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REPRODUCTIVE MEDICINE

Coenzyme Q10 content in follicular fluid and its relationship with oocyte fertilization and embryo grading

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Abstract
Background No data are available on the presence and content of Coenzyme Q10 (CoQ10) in human follicular fluid and its role.
Objective To assess the presence and concentration of CoQ10 in human follicular fluid in relation to oocyte fertilization.
Methods CQ10 content was measured in follicular fluid obtained from 20 infertile women undergoing ovarian stimulation program for in vitro fertilization. CoQ10 levels were assayed by high-performance liquid chromatography system and normalized for follicular cholesterol and protein levels. Oocyte morphology and embryo grading were assessed.
Results CoQ10/Protein levels resulted significantly in mature versus dysmorphic oocytes. Similarly, CoQ10/Cholesterol was significantly higher in grading I–II versus grading III–IV embryos.
Conclusions This study is the first demonstration of the presence of CoQ10 in the human follicular fluid. Although the biological and endocrine mechanism of CoQ10 in the follicular fluid and its correlation with oocyte and embryo development is unclear, a new step may be the administration of CoQ10 in infertile women to evaluate the biological and reproductive outcomes.

Keywords Coenzyme Q10 · Follicular development · Oocyte maturation · Embryo grading · Oxidation

Abbreviations

CoQ10	Coenzyme Q10
IVF–ET	In vitro fertilization and embryo transfer
SOD	Superoxide dismutase
SeGPX	Glutathione peroxidase
QH_2	Ubiquinol
HPLC	High-performance liquid chromatography system
ECD	Electrochemical detector

Introduction

Oocyte quality is one of the most important factors associated with successful pregnancy in in vitro fertilization and embryo transfer (IVF–ET). The microenvironment of the follicle is vital for normal oocyte development, folliculogenesis, and timely ovulation. The oocyte resides in a metabolically active environment containing of steroid hormones, growth factors, cytokines, granulosa cells, and leukocytes.

In the past 60 years, reactive oxygen species (ROS) and oxygen radicals were shown to be involved in human reproduction [1].

In vivo the damaging effects of oxygen radicals are usually prevented or limited by endogenous antioxidants (or scavengers of free radicals). These include enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase (SeGPX) as well as lipid- and water-soluble antioxidants such as vitamins C, E and uric acid [2]. Decreased

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levels of SeGPX were found in the follicular fluid of women with unexplained infertility, and reported increased antioxidant consumption during the incubation of poor quality embryos [3]. Yang et al. [4] found higher levels of hydrogen peroxide in fragmented compared with non-fragmented embryos and unfertilized oocytes.

Coenzyme Q10 (CoQ10) is a component of the mitochondrial respiratory chain and is present in other biological membranes, playing a crucial role both in energy metabolism and as liposoluble chain-breaking antioxidants for cell membranes and lipoproteins [5, 6]; the role of CoQ10 as gene inducer has also been investigated [7]. CoQ10 biosynthesis is markedly active in testis [8], and high levels of its reduced form ubiquinol (QH_2) are present in sperm [9, 10], suggesting a protective role as antioxidant. Some data from our group demonstrated that reduced levels of CoQ10 and its reduced form QH_2 in seminal plasma and sperm cells of infertile men with idiopathic and varicocele-associated asthenospermia [11]. No data are available on presence and content of CoQ10 in follicular fluid. The present study was designed to assess the presence and concentration of CoQ10 in human follicular fluid in relation to oocyte fertilization.

Materials and methods

This study was conducted on 20 patients undergoing IVF–ET program in the Department of Obstetrics and Gynecology of Salesi University Hospital of Marche Polytechnic University (Italy). The study was approved by the local Institutional Review Board; an informed consent was subscribed by all women enrolled in the study. All patients had both ovaries and regular menstrual cycles every 27 to 32 days, and normal ovulatory function as shown by mid-luteal plasma progesterone and ultrasonographic scanning. They were of Caucasian race, aged 31-41 years, had normal blood pressure and body mass index, were non-smoking and not taking any medication, and were not involved in intensive exercise. Infertility diagnosis included tubal disease and idiopathic infertility; patients with concomitant diseases such as uterine anomaly, fibroids, ovarian cyst or pelvic inflammatory disease were excluded from the study. All women recruited were not taking any supplements at the time of the study. Controlled ovarian hyperstimulation was performed by a standard protocol: 3.75 mg/day of gonadotropin-releasing hormone agonist (Triptorelin) was administrated on day 21 of the menstrual cycle preceding oocyte retrieval. After attainment of pituitary desensitization, indicated by serum 17beta-estradiol levels, stimulation was initiated with recombinant FSH (Puregon®, Organon, The Netherlands). The daily dose of gonadotrophins was continued on an individual basis, depending upon follicular growth. When the leading follicle reached 17 mm in diameter, FSH administration was discontinued, and 10,000 IU of hCG were administered (Gonasi HP®, Amsa, Italy). After 34–36 h following the hCG injection, oocytes were recovered by transvaginal ultrasound-guided follicle aspiration (range 2–4 oocytes for each woman). In each patient, the follicular fluid was individually aspirated in conjunction with oocyte retrieval. The follicular fluid was aspirated without the contamination of flushing medium, and new pipettes being used for each aspiration to eliminate contamination. At each collection time, the volume of fluid and the presence of oocytes were recorded for each follicular fluid sample. After oocyte isolation, the follicular fluid was centrifuged at $3{,}000 \times g$ for 10 min at 4°C to remove debris, blood and granulosa cells, and was then frozen at −80°C until assayed. Follicular fluids that were contaminated with significant quantities of blood cells were not used for analysis. A total of 40 follicular fluid samples were obtained, and samples that contained blood, did not have oocyte, or contained more than a single oocyte were excluded from the study. After the oocyte retrieval, oocyte classification was performed. The maturational status of the oocytes and the embryo grading were recorded according to the published criteria [12]. Embryo quality was assessed morphologically 2 days after fertilization by using a modified grading system [12]: grade I and II embryos have no or very few fragments in the cytoplasm with equal size of blastomeres and therefore are considered the best embryos; grade III and IV embryos have significant or severe fragmentation, little cytoplasmic fragmentation with blastomeres of distinctively unequal size.

CoQ10 levels were assayed in follicular fluids fractions with the use of a dedicated high-performance liquid chromatography system (HPLC) with electrochemical detector (ECD) by Shiseido Co. Ltd., Tokyo, Japan. Mobile phases were as previously described. A peculiarity of the system was the use of a post-separation reducing column (Shiseido CQR; 20 × 2.0 mm) capable of fully reducing the peak of oxidized CoQ10. The oxidation potential for ECD was 650 mV. Follicular fluids levels of total CoQ10 was expressed as μg/ml. Values were also normalized for cholesterol (nM CoQ10/mM Chol.) and for total protein content assessed by the Bradford method [13] (μg CoQ_{10}/mg proteins).

The various biological parameters germane to IVF cycles for these two groups of patients were compared by Students' t test or Mann–Whitney U test. A confidence level of $p < 0.05$ indicated statistical significance.

Results

The characteristics of the patient groups and various CoQ10-associated parameters are summarized in Table 1. There was

Table 1 Characteristics, hormone levels and CoQ10 levels in groups studied

	Mature oocyte ($N = 20$)	Dysmorphic oocyte ($N = 20$)	$p<$	Grading I–II ($N = 21$)	Grading III–IV ($N = 12$)	$p<$
Age (years)	35.6 ± 4.7	37.1 ± 2.9	N.S.	35.2 ± 4.6	38.2 ± 3.9	N.S.
Body Mass Index	23.9 ± 3.8	24.2 ± 4.0	N.S.	23.7 ± 3.1	23.8 ± 4.2	N.S.
Day 3 basal FSH (mlU/mL)	6.88 ± 2.46	7.90 ± 2.89	N.S.	8.08 ± 2.63	8.88 ± 3.43	N.S.
Estradiol (pg/ml)	1779 ± 943.29	1348.58 ± 658.68	N.S.	1298.48 ± 733.22	2028.68 ± 884	<0.05
Pregnancy rate (%)	9/20 (45)	3/20 (15)	<0.05	11/21 (52.3)	3/12 (25)	<0.05
No. of oocytes recruited	2.9 ± 0.9	2.8 ± 0.7	N.S.	3.1 ± 0.9	3.1 ± 0.5	N.S.
CoQ10 Total (ug/ml)	0.11 ± 0.03	0.12 ± 0.02	N.S.	0.10 ± 0.02	0.12 ± 0.03	N.S.
CoQ10/Proteins (ug/mg)	0.0026 ± 0.0008	0.0024 ± 0.0006	<0.05	0.002 ± 0.0008	0.002 ± 0.0006	N.S.
CoQ10/Cholesterol (nM/mM)	174.75 ± 28.61	189.47 ± 50.02	N.S.	184.27 ± 29.97	159.43 ± 31.19	<0.05

Data are expressed as mean ± standard deviation

N.S. not significant

a significant difference between the group of women with mature versus women with dysmorphic oocytes in terms of CoQ10/proteins levels (0.0026 ± 0.0008 vs. 0.0024 ± 0.0006 ug/mg; $p < 0.05$). Moreover, significant differences were also observed between the groups of women with grading I–II versus women with grading III–IV embryos in terms of CoQ10/Cholesterol (184.27 ± 29.97 vs. 159.43 ± 31.19 nM/mM; $p < 0.05$).

Discussion

This study is the first demonstration of the presence of CoQ10 in the human follicular fluid.

Previous studies in animals have reported that free radicals have detrimental effects on cell membranes and lead to defective growth [14] or limited cell growth of embryos [15]. CoQ10 scavenges free radicals generated chemically within the liposomal membranes preventing per oxidative damage [16] and stimulates cell growth [17].

Stojkovic et al. [18] reported that CoQ10 supports the development and viability of bovine embryos: in a chemically defined culture system, noncrystalline CoQ10 in submicron-sized dispersion increases, in a concentration-dependent manner, the proportion of oocytes developing to five to eight cell and blastocyst stages in vitro.

In our series total CoQ10 levels were higher in follicular fluids associated with mature oocyte and high grade embryos, suggesting a possible correlation to the mechanisms of control and growth in follicular ambient. As reported in sperimental in vitro cultures of myocardial cells, the CoQ10 stimulated the formation of ATP [19] that in reproductive biology could accelerate formation of the blastocoels cavity and consequently the hatching process [20, 21], second the presence of CoQ10 may correct ionic imbalance that exists in embryos cultures [21].

A criticism of our result may derivate from the observation that CoQ10/protein level was significantly higher in mature compared to dysmorphic oocytes but was not significantly different with embryo grade, whereas CoQ10/cholesterol level was not significantly different between mature or immature oocytes but was significantly higher in better embryo grading. It might be intuitive that the same marker that would reflect improved antioxidant activity associated with mature eggs would also predict better quality embryos. If the analysis of oocyte maturation might be of great importance in predicting successful fertilization and embryo development, on the other hand the correlation between oocyte quality and embryo grading is still controversial. De Sutter et al. [22] reported that oocyte morphology does not correlate with fertilization rate and embryo quality after ICSI. In fact some oocytes have one or more biological characteristics which make it difficult to define the relationship between the oocyte morphology and its development.

A recent double-blind, randomized placebo-controlled trial demonstrated that the exogenous administration of CoQ10 produced an increase in its semen level both of the oxidised and reduced form. This data were associated to an improvement of sperm kinetic features in the patients affected by idiopathic asthenozoospermia [23].

A meta-analysis that reviewed RCTs with CoQ10 administration reported no side effects or adverse events in any of the studies [24].

Although the biological and endocrine mechanism of CoQ10 in the follicular fluid is unclear, in fact even if biosynthesis of CoQ10 is localized to the inner mitochondrial membrane, individual enzymes are also present in the endoplasmic reticulum, golgi system and peroxisomes. Such creation of local pools of CoQ10 for specific functions may not be reflected in the total tissue or cellular level and in the follicular environment we have to study the mechanism of biosynthesis and regulation of CoQ10 between the cells and

extracellular fluid. At present our data need to be confirmed in a larger study, a new step may be the administration of CoQ10 in infertile women to evaluate the biological and reproductive outcomes.

Mitochondrial nutrients are naturally occurring vitamins that have been used successfully to treat the conditions associated with diminished energy production from mitochondria, and appear to be very safe in the doses studied.

A new trial on the role of CoQ10 administration to improve oocyte and embryo quality is needed and it is in progress in our Department, a randomized placebo-controlled study to assess this hypothesis in women undergoing fertilization procedures.

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Conflict of interest All authors declare any financial and personal relationships with other people or organizations that could influence their work.

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