamydial infection[11].

Coenzyme Q10 (CoQ10) has recently been reported as one of the promising antioxidants for improving semen quality. The meta-analysis of three randomized controlled trials (RCTs) by Lafuente et al in 332 infertile men with idiopathic astheno or oligoastheno-teratospermia showed the global improvement of the sperm parameters after using daily CoQ10 200 to 300 mg for at least three months[12]. The biggest RCTs in the meta-analysis (n=212) showed that the reproductive hormone profiles improved from the fourth week onwards[13]. The main function of this compound is cellular bioenergetics. It is an important factor in mitochondrial electron transport chain for cellular respiration and the production of adenosine triphosphate (ATP). When CoQ10 is absorbed into human body, it will turn into a reduced form called ubiquinol, which is a potent antioxidant and is capable of recycling other antioxidants. Another remarkable function is to help protect from damaging the sperm membrane from reactive oxygen species (ROS) that are produced from infection and inflammatory process[14]. There is a high correlation of its extra- and intra-cellular level, especially in the organs with high energy required, including testis. CoQ10 is a bioenergetics antioxidant that has the peak level at 24 hours and the subcellular distribution is mainly in mitochondria[14]. Because of this, the addition of CoQ10 for four weeks to regular antibiotics for treating men with pyospermia and borderline sperm parameters may be propitious. The present study aims to demonstrate the effects of a 30-day course of CoQ10 on sperm motility, as well as other sperm parameters, of Thai infertile men with pyospermia treated with a 14-day course of doxycycline and to determine the prevalence of *C. trachomatis* infection among infertile men with pyospermia.

Materials and Methods

The double-blinded, randomized, placebo-controlled trial was conducted between March 2014 and February 2015 at Siriraj Infertility Clinic, Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj Hospital after obtaining the ethical approval from Siriraj Institutional Review Board, Mahidol University (COA Si133/2014). The clinical registration trial number is TCTR20140402002.

Patient selection

The eligible participants were men who attended Siriraj Infertility Clinic, had pyospermia (WBC >1 $\times 10^6$/mL) on the initial semen analysis (SA), and had normal complete urological examination excluding urethritis, epididymitis, orchitis, and varicocele. Those with the following conditions were excluded, azoospermia, symptomatic genital tract infections, history of vasovasostomy, regular smoker (smoke three days per week or more), regular alcohol consumer (drank three days per week or more), being on any antibiotics or fat soluble vitamin, and allergy to doxycycline or CoQ10.

Study procedure

After obtaining the written informed consent, the initial semen was collected for *C. trachomatis* detection by polymerase chain reaction (PCR) method. All subjects were randomized into two groups by block-of-four randomization with allocation ratio 1:1. Group A was given doxycycline (Unison Laboratories Co., Ltd., Thailand) 100 mg capsule per oral twice daily for two weeks and CoQ10 (Alerten, MEGA We Care, Thailand), 200 mg soft gel per oral twice a day for 30 days and group B was given the same dose of doxycycline and the similarly packaged placebo soft gel (MEGA We Care, Thailand) for 30 days. All medications were prepared in sealed envelopes following computerized randomization number by a nurse who was not involve in the study. The participants, the investigators, and scientists in SA lab were all blinded to the allocation. The blinding remained until analysis. At baseline, demographic data and pyospermia-related social history were collected. All participants were contacted by telephone interview weekly to ask about their compliance to medication and side effects of the study medications. Each patient was scheduled for SA at 30±7 days (T1) and 60±7 days (T2) after initiating the treatment (T0).

A one-week course of twice daily oral doxycyclines 100 mg is effective for treating three common asymptomatic male lower genital infections, including *C. trachomatis*, *Mycoplasma genitalium*, and *Ureaplasma urealyticum*[15]. However, to cover asymptomatic epididymo-orchitis that was most probably due to sexually transmitted pathogens, the

authors extended the course of doxycycline to two weeks[16]. During the treatment period, the authors asked all participants to use condom until receiving the PCR results for *C. trachomatis*.

Semen collection and analysis were performed in the same laboratory at the clinic in accordance with WHO 2010 standard. The abstinence time was limited to three to five days. The semen collection was done by masturbation into a sterile container. The results of SA were confirmed by computer-assisted sperm analysis (CASA) (Hamilton-Thorne Bioscience, USA). The semen parameters were reported as volume (mL), sperm concentration ($\times 10^6$/mL), motility (%), normal morphology (%), and WBC ($\times 10^6$/mL). Pyospermia is defined as WBC greater than 10^6/mL of seminal fluid according to WHO criteria 2010[4]. Pyospermia was determined by counting all round cells in the counting chamber, at least 200 round cells were counted. If the number of round cells was more than 1 $\times 10^6$/mL, peroxidase staining was used to differentiate leukocyte from other round cells[17].

Microbiological analysis

Nucleic acid of *C. trachomatis* was tested by genesig® Standard Kit real time (RT) PCR for *C. trachomatis* (Primerdesign Ltd., Southampton, United Kingdom) as described by the manufacturer's protocol. Briefly, a 20 µl reaction was prepared with 10 µl of oasig™ 2x qPCRMasterMix, 1 µl of *C. trachomatis* primer/probe mix (BROWN), 4 µl of RNAse/DNAse free water and 5 µl of DNA template. The RT-PCR was performed and analyzed by comparing with positive and negative controls using a standard curve method.

Sample size and statistical analysis

The sample size was calculated based on the data of the pilot study in 50 Thai men with pyospermia at the same clinic. The sperm motility was 42.4±13.2%. The authors expected that the difference of total motility between groups was at least 10%, determined 2-sided alpha error at 0.05, and statistical power at 80%. The sample size calculation yielded 28 per group with an additional 15% loss to follow-up, so it added to 35 participants per group.

Stata, version 12.1 (StataCorp LP, College Station, TX, USA) was used for the statistical analysis. Descriptive statistics was used to describe participants' characteristics. All the sperm parameters were non-parametric. Wilcoxon rank sum test were used to compare the median of the sperm parameters between groups; and Wilcoxon signed rank test were used to compare the changes of all sperm parameters within group at each visit. A p-value less than 0.05 was considered statistically significant.

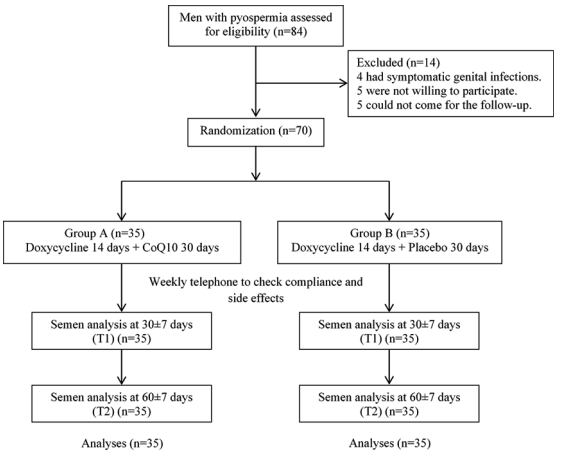

Figure 1. Study schedule and participant flow diagram.

Results

Eighty-four men with pyospermia were assessed. Four men had symptomatic genital infections, five men were not willing to participate, and five men could not come for any follow-ups. Seventy men were equally randomized into two groups (35 men in each group). All of the participants had a 100% adherence to the protocol and underwent SA at T1 and T2 (Figure 1). At the end of the study, no severe side effects of all treatment regimens were reported. None of the specimen was tested positive for *C. trachomatis*.

The age of the participants ranged from 26 to 52 years. There was no difference in age, education level, monthly income, body mass index (BMI), and female infertility factors between Group A and Group B ($p>0.05$ for all) (Table 1). The average age was 37.4±6.3 years in Group A and 35.4±4.1 years in Group B. They were not obese (BMI 24.5±2.7 kg/m² in Group A and 23.6±3.0 kg/m² in Group B). Most of them finished university and had an income of more than 25,000 Baht per month. There was no difference of the female infertility factors between groups.

Table 2 shows the comparison of the median of semen parameters between the two groups and the changes within each group. At all visits, there was no difference of all sperm parameters between Group A and Group B ($p>0.05$ for all). There was no difference of total sperm motility between Group A and Group B (46%; 32% to 55% versus 43%; 33% to 50% at T1 and 46%; 32% to 57% versus 45%; 32% to 53% at T2, $p>0.05$ for all). Number of WBC declined

Table 1. Baseline characteristics of the participants

	Group A n (%)	Group B n (%)	p-value
Age (years); mean±SD	37.4±6.3	35.4±4.1	0.120
Education level			0.077
< Bachelor degree	4 (11.4)	0 (0.0)	
Bachelor degree	26 (74.3)	32 (91.4)	
> Bachelor degree	5 (14.3)	3 (8.6)	
Monthly income (Baht)			0.070
<25,000	12 (34.3)	5 (14.3)	
25,000 to 50,000	14 (40.0)	23 (65.7)	
>50,000	9 (25.7)	7 (20.0)	
BMI (kg/m^2); mean±SD	24.5±2.7	23.6±3.0	0.149
Female infertility factors			
Tubal factor	6 (17.1)	6 (17.1)	1.000
Uterine factor	1 (2.3)	2 (5.7)	0.555
Ovarian factor	4 (11.4)	3 (8.6)	0.690

BMI=body mass index; SD=standard deviation

Group A: doxycycline plus coenzyme Q10, Group B: doxycycline plus placebo

significantly from T0 to T1 and the improvement persisted to T2 in both groups (p T0-T1 <0.001 and p T0-T2 <0.05 in both groups). Total sperm motility, morphology, volume, and concentration appeared unchanged over time (p>0.05). At T1, there were 23 (65.7%) in Group A and 25 (71.4%) in Group B that became non-pyospermic (p=0.607). There was no significant difference of sperm parameters between men with resolved pyospermia and unresolved pyospermia in both groups (p>0.05 for all) (Table 3). None of the participants reported any adverse effects of the study drugs.

Discussion

Daily oral intake of 200 mg CoQ10 for 30 days shows no additional benefit to a 14-day course of doxycycline on any of the sperm parameters in Thai men with pyospermia. The present study supports that infection is an important etiology in this population because more than two thirds of the participants had resolved pyospermia at T1 and persisted to

Table 2. The comparison of the median of the semen parameters between doxycycline plus CoQ10 (Group A) and doxycycline plus placebo (Group B)

Sperm parameters	T0 Median (range)	T1 Median (range)	T2 Median (range)	p-value[b] (T0-T1)	p-value[b] (T1-T2)	p-value[b] (T0-T2)
Volume (mL)						
Group A	1.7 (1.2 to 2.3)	2.0 (1.3 to 2.5)	2.0 (1.2 to 2.5)	0.081	0.182	0.984
Group B	2.3 (1.2 to 3.0)	2.3 (1.5 to 3.0)	2.0 (1.5 to 2.9)	0.316	0.110	0.748
p-value[a]	0.274	0.271	0.693			
Total motility (%)						
Group A	46 (42 to 54)	46 (32 to 55)	46 (32 to 57)	0.377	0.840	0.464
Group B	45 (40 to 56)	43 (33 to 50)	45 (32 to 53)	0.112	0.305	0.543
p-value[a]	0.746	0.254	0.855			
Concentration (×10^6/mL)						
Group A	36 (17 to 60)	34 (15 to 52)	33 (13 to 50)	0.494	0.948	0.440
Group B	40 (25 to 70)	31 (15 to 61)	38 (20 to 61)	0.212	0.846	0.333
p-value[a]	0.310	0.925	0.609			
Morphology (%)						
Group A	9 (6 to 13)	8 (6 to 12)	8 (4 to 11)	0.477	0.312	0.122
Group B	10 (8 to 12)	12 (8 to 14)	10 (9 to 12)	0.228	0.410	0.579
p-value[a]	0.366	0.663	0.814			
WBC (×10^6/mL)						
Group A	1.6 (1.2 to 2.6)	0.8 (0.3 to 1.2)	0.7 (0.3 to 1.5)	<0.001	0.430	0.021
Group B	1.5 (1.2 to 2.0)	0.7 (0.4 to 1.2)	0.7 (0.5 to 1.2)	<0.001	0.630	<0.001
p-value[a]	0.250	0.663	0.814			

WBC=white blood cell

[a] Wilcoxon rank sum test between Group A and Group B, [b] Wilcoxon signed rank test

T0: initial semen analysis, T1: semen analysis at 30±7 days after initiating the treatment, T2: semen analysis at 60±7 days after initiating the treatment (30 days without any medications)

Table 3. The comparison of the sperm parameters when completing the treatment (T1)

	Group A; median (range)			Group B; median (range)		
	Pyospermia (n=12)	No pyospermia (n=23)	p-value	Pyospermia (n=10)	No pyospermia (n=25)	p-value
Volume (mL)	2.0 (1.7 to 3.2)	2.0 (1.2 to 2.5)	0.472	1.7 (1.5 to 4)	2.6 (2.0 to 3.0)	0.701
Concentration ($\times 10^6$/mL)	35.0 (12.5 to 61.5)	34.0 (16.0 to 52.0)	0.945	34.5 (17.0 to 80.0)	29.0 (15.0 to 58.0)	0.432
Total motility (%)	47.0 (26.5 to 57.0)	46.0 (38.0 to 54.0)	0.917	43.0 (40.0 to 56.0)	43.0 (29.0 to 48.0)	0.207
Morphology (%)	8.0 (5.5 to 10.0)	10.0 (7.0 to 12.0)	0.336	10.0 (8.0 to 13.0)	12.0 (9.0 to 14.0)	0.593

Group A: doxycycline plus coenzyme Q10, Group B: doxycycline plus placebo

T2. The lesser number of WBCs result in lower level of ROS, which should enhance sperm energy production as being partly demonstrated by sperm motility[2]. However, the authors found no increase in sperm motility or other parameters in men with resolved pyospermia. The present study finding is in contrasts to the study by Cai et al[11] in that adding antioxidative agent to antibiotics did not result in any better outcomes overall.

Sperm motility is one of the predictors of successful natural conception[18]. Sperm requires ROS to optimally function, including capacitation, hyper-activation, and sperm-oocyte fusion[18]. However, the excess of ROS damages the cell membranes and DNA resulting in the impairment of other functions[19]. Given that the infection occurs in the upper genital tract such as epididymo-orchitis and prostatitis, sperm morphology may be permanently damaged resulting in poor sperm morphology and motility. The impaired sperm function should be more reversible in men with lower genital tract infection as only sperm function is affected.

Pyospermia associates with not only the sperm function but also sperm morphology. Aziz et al reported the correlation between pyospermia and sperm tail defects, acrosomal damage, and high sperm deformity index[20]. All of the participants in previous studies evaluating the efficacy of the treatment for men with pyospermia had markedly impaired sperm parameters[3,11,12]. On the contrary, the present participants were asymptomatic healthy men of infertile couples. They had pyospermia and only borderline sperm parameters at baseline. This implies that treating men with pyospermia and normal or borderline sperm parameters is beneficial only in terms of declining the ROS-producing sources like WBC, but the clinical significance remains unclear.

In the present study, at T1, the number of WBC declined significantly in both groups but neither Group A nor Group B had improved sperm motility. After the subgroup analysis, men with resolved pyospermia also had similar sperm parameters to the other. The finding is contrasting to the study by Pajovic et al in asymptomatic Montenegro men with pyospermia who had been proven as having either computed tomography (CT) or *U. urealyticum* in that they found significant improvement of sperm concentration and sperm motility from the tenth day after initiating treatment[3]. Another consideration is that spontaneous variation per se may affect our outcomes as Lackner et al demonstrated that, in around three months apart, there could be 30% downward and 17% upward variation of sperm motility, and 28% downward, and 4% upward variation of sperm morphology[17].

There are limited data on the prevalence of infectious etiologies of pyospermia. Bezold et al reported comparable prevalence of STIs at around 20% in American men with and without pyospermia[5]. However, the studied etiologic organisms were all virus except for only CT. Another study in infertile and fertile men in Kuwait showed that around one third of men in both groups had bacterial infection[6]. Infection caused by *Mycoplasma* spp. and CT appeared to associate with the increased WBC in seminal fluid. The prevalence of CT was 2.5% and 3.9% in the study by Bezold et al and that by Al-Sweih et al, respectively[5,6]. In the present study, most of the non-infectious causes had been excluded by history-taking and physical examination. Therefore, the lower genital tract infection seems to be the most likely etiology. Surprisingly, none of the specimen in the present study tested positive for CT despite the dramatic resolution of pyospermia after receiving doxycycline. All the mentioned studies, including ours, used semen as the tested specimen, which is well-accepted[21].

CoQ10 has also been proven to significantly improve sperm concentration and motility[12]. There are three typical commercially available formulations of CoQ10 as follows, powder based, oil based, and solubilized form. CoQ10 that was used in the present study was a lipid-based formulation, one of the solubilized forms, which formed bile salt-mixed

micelle with dietary triglyceride after digestion called "colloidal-Q10". A previous study by Bhagavan et al[14] compared serum level of CoQ10 in all three forms following daily supplement 120 mg per day for three weeks. The result showed that the solubilized form resulted in the highest serum CoQ10 level with 3.25 μmol/L (2.8 μgl/mL) followed by powder based and oil based, respectively[14]. Moreover, the CoQ10 serum level of 2.8 μg/mL from 120 mg per day for three weeks was higher than 2.0 μgl/mL from the study of Safarinejad et al[13], which used 300 mg per day for 26 weeks. The latter study demonstrated the significant improvement of sperm concentration and motility[13,22,23]. From all of these data, the solubilized form of CoQ10 at the dose 200 mg per day used in the present study for 30 days should be adequately effective to improve sperm motility as compared to the serum level of Bhagavan et al[14] and Safarinejad et al[13].

Theoretically, spermatogenesis process takes approximately 70 days to complete from spermatocyte stage and another 12 to 21 days require for the transport of sperm from testis through epididymis to ejaculatory duct[18]. According to a meta-analysis, Balercia et al hypothesized that CoQ10 diffuse through phospholipid bilayer of cellular membrane in their transport from the peripheral blood to testicular and accessory male genital glands[22]. Therefore, the authors assume that 30 days of CoQ10 is absorbed into male genital tract and acts during this transportation of sperm from the testis to the ejaculatory duct. Naturally, in this passage, sperm itself also matures further to develop the capacity for sustained motility[18], so the authors expect the improvement in sperm parameter, especially sperm motility as reported in previous studies[10-13]. But somehow, the increased CoQ10 level was not high enough to overwhelm the deleterious effects of oxidative stress on sperm and improve sperm parameters. There is limited data on the correlation of the level of CoQ10 in seminal fluid and serum in Asian men. Further studies on the optimal dosage and duration of CoQ10 given in this population should be performed.

Currently, there is no evidence that CoQ10 increases either live birth rate or pregnancy rate[12]. The probability of natural conception rises with increasing sperm motility up to approximately 60% as well as pregnancy rate from intrauterine insemination (IUI) in infertile couple with male factor[18]. Thus, if infertile men can improve sperm motility from taking oral CoQ10 in proper dosage and duration, the pregnancy rate either from natural conception or IUI may also increase and the possibility of attending assisted reproductive technology may be lessened.

The strength of the study is that it is an RCT aiming at evaluating the additive effect of CoQ10 on the regular antibiotics given for men with asymptomatic pyospermia. There is a 100% adherence to the protocol and no loss-to-follow-up case. However, there are also a few limitations. First, the etiologies of pyospermia were not thoroughly explored. As infectious causes appeared to play a major role, molecular diagnosis of more probable pathogens should be studied. Second, the duration of follow-up period may be too short. The longer study period as well as the longer course of CoQ10 should be further examined.

Conclusion

A 30-day course of daily oral intake of 200 mg CoQ10 appears to have no additive benefit on total sperm parameters in Thai men with pyospermia treated with a 14-day course of doxycycline.

What is already known on this topic?

Pyospermia is a multifactorial disease. There is no ideal treatment, but the most promising treatment modalities are antibiotics and antioxidants.

What this study adds?

Doxycycline for 14 days is effective for relieving pyospermia. The addition of CoQ10 for one month does not seem to be beneficial.

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Conflicts of interest

The authors declare no conflict of interest.

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