The impact of coenzyme Q10 on seminal oxidative stress markers, sperm DNA fragmentation, and predictors of pregnancy in men with idiopathic infertility

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Dedication

To my beloved family for their great help and support to complete my Ph.D.

Acknowledgments

I am grateful to all the participants in the studies for their invaluable contribution to the conduction of this work. I am very grateful to my supervisor Prof. Naemi for his great help and guidance throughout my Ph.D. journey. I am also very thankful for my co-authors Prof. Calogero, Dr. Singh, Dr. Sengupta, Dr. Dutta, Dr. Vishvkarma, Dr.Gupta, and Dr. Cannarella, for their valuable contribution to the revision of the manuscripts and provision of insights throughout the research period of the previous articles.

Declaration

I declare that I will not submit material, which has been or is being submitted in respect of another research degree at this or any other University.

List of abbreviations

ART: Assisted Reproductive Techniques

AS: Asthenospermia

AT: Asthenoteratospermia

AVP1: Acrosomal Vesicle Protein 1

BFS: British Fertility Society

BMI: Body Mass Index

CAT: Catalase

CoQ10: Coenzyme Q10

CV: Coefficient of Variation

ECM1: Extracellular Matrix Protein 1

FSH: Follicle-Stimulating Hormone

GPx: Glutathione Peroxidase

GST: Glutathione-S-Transferase

ICSI: Intracytoplasmic Sperm Injection

IMI: Idiopathic Male Infertility

IUI: Intrauterine Insemination

IVF: *In Vitro* Fertilization

LH: Luteinizing Hormone

L-PGDS: Lipocalin-Type Prostaglandin D Synthase

MOSI: Male Oxidative Stress Infertility

OA: Oligoasthenospermia

OAT: Oligoasthenoteratospermia

ORP: Oxidation Reduction Potential

OS: Oxidative Stress

P1: Paper 1

P2: Paper 2

P3: Paper 3

P4: Paper 4

P5: Paper 5

P6: Paper 6

P7: Paper 7

P8: Paper 8

P9: Paper 9

P10: Paper 10

PICO: Patients Intervention Comparison Outcome

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

RCP: Royal College of Physicians

RCT: Randomized Controlled Trials

ROS: Reactive Oxygen Species

SARSCOV2: Severe Acute Respiratory Syndrome–Related Coronavirus2

SDF: Sperm DNA fragmentation

SOD: Superoxide Dismutase

SRF: Society for Reproduction and Fertility

TAC: Total Antioxidant Capacity

TEX1: Transcription/Export 1

TSH: Thyroid-stimulating hormone

TTP: Time To Pregnancy

WHO: World Health Organisation

Abstract

Oxidative stress (OS) and sperm DNA fragmentation (SDF) have been linked to idiopathic male infertility (IMI) but the underlying mechanisms remain unknown. Oral antioxidants including coenzyme Q10 (CoQ10) have been tried to treat IMI with inconsistent outcomes and lack of agreement on a standardized treatment regimen. In addition, data on the impact of CoQ10 on oxidative stress markers, SDF, and pregnancy outcomes are limited. Therefore, in this research portfolio, I led several clinical studies to explore the impact of CoQ10 therapy (200 mg/day for 3-6 months) on seminal antioxidant markers, SDF, pregnancy outcome, time to pregnancy (TTP), and their predictors. The submitted work represents the outcome of my research experience from working for sixteen years as a lecturer, researcher, and specialist doctor at the College of Medicine, University of Babylon, Iraq. I have published 10 papers in specialized international journals with impact factor. I have used different clinical study designs, established and validated research methodologies including systematic reviews, meta-analysis, narrative reviews, prospective randomized and controlled clinical studies. These papers represent a coherent work that is focused on the seminal antioxidant status, SDF, and determinants of pregnancy and TTP outcomes in men with IMI before and after CoQ10 therapy. To explore this theme, different parameters including reactive oxygen species (ROS), total antioxidant capacity, catalase, superoxide dismutase, glutathione peroxidase, SDF, seminal CoQ10 level, and sex hormones were assessed in men with IMI and fertile controls in follow-up studies. I have also compared different doses of CoQ10, CoQ10 with selenium, and CoQ10 with Centrum multivitamin. In addition, the main paper (P10) included the largest number of participants (178 patients and 84 controls) who received CoQ10 for an extended period of 6 months and a total follow-up of 24 months. This paper further explored the pregnancy rate, TTP, and their clinical and biochemical predictors in patients with idiopathic oligoasthenospermia (OA) using binomial logistic regression and Cox regression (survival analysis). Overall, the studies demonstrated that 3-6 months of CoQ10 therapy (200 mg/day) significantly increased seminal CoQ10 levels, semen parameters, antioxidant capacity, and reduced SDF and ROS with a pregnancy rate of 24.2% in men with idiopathic OA after 24 months. In addition, the concluding study has identified several independent clinical and biochemical reproductive predictors. CoQ10 level, sperm concentration,

motility, and ROS could be independent predictors of pregnancy outcome. CoQ10 level, male age, sperm concentration, motility, ROS, and glutathione peroxidase could be independent predictors of TTP in men with idiopathic OA. The studies have been cited in journals with impact factor and represent a significant contribution to the understanding of the underlying mechanisms and oxidative stress and SDF contribution for IMI. Further, the identified predictors could be used as a diagnostic and therapeutic target in men with IMI, and CoQ10 therapy (200 mg/day) for 6 months could be a potential therapy for the challenging group of men with IMI. Therefore, the studies provide a set of potential clinical and biochemical markers and an optimized targeted therapy that may facilitate the management of men with IMI in the clinical setting and may enhance their fertility potential. Thus, our findings may improve the diagnosis and treatment of IMI, OS, and SDF testing, indication, pregnancy outcome, provide prognostic data, and may reduce the psychological, and financial burden on infertile couples with improved quality of health care. The observed reduction of SDF may also be associated with enhanced wellbeing of the offspring. In addition, the current work will also enhance my career as a lecturer, researcher, and specialist doctor at the University of Babylon through incorporating the current knowledge into the clinical practice and management of infertile men with IMI. Further, the findings of the studies will motivate me to conduct further large-scale studies to consolidate the evidence on the impact of CoQ10 and other antioxidants on fertility biomarkers and determinants of pregnancy and ART outcomes in IMI. Finally, the current programme will also help me to supervise the research projects of postgraduate students at the University of Babylon.

List of contents

Table 1. List of submitted papers.

No.	List of authors (In the order shown in publication)	Title of the paper and web link	Name of the journal	Impact factor/ CiteScore	Journal Index	No. of Citations
1	**-Ahmed T Alahmar**(1)	Role of oxidative stress in male infertility: an updated review (Review article) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6472207/	Journal of Human Reproductive Sciences	1.4/ 2.1	PubMed, Scopus, Clarivate (Web of Science)	151
2	**-Ahmed T Alahmar**(2)	The effects of oral antioxidants on the semen of men with idiopathic oligoasthenoteratozoospermia (Review Article) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6030611/	Clinical and Experimental Reproductive Medicine	2.2/ 2.6	PubMed, Scopus, Clarivate (Web of Science)	44
3	**-Ahmed T Alahmar** -Pallav Sengupta(3)	Impact of coenzyme Q10 and selenium on seminal fluid parameters and antioxidant status in men with idiopathic infertility https://pubmed.ncbi.nlm.nih.gov/32572802/	Biological Trace Element Research	3.7/ 5.0	PubMed, Scopus, Clarivate (Web of Science)	20
4	**-Ahmed T Alahmar**(4)	The impact of two doses of coenzyme Q10 on semen parameters and antioxidant status in	Clinical and Experimental	2.2/ 2.6	PubMed, Scopus, Clarivate	17

		men with idiopathic oligoasthenoteratozoospermia https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6736512/	Reproductive Medicine		(Web of Science)	
5	-Rahul Vishvkarma **-Ahmed T. Alahmar** -Gopal Gupta - Rajender Singh(5)	Coenzyme Q10 effect on semen parameters: Profound or meagre? (Metanalysis) https://pubmed.ncbi.nlm.nih.gov/32271472/	Andrologia	2.7/ 3.4	PubMed, Scopus, Clarivate (Web of Science)	4
6	**-Ahmed T Alahmar** -Aldo - Calogero -Rajender Singh -Rossella Cannarella -Pallav Sengupta -Sulagna Dutta(6)	Coenzyme Q10, oxidative stress, and male infertility: A review (Review Article) https://pubmed.ncbi.nlm.nih.gov/34078005/	Clinical and Experimental Reproductive Medicine	2.2/ 2.6	PubMed, Scopus, Clarivate (Web of Science)	4
7	**-Ahmed T Alahmar**	Coenzyme Q10, oxidative stress markers, and sperm DNA damage in men with idiopathic oligoasthenoteratospermia	Clinical and Experimental	2.2/ 2.6	PubMed, Scopus, Clarivate	4

	-Pallav Sengupta -Sulagna Dutta -Aldo E Calogero(7)	https://pubmed.ncbi.nlm.nih.gov/34078008/	Reproductive Medicine		(Web of Science)	
8	**-Ahmed T Alahmar** -Aldo E - Calogero -Pallav Sengupta -Sulagna Dutta(8)	Coenzyme Q10 improves sperm parameters, oxidative stress markers, and sperm DNA fragmentation in infertile patients with idiopathic oligoasthenozoospermia https://pubmed.ncbi.nlm.nih.gov/32009311/	The World Journal with Men's Health	5.4/ 3.1	PubMed, Scopus, Clarivate (Web of Science)	24
9	**-Ahmed T Alahmar** - Rajender Singh (9)	Comparing the effects of coenzyme Q10 and Centrum multivitamin on semen parameters, oxidative stress markers, and sperm DNA fragmentation in infertile men with idiopathic oligoasthenospermia https://pubmed.ncbi.nlm.nih.gov/35255658/	Clinical and Experimental Reproductive Medicine	2.2/ 2.6	PubMed, Scopus, Clarivate (Web of Science)	
10	**-Ahmed T Alahmar** -Roozbeh Naemi(10)	Predictors of pregnancy and time to pregnancy in infertile men with idiopathic oligoasthenospermia pre- and post-coenzyme Q10 therapy https://pubmed.ncbi.nlm.nih.gov/35102599/	Andrologia	2.7/ 3.4	PubMed, Scopus, Clarivate (Web of Science)	

1. Introduction

The submitted work represents the outcome of my research experience from working for sixteen years as a lecturer, researcher, and specialist doctor at the College of Medicine, University of Babylon, Iraq (2005 till present). I received MBChB (Hons.) in Medicine and MSc (Hons.) in Medical Physiology degrees from the University of Babylon, Iraq. Throughout my career period, my main duties were teaching undergraduate students in the Medical Physiology course as well as conducting and publishing original research studies and reviews in reproductive physiology and male infertility. I have also practiced the clinical management of infertility at the Fertility Clinic and Babylon Teaching Hospital, Babyl, Iraq. My area of research interest is the role of oxidative stress (OS) and sperm DNA fragmentation (SDF) in male infertility including idiopathic male infertility (IMI). So far, I have 18 papers published in impact factor international journals indexed in PubMed, Scopus, and Clarivate Web of Science SCI (Impact factor 1.4-5.4) which are listed in my ORCID and ResearchGate profile (https://orcid.org/0000-0003-2100-5807). I have also presented many of my papers at local and international medical conferences as well as conducting and attending many workshops and seminars on infertility research. The objective for joining the current Ph.D. by publication programme at Staffordshire University was to enhance my research skills, experience, and methodological approaches with a view of collating a reflective account of my research over the past 16 years in the area of male infertility and the role of OS and SDF in men with IMI. Another objective was to be able to supervise Masters and Ph.D. postgraduate students at the University of Babylon and conduct further collaborative local and international research.

The submitted work consists of 10 papers (6 original research articles and 4 reviews including systematic reviews and meta-analysis) (Table 1). All the submitted papers have been published in international journals with impact factor. I am the lead author in all papers except paper 5 (P5) in which I am the second author. Some papers have been co-authored by international authors working in the same research area, however, in all studies except P5 I led authorship, conceptualization, formulation, execution, analysis and I have designed the study, searched the

literature, collected and analysed data, wrote the manuscript draft and handled the submission, revisions, and publications. This portfolio work aimed to explore the impact of coenzyme Q10 (CoQ10) on semen parameters, antioxidant markers, SDF, pregnancy rate, time to pregnancy (TTP), and their clinical as well as biochemical predictors in infertile men with IMI. I have used different methodologies including systematic reviews and meta-analysis, randomized and controlled prospective clinical studies with a period of follow-up of 3-24 months. I have also used different types of controls including active and negative controls and different doses of CoQ10 (200 versus 400 mg/day) and comparison with an individual (selenium) and combined antioxidants (Centrum multivitamin). In our main study P10, I have included the largest number of participants (178 patients and 84 controls) who received CoQ10 for an extended period of 6 months and additional 18 months of follow-up to detect pregnancy, TTP, and their predictors before and after CoQ10 therapy. I have also used different statistical analysis tests including normality tests, descriptive, t-test, Mann-Whitney test, Wilcoxon signed-rank test, correlation as well as binomial logistic regression and survival analysis (Cox regression and Kaplan-Meier curve) to explore the clinical and biochemical predictors of pregnancy and TTP in infertile men with IMI before and after CoQ10 treatment.

1.1. Originality and significance of the submitted work

- Although several potential mechanisms have been suggested for IMI, the exact molecular mechanisms underlying IMI remain unknown. These findings are reinforced by the fact that several therapies have been tried to treat male patients with IMI with inconsistent results. Further, there is a lack of agreement on types, dose, duration of treatment, the use of individual or combined antioxidants in patients with IMI (P1, 2, 5, and 6).
- Data on the impact of CoQ10 on antioxidant markers, SDF, pregnancy, and TTP in men with IMI are limited. To my knowledge, all the submitted original articles represent the first study that explored the effects of CoQ10 on these measures in men with IMI (P3, 4, 7-10).
- We have explored multiple OS markers, CoQ10 level, SDF, and predictors of pregnancy and TTP in men with IMI which represent promising diagnostic biomarkers as well as a therapeutic target in male patients with IMI (P3, 4, 7-10).
- The observed reduction in SDF in our studies may also reduce the incidence of congenital diseases, malignancies, and neuropsychiatric diseases in the offspring (P7-10).
- We have compared CoQ10 efficacy with another antioxidant (selenium), different doses of CoQ10 (200 vs 400 mg/day), and combined antioxidants (Centrum multivitamin) on study measures in men with IMI. We have also explored the effects of CoQ10 on hormonal profiles in men with IMI (P3,4 and 9).
- Study measures as well as pregnancy rate, TTP, and their predictors, were also compared between patients and fertile controls (P3,4, 7-10).
- Many previous clinical studies on the effect of CoQ10 therapy in men with IMI had semen parameters improvement but not pregnancy as a primary endpoint. Further, the results of these studies were limited by a small number of participants, heterogeneity of the patients' groups, a short period of follow-up, and the lack of exploration of the predictors of pregnancy outcomes. Therefore, our final and large study P10 included an extended period of CoQ10 therapy (6 months) in a larger number of participants (178 patients and 84 controls). We have explored CoQ10 level, multiple enzymatic and non-enzymatic antioxidant markers, SDF, BMI, pregnancy, and TTP and their predictors in patients with

OA before and after CoQ10 therapy using binomial logistic regression and survival analysis over 24 months period of follow-up (P10).

- The studies provide a better understanding of the mechanisms underlying IMI. We have also identified independent predictors of pregnancy and TTP which could be used as diagnostic markers and therapeutic targets in IMI (P1-10).
- CoQ10 could be a potential therapy to IMI which represents a challenge for science and clinical practice (P3, 4, 7-10).
- The prospective study design and multiple regression model allow for accurate, time-specific, and unbiased data collection related to the exposure, covariates, outcome, and independent predictors (P3, 4, 7-10).
- Our review articles search covered a long period (2000-2020) with systematic search, critical review, and updated evidence on the molecular mechanism OS, OS markers, tests, oral antioxidants, the impact of OS on male fertility, pregnancy, and assisted reproductive techniques (ART) outcome with comprehensive summary tables. We have also provided a critical review of the findings, strengths, and limitations of the relevant body of literature (P1, 2,5, and 6).
- The papers were published in specialized impact factor journals indexed in PubMed, Scopus, and Clarivate (Web of Science) databases and cited by many articles (Table 2).

1.2. Impact of the work:

Infertility has medical, psychological, social, and financial dimensions affecting approximately 10-15% of couples globally. The current studies provide a better understanding of the molecular mechanisms and the contribution of OS and SDF to infertility in men with IMI. A role for CoQ10 as an OS- and SDF-modifier and a potential therapy for the challenging group of men with IMI is also highlighted with the comparison of different doses of CoQ10, CoQ10 with selenium, and CoQ10 with Centrum multivitamins in follow-up studies. Our main study (P10) has collated data from the largest number of patients and controls and explored pregnancy rate, TTP, and their predictors after an extended period of CoQ10 therapy and 24 months of follow-up. The papers have been cited several times by many international specialized infertility and reproductive medicine journals with impact factor (1.4-5.4) indexed in PubMed, Scopus, Clarivate (Web of Science) and published by international publishers (Table 2).

The results of the current studies provide a solution for the critical diagnostic shortcomings of routine seminal fluid analysis by suggesting complementary biomarkers for IMI such as OS markers and SDF. Further, the identified clinical and biochemical predictors of pregnancy and TTP can facilitate future investigations and may augment management protocol for men with IMI also can provide prognostic data on whom will benefit most from antioxidants and ART treatment. Thus, our findings may enhance IMI diagnosis and may reduce the medical, psychological, and financial burden on infertile couples with IMI, and may improve the quality of health care and management. In addition, the observed reduction of SDF may be associated with improved pregnancy outcomes as well as a reduction of the incidence of congenital anomalies, malignancies, and neuropsychiatric disorders in the offspring.

OS represents a key player in IMI and the term MOSI (Male Oxidative Stress Infertility) has been coined for this contributing factor(11). Antioxidant supplementation to culture, incubation/handling, and sperm cryopreservation media has been proposed as a way to overcome ROS production and OS status in spermatozoa during ART treatment(12). Therefore, the findings of the current work reinforce previous reports on the beneficial effects of antioxidants including CoQ10 on semen parameters, antioxidant status, SDF, pregnancy

outcome, ART, and male fertility potential. The identification of an effective and optimized regimen of CoQ10 therapy by our studies may render the treatment of men with IMI more effective as evident from improved seminal parameters, SDF, and pregnancy outcome in this challenging group of patients. Further, excessive and unoptimized use of antioxidants and multivitamins should be avoided as recent studies have reported the development of reductive stress and antioxidant paradox. Thus, our results may contribute to the development of universal guidelines on OS and SDF testing, indication, and optimized treatment recommendations for the management of IMI in the clinical setting.

Establishing individualized antioxidant therapy as well as conducting further double-blind RCT are recommended to explore whether pregnancy and live birth rates are improved in couples with IMI and various OS levels following CoQ10 treatment. In addition, the current work will also enhance my career as a lecturer, researcher, and specialist doctor at the University of Babylon through incorporating the current knowledge into my clinical practice and management of infertile men with IMI. Further, the findings of the studies will motivate me to conduct further large-scale studies to consolidate the evidence on the impact of CoQ10 and other antioxidants on fertility biomarkers and determinants of pregnancy and ART outcomes in IMI. Finally, the current programme will also help me to supervise the research projects of postgraduate students at the University of Babylon.

Table 2. List of papers citations

Paper number	Number of citations	Link to citations
1	151	https://scholar.google.com/scholar?cites=4178754484259686749&as_sdt=2005&sciodt=0,5&hl=en
2	44	https://scholar.google.com/scholar?cites=8478009402218103888&as_sdt=2005&sciodt=0,5&hl=en
3	20	https://scholar.google.com/scholar?cites=14889864768684780341&as_sdt=2005&sciodt=0,5&hl=en
4	17	https://scholar.google.com/scholar?cites=16077556777709038824&as_sdt=2005&sciodt=0,5&hl=en
5	4	https://scholar.google.com/scholar?cites=14108747563119488925&as_sdt=2005&sciodt=0,5&hl=en
6	4	https://scholar.google.com/scholar?cites=75032244261023214 28&as_sdt=2005&sciodt=0,5&hl=en
7	4	https://scholar.google.com/scholar?cites=3759382317799184492&as_sdt=2005&sciodt=0,5&hl=en
8	24	https://scholar.google.com/scholar?cites=9231938819716635 07&as_sdt=2005&sciodt=0,5&hl=en
9		https://scholar.google.com/citations?view_op=view_citation&hl=en&user=J63nJNYAAAAJ&sortby=pubdate&citation_for_view=J63nJNYAAAAJ:UebtZRa9Y70C
10		https://scholar.google.com/citations?view_op=view_citation&hl=en&user=J63nJNYAAAAJ&sortby=pubdate&citation_for_view=J63nJNYAAAAJ:Se3iqnhoufwC

1.3. Contribution

In all submitted papers, except for P5, I led the authorship and directed the studies as the first author, or I was the sole author of the paper. I have undertaken conceptualization, formulation, execution, analysis, and I have designed the study, searched the literature, collected and analyzed data, wrote the manuscript draft, and handled the submission, revisions, and publication. In P5, I led authorship as a second author and I have undertaken conceptualization, formulation, execution, data collection, and analysis. In our last and main paper P10, I have undertaken study design, execution, analysis, searched the literature, collected and analyzed data, wrote the manuscript draft, and handled the submission and revisions. My supervisor Prof. Naemi has undertaken conceptualization, formulation, and revised the manuscript.

1.4. Scope and boundaries

The studies were focused on the impact of CoQ10 on the seminal OS, antioxidant status, SDF, and determinants of pregnancy and TTP outcomes in men with IMI before and after CoQ10 therapy. I have used different clinical study designs, systematic reviews, meta-analysis, narrative reviews, prospective randomized and controlled clinical studies. The participants included were patients with IMI (idiopathic OA and idiopathic OAT) and fertile controls who were recruited from Fertility Clinic in Babyl Governorate, Iraq. Participants were carefully selected using a set of inclusion and exclusion and those presented with factors affecting male fertility and OS were excluded.

In the studies, I have explored several parameters including ROS, TAC, catalase, superoxide dismutase, glutathione peroxidase, SDF, seminal CoQ10 level, and sex hormones (FSH, LH, testosterone, and prolactin) were assessed in men with IMI and fertile controls in follow-up studies. I have also compared different doses of CoQ10 (200 versus 400 mg/day), CoQ10 with selenium, and CoQ10 with Centrum multivitamin treatment for three months. In addition, the main paper (P10) explored the pregnancy rate, TTP, and their clinical and biochemical predictors in patients with idiopathic OA using binomial logistic regression and Cox regression (survival

analysis). The primary endpoint was pregnancy rate and TTP and the secondary endpoint was the alteration in semen parameters, seminal antioxidant status, and SDF.

As our target patient group was IMI, we have not assessed OS in infertile men with other causes of OS such as varicocele, infection, systemic disease, chemotherapy, radiotherapy, smoking, and alcoholism. The patients and controls were recruited from a single region in Iraq from May 2018 to February 2019 and were followed up for a period of 3 to 24 months. The studies were focused on the impact of CoQ10 in IMI and I have not included live birth rate as an additional endpoint due to the consent, time, and cost constraints in the follow-up studies.

1.5. List of other publications, presentations, workshops, scientific and community engagement activities

- Alahmar AT, Dutta. S, Pallav S. Thyroid hormones in male reproduction and infertility. Asian Pacific Journal of Reproduction. 2019; 8(5):203-210
- Alahmar AT, Ali Z, Muhsin Z and Qasim H. The impact of obesity on seminal fluid in men with infertility. Middle East Fertility Society Journal. 2018; 23(4): 346-394
- Alahmar AT. Effect of Vitamin C, Vitamin E, Zinc, Selenium, and Coenzyme Q10 in Infertile Men with Idiopathic Oligoasthenozoospermia International Journal of Infertility and Fetal Medicine.2017; 8(2):45-49
- Al-Araji SM, Mahmoud IM, Neamah AT. Seminal fluid & hormonal profiles among Iraqi patients with male infertility. Jordan Medical Journal,2010;44(2):198-207
- The impact of obesity on seminal fluid in men with infertility (Presentation, Al-Qadisiya University International Scientific Conference, April 2018).
- Update on the role of oxidative stress and SDF in idiopathic male infertility (Lecture, College of Medicine, University of Babylon, Iraq, December 2020).

- Establishing and managing a scientific online platform for free online local and international lectures and webinars in 2019 (Tatweer Global Platform, https://www.tatweer-training.net/english)
- Epidemiology and preventive measures of COVID-19 (Online lecture for public, Tatweer Global Platform, May 2020)
- Research Methodology course (University of Babylon, Iraq, January 2010)
- Publication and peer-review for Scopus and Clarivate journals. (Workshop, Second International Conference Creativity in Scientific Research, College of Pharmacy, Almustansiriyah University, February 2021)
- How to publish and review articles in journals with high impact factor (Workshop, University of Sumer, March 2020).
- Organizing and moderating 'Unboxing the future: Education for new times' (International online webinar, Prof. Mark Brown, Tatweer Global Platform, December 2020, https://youtu.be/lYjFEMWWuZY)
- Organizing and moderating 'COVID-19 and male infertility ' (International online webinar, Dr. Pallav Sengupta, Tatweer Global Platform, November 2020, https://youtu.be/zTPCVbNk3yE)
- Immunohistochemistry (IHC) workshop (College of Pharmacy, University of Babylon, Iraq, March 2018)
- PCR principles and applications workshop (College of Pharmacy, University of Babylon, Iraq, March 2018)
- ELIZA and HPLC principles and applications workshop (College of Pharmacy, University of Babylon, Iraq, January 2018)
- Nanotechnology in pharmacy workshop (College of Pharmacy, University of Babylon, Iraq, January 2018)
- Many online lectures and workshops during COVID-19 period on scientific research methodology, publications in international journals, and improving teaching methods and online learning (College of Medicine, University of Babylon, February 2020- present)
- Statistical analysis tests and SPSS (Workshop, August 2020, University of Sumer, Iraq)

- Conducting peer-review for 60 papers of international journals with impact factor (https://publons.com/researcher/1393271/ahmed-t-alahmar/)
- A member of the Royal College of Physicians (RCP) UK, British Fertility Society (BFS) UK, Society for Reproduction and Fertility (SRF) UK, Physiological Society UK, and Iraqi Medical Association.
- I had undertaken IELTS test (score 7.0) and also I have significant experience with statistical analysis software such as SPSS, StatsDirect, and GraphPad Prism.
- Toward prosperous higher education and research (College of Medicine, College of Science for Girls, University of Babylon, December 2016)

2. Literature review

2.1 Male infertility and idiopathic male infertility

Infertility is defined as the inability to achieve pregnancy after 12 months of regular unprotected sexual intercourse, affecting approximately 10 to 15% of couples globally (13). It is estimated that at least 48 million men are infertile worldwide and male infertility accounts for approximately 50% of all cases of infertility(14). Male infertility encompasses complex pathophysiology and has been linked to several conditions, including varicocele, cryptorchidism, hypogonadism, genital tract infection, endocrine abnormalities, testicular cancer, environmental toxins, systemic disease, exogenous drugs, and genetic factors(3). Nevertheless, in approximately 30%–40% of cases, the underlying cause for semen abnormalities is unknown, and this condition is referred to as IMI(15). Idiopathic reduction in semen parameters such as idiopathic oligoasthenoteratospermia (OAT) and idiopathic OA is defined as a combination of low sperm concentration (< 15 × 10^6 million/ml), reduced motility (progressive motility < 32% or total motility < 40%), and abnormally shaped spermatozoa (< 30% normal morphology by the 2010 World Health Organization (WHO) criteria or < 4% by the Kruger strict criteria) in men who do not have any disease that could affect their fertility(16). Potential mechanisms for IMI include genetic, epigenetic, posttranslational modification, OS, and SDF(17–19).

2.2. Oxidative stress, sperm DNA fragmentation, and idiopathic male infertility

OS is defined as an imbalance between the production of reactive oxygen species (ROS) and the scavenging capacity of available antioxidants resulting in a redox paradox(4). OS is a key contributing factor in 30–80% of male infertility cases. ROS are highly reactive molecules produced as a by-product of cellular metabolism and play important roles in cell signalling and homeostasis. Sources of ROS can be endogenous (cellular metabolism, immature sperm, leukocytes, and aging) or exogenous (varicocele, infection, malignancies, toxins, oestrogen, stress, smoking, alcohol, obesity, radiotherapy, and chemotherapy). Ultimately, OS may result in altered sperm function, sperm membrane lipid peroxidation, reduced sperm motility, impaired fertilization, and recurrent pregnancy loss. Further, mDNA damage and sperm DNA fragmentation which may ultimately lead to increased incidence of genetic diseases and

childhood malignancies in the offspring (Figure 1). The maintenance of redox homeostasis is governed by a balance between ROS production and antioxidants in the body(20). Seminal fluid is a major source of antioxidants that play key roles in protecting sperm from oxidative injury and both enzymatic and nonenzymatic antioxidant protective systems have been identified(21). Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), and glutathione S-transferases (GST) form the main enzymatic antioxidant system in semen. Numerous nonenzymatic antioxidants are present in semen, including vitamins A, E, C, and B complex, glutathione, CoQ10, carnitine, urate, and minerals such as zinc, copper, selenium, and chromium(1). Certain levels of ROS are required for the maturation of spermatozoa, acrosome reaction, capacitation, hyperactivation, and sperm oocyte fusion. However, excessive ROS production overwhelms the neutralizing capability of antioxidants in the seminal plasma resulting in multiple detrimental effects. The adverse effects of ROS on sperm encompass lipid peroxidation, loss of membrane integrity with increased permeability, reduced sperm motility, impaired hyperactivation and oocyte fusion, apoptosis, and structural damage to DNA resulting in SDF(22). Therefore, OS and antioxidant markers may serve as a potential diagnostic biomarker as well as a therapeutic target for IMI (9). Both direct and indirect tests are available to assess oxidative stress level (Table 3).

Another potential mechanism for IMI is SDF. SDF can be caused by extrinsic factors such as infection, systemic diseases, smoking, gonadotoxins, heat exposure, environmental pollutants, radiation, and chemotherapy as well as intrinsic factors such as defective germ cell maturation, abortive apoptosis, protamine imbalances, and OS(23). ROS alter DNA integrity in the sperm nucleus by inducing breakage of DNA strands, base modifications, and chromatin cross-linking. Moreover, sperm have limited defence mechanisms against ROS- induced DNA damage(6,24). High SDF levels can alter sperm function leading to decreased fertilization, poor embryonic development, pregnancy loss, and infertility. Recent studies have also reported that SDF may increase the risk of autosomal dominant disorders, neuropsychiatric disorders, and childhood cancers affecting the wellbeing of the offspring(8,25).

Several studies have demonstrated increased OS, reduced seminal antioxidant capacity, and high SDF levels among infertile men as compared to their fertile counterparts(4,26,27). Further, many studies have reported an improvement in these parameters following antioxidant therapy(1,4,8,13,26,28,29). Oral antioxidant therapy helps to scavenge seminal ROS and restore the redox balance. The different oral antioxidants available belong to the exogenous antioxidant category include vitamin C, vitamin E, CoQ10, N-acetylcysteine, carnitines, trace elements such as zinc, selenium, pentoxifylline, and a combination of these oral antioxidants(12,30,31). The treatment of men with IMI; however, remains a challenge as different medications including antioxidants have been tried individually or in combination with inconsistent results (2,6,32,33). Some studies have reported that antioxidant therapy may be beneficial and improve several sperm parameters, antioxidant capacity, and SDF(3,8,28). Other studies, on the other hand, reported no improvements in semen parameters (2,34). Further, there is a lack of consensus on the type, dosing, duration of treatment, target patient groups, and the use of individual or combined antioxidants (35). Further, data on the impact of CoQ10 on oxidative stress markers, SDF, and pregnancy outcome in IMI are limited. Therefore, we have decided to focus on the role of CoQ10 on these measures and male fertility potential in IMI.

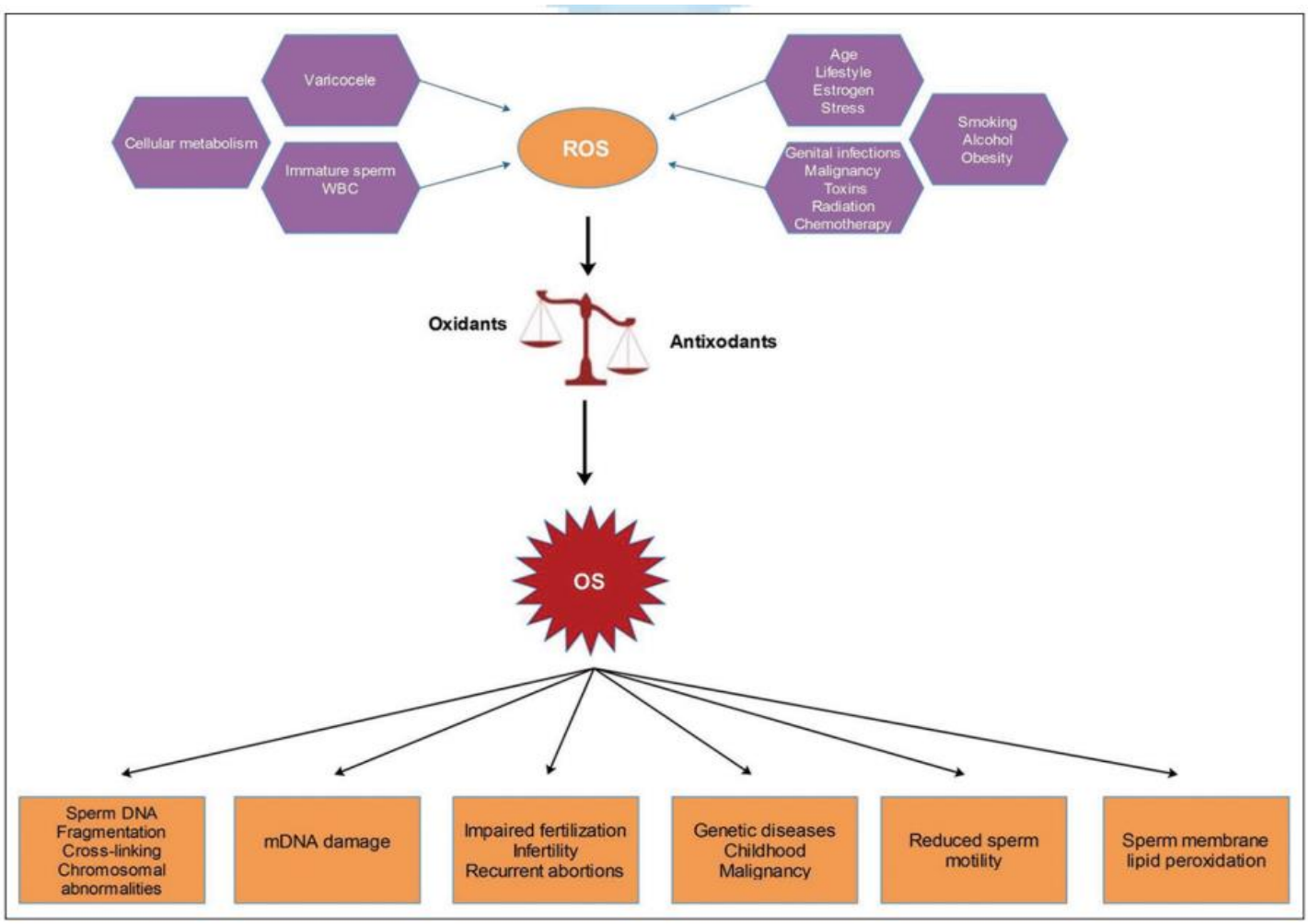

Figure 1. Sources of reactive oxygen species in the body and their pathological consequences on semen, fertility, and health. Various physiological and pathological processes lead to excessive reactive oxygen species and oxidative stress. The consequences of oxidative stress include altered sperm function, sperm membrane lipid peroxidation, reduced sperm motility, impaired fertilization, and recurrent pregnancy loss. Additional genetic alterations encompass mDNA damage and sperm DNA fragmentation which may ultimately lead to increased incidence of genetic diseases and malignancies in the offspring. ROS, Reactive oxygen species; OS, Oxidative stress

Table 3. Direct and indirect tests of oxidative stress

Direct tests	Indirect tests
-Chemiluminescence assay -Flow cytometry -Electron spin resonance -Cytochrome c reduction -Nitroblue tetrazolium test -Thiobarbituric acid assay -Xylenol orange-based assay -Fluorescein isothiocyanate-labelled lectins	-Myeloperoxidase or Endtz test -Lipid peroxidation levels -MiOXSYs system for oxidation-reduction potential -Total antioxidant capacity -Glutathione peroxidase activity -Comet test

2.3. Coenzyme Q10 and IMI

CoQ10 is a respiratory chain component with antioxidant properties that protect cells against lipid peroxidation-induced damage and OS and exists in reduced form (ubiquinol) or oxidized form (ubiquinone). CoQ10 transports electrons from complexes I and II to complex III in the mitochondrial respiratory chain–regulating cytoplasmic redox potential-protecting cell membrane OS and regulating the mitochondrial permeability transition pores(36). CoQ10 may also modulate gene expression, cell signalling, transport, and metabolism(37). Adequate CoQ10 levels are necessary for proper spermatozoa function given the role of CoQ10 in the mitochondrial respiratory chain and its antioxidant properties. In particular, mitochondrial dysfunction in spermatozoa has been associated with reduced sperm motility and mitochondrial DNA deletions(38).

Previous studies have also reported reduced CoQ10 level, antioxidant capacity, and high SDF levels in infertile men (7,39,40). We and others have reported improvement in sperm concentration and motility following CoQ10 therapy (4,8,41,42). Further, Our recent meta-analysis (5) and another meta-analysis (15) of three randomized controlled trials confirmed improvement of semen parameters but not pregnancy rates. A recent systematic review on the impact of CoQ10 on male fertility has also confirmed the improvement of semen parameters (28). Further, CoQ10 therapy is associated with improvement in antioxidant capacity and reduced SDF levels in men with IMI (8,43). Other studies, however; demonstrated no improvement in one or more of the seminal fluid parameters following CoQ10 therapy(44).

2.4. Aims and Objectives

The overall aim of the studies was to explore the impact of CoQ10 therapy (200 mg/day for 3-6 months) on semen parameters, seminal antioxidant markers, SDF, pregnancy rate, TTP, and their predictors in follow-up clinical studies (of 3-24 months). This aim encompasses the following objectives:

- To review the current evidence regarding the molecular mechanism of OS and ROS production, pathological effects of OS, OS tests, antioxidants, and the impact of OS on fertility, pregnancy, and ART outcome in male infertility and IMI (P1)
- To review the role of OS and reduced seminal antioxidant capacity as a potential mechanism for IMI as well as a role for oral antioxidants as a promising therapy for IMI (P2)
- To explore the effects of CoQ10 on semen parameters and seminal antioxidant markers in men with IMI (P3, 4, 7, 8, 9, and 10)
- To review the current evidence on the effects of CoQ10 on male semen parameters, seminal antioxidant status, SDF, pregnancy, and ART and to conduct a meta-analysis for the available CoQ10 RCT (P5 and P6)
- To compare the effects of different doses of CoQ10, CoQ10 versus an individual or combined antioxidants on semen parameters, seminal antioxidant status, and SDF in IMI (P3, 4, and 9)
- To assess the impact of CoQ10 on seminal CoQ10 level, sex hormones, and SDF in men with IMI (P7, 8, 9, and 10)
- To estimate pregnancy rate and TTP in infertile men with idiopathic OA after CoQ10 therapy (P10)
- To assess the clinical and biochemical predictors of pregnancy and TTP in men with idiopathic OA before and after CoQ10 treatment in men with idiopathic OA (P10).

-The objective of the P1 was to provide a systematic search and update on the current evidence regarding the molecular mechanism of OS and ROS production, pathological effects of OS, OS tests, antioxidants, and the impact of OS on fertility, pregnancy, and ART outcome in male infertility and IMI. In the next paper (P2), I explored the evidence provided by studies published from 2002 to 2017 on the impact of oral antioxidants (vitamin C, vitamin E, LC, CoQ10, zinc, selenium, and PTX) on seminal fluid parameters, antioxidant markers, SDF, and pregnancy outcome in men with idiopathic OAT.

Data on the impact of CoQ10 on OS markers, male fertility potential, and pregnancy outcome are limited. In addition, there is lack of agreement on a standardized treatment regimen. Therefore, I have decided to focus on the impact of CoQ10 treatment in IMI in our subsequent studies (comparing CoQ10 with other antioxidants and comparing different doses of CoQ10). Therefore, in P3 I have compared the effects of CoQ10 and selenium on seminal fluid parameters and antioxidant status in infertile men with idiopathic OAT. Another issue with CoQ10 therapy is the lack of agreement on the dose of CoQ10 to be used in the treatment of IMI as previous studies have used different doses of CoQ10 in male infertility ranging from 100 to 400 mg/day (table 4). As a result, in our next paper (P4) we have compared the impact of two doses of CoQ10 (200 versus 400 mg/day) on semen parameters, oxidative stress markers, and sperm DNA fragmentation in patients with idiopathic male infertility and fertile controls.

Previous studies on the impact of CoQ10 on male fertility and pregnancy outcome have been inconsistent. Therefore, there was a need to conduct a systematic review and meta-analysis to assess the strength of the current evidence from pooled randomized controlled trials (RCT) exploring the impact of CoQ10 on semen parameters in infertile men. Thus, in P5 I undertook a quantitative meta-analysis by pooling data from three RCT to evaluate the efficacy of CoQ10 in improving semen parameters. Additionally, in P6 I have undertaken a systematic review of the current body of literature (studies from 2000-2020) on the impact of CoQ10 semen parameters, OS markers, SDF, pregnancy, and ART outcomes.

As previous reviews have shown that SDF has many detrimental effects on sperm function, fertilization, male fertility potential, abortions, ART outcome, and offspring wellbeing, I have

decided to explore SDF in subsequent papers. Therefore, in P7, I have investigated the effects of CoQ10 therapy on semen parameters, seminal OS marker, CoQ10 level, and SDF in infertile men with idiopathic OA. Patients with idiopathic OAT might also demonstrate alteration in SDF following CoQ10 therapy. In addition, CoQ10 treatment may alter the hypothalamic-pituitary-gonadal axis. As a result, in P8 I have assessed the impact of CoQ10 therapy on semen parameters, another set of antioxidant markers, CoQ10 level, hormonal profile, and SDF in infertile men with idiopathic OAT. Another issue with oral antioxidants therapy is that previous studies have also shown the lack of consensus on the use of the individual or combined antioxidants regimen in men with IMI. We had previously established that CoQ10 was more effective than another individual antioxidant (namely selenium) in men with idiopathic OAT (P3). Therefore, in P9 I have compared the effects of CoQ10 and Centrum multivitamin on semen parameters, antioxidant markers, CoQ10 level, hormonal profile, and SDF in infertile men with idiopathic OA.

The results of studies P3, 4,5, 7-9 showed beneficial effects for CoQ10 therapy in IMI but the findings of our studies, however; were limited by the small number of participants, lack of long duration of treatment and follow-up, and lack of pregnancy outcome as an endpoint. In addition, data on the impact of CoQ10 therapy on seminal antioxidant capacity, SDF, pregnancy outcomes, time to pregnancy and their predictors are limited (Table 5). The identification of such predictors could have both diagnostic and therapeutic implications in men with IMI. Therefore, in our main study (P10) I have combined the data of a large number of men with idiopathic OA (178 patients and 84 controls) who were treated with CoQ10 for an extended period of 6 months, compared with fertile controls, and followed up for another 18 months. This study aimed to determine the clinical and biochemical predictors of pregnancy outcome and time to pregnancy TTP in infertile men with idiopathic OA pre- and post-CoQ10 therapy after 24 months of follow-up.

3. Materials and Methods

3.1. Systematic Review and meta-analysis studies

Our review articles provided an update on the molecular mechanism of OS, OS tests, the impact of OS on semen parameters, antioxidant markers, SDF, pregnancy, and ART outcomes. Further, the impact of oral antioxidants and CoQ10 on these parameters in men with IMI was reviewed. I have used specific search keywords, inclusion and exclusion criteria and several databases search including PubMed, Medline, Embase, Science Citation Index, Google Scholar, and Cochrane databases to explore relevant published articles for an extended period from 2000 to 2020. For systematic reviews and meta-analysis, we adopted the ideal reporting flow chart for Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) to report the results of this systematic assessment. Inclusion and exclusion criteria, data extraction, and quantitative data analysis were used. Pooled data from the available three RCT were presented in the meta-analysis. Comprehensive Meta-Analysis Software version 2 was used to perform all statistical analyses for this study. The reviews also provided comprehensive summary tables for the clinical studies included in these papers.

3.2. Original research methodologies

Participants were recruited from the Fertility Clinic, Babyl Governorate, Iraq from May 2018 to February 2019. Data were collected using a questionnaire designed for the studies. Medical history, physical examination as well as laboratory and radiological tests were performed to ascertain the presence of known factors that contribute to male infertility. Patients were carefully selected using a set of inclusion and exclusion criteria to identify patients with IMI and to exclude those with known medical conditions that affect male fertility. Fertile controls were also selected using a selection criterion to confirm fertility. I have used active controls to compare different antioxidants as well as no treatment fertile controls. When patients were allocated to receive one of two treatments, a simple randomization technique was used for randomization.

Patients received a CoQ10 dose that has been used in previous studies (13,45,46) for three months. Our main study P10 included 178 patients with idiopathic OA and 84 fertile controls. The participants and their partners underwent comprehensive fertility assessment by fertility specialists at the Fertility Clinic, Babyl Governorate, Iraq at baseline as well as during follow-up visits. All patients received a daily dose of 200 mg of CoQ10 (as ubiquinol) (America Medic and Science AMS, WA, USA) as a single oral dose for 6 months. Clinical demographics, weight, height, body mass index (BMI), semen parameters, seminal CoQ10 level, ROS, TAC, GPx, CAT, and SDF were measured compared at baseline and after 6 months. All participants were followed up for additional 18 months for pregnancy outcome and TTP and follow-up visits which were scheduled at 3 months intervals (a total of 24 months follow-up study). Patients who dropped out of the studies were excluded. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Study approval was obtained from the University of Sumer local research ethical committee (EC/2018/8866/8876/8878/8879). All the participants provided informed consent before enrollment in the studies.

Semen analysis was performed according to WHO guidelines (16). This method is the most widely accepted method worldwide as the standard method to perform semen analysis both in research and clinical practice settings although it has some limitations (47,48). All semen analyses were performed by the same investigator to ensure data consistency. Further, two semen analyses were performed at baseline and the end of the study, and the mean value of the two tests was used in the study. We have also optimized the abstinence period, handling time, and temperature to reduce technical errors. ROS, TAC, SOD, CAT, and GPx levels were assessed using colorimetric and chemiluminescence methods either manually using a previously published method or using kits (Elabscience, Texas, USA) following the recommended and validated protocols(49–53). The manufacturer Elabscience reported an inter-assay coefficient of variation (CV) (3.7-5.6%) and an intra-assay CV (2.72-4.8%)(54). The seminal level of CoQ10 was estimated using High-

performance liquid chromatography (HPLC using) reversed-phase HPLC method with UV detection method as described previously(26,55). HPLC method has the advantages over the colorimetric method of being more sensitive and specific, rapid and accurate for detection of CoQ10 level in semen(26). A sperm chromatin dispersion (SCD) test was performed using the Halosperm kit (Halotech DNA, S.L., Madrid, Spain) using a previously published method (56,57). Serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, and prolactin were measured using enzyme-linked fluorescent assays (mini-VIDAS; Biomerieux, Lyon, France)(58). Study variables were measured at baseline and the end of the studies and compared between patients and control groups.

3.3. Statistical Analysis

Statistical analysis was performed using IBM SPSS v.24 (IBM Corp., Armonk, NY, USA). Results were expressed as mean± SD. The data were assessed for normality using Shapiro- Wilk, and Kolmogorov–Smirnov tests. Appropriate statistical tests were then applied based on the normality of the data. For normally distributed data, an unpaired t-test was used to compare the means of two independent patients and controls groups and paired t-test was used to compare those of dependent groups (pre- and post-therapy). Pearson's correlation coefficient was used to estimate correlations between semen parameters and other study variables. For non-normally distributed data, Mann–Whitney U was used to compare the means of two independent groups and Wilcoxon signed-rank test was used to compare those of dependent groups. Spearman's correlation coefficient was used to correlate semen parameters with other variables. Chi-square test was used to compare variables presented as proportions. Univariate and multivariate logistic regression were used to explore the predictors of pregnancy outcome in patients and controls (by estimating pre-and post- values for each group). Univariate and multivariate cox regression was used to perform survival analysis to estimate the predictors of TTP in patients and controls (by estimating pre-and post- values for each group). Kaplan Meier Curve was used to examine the survival analysis between family history and education with TTP in patients and controls. A false discovery rate (FDR) correction for multiple comparisons was performed using a Benjamini–

Hochberg (BH) procedure. Sample size calculation was performed using 80% power and 5% level of significance. A P-value lower than 0.05 was considered statistically significant.

4. Results

P1 provided an update on OS, OS tests, SDF, pathological effects of OS and SDF on semen parameters, antioxidant markers, male fertility potential, pregnancy, and ART outcomes. The systematic review highlighted the impact of OS and reduced seminal antioxidant capacity on semen parameters, male fertility potential, pregnancy, and ART outcome in IMI. The review also demonstrated the current knowledge gap in the use of oral antioxidants and the different regimens tried for the treatment of IMI. Therefore, in the second systematic review (P2) I have explored the clinical studies that assessed the effect of different oral antioxidants in infertile men including IMI. The review confirmed the beneficial role of oral antioxidants including CoQ10 in improving semen parameters, antioxidant status, SDF, and male fertility potential in men with IMI. Comprehensive summary tables of the clinical studies including RCT on the impact of oral antioxidants and CoQ10 in IMI were also provided to summarize the PICO (patients, intervention, comparison, and outcome) findings of these studies. The review also demonstrated the lack of consensus on the dose, duration of treatment, target group, and the use of individual or combination of antioxidants. Further, data on the impact of CoQ10 on seminal antioxidant markers, SDF, male fertility potential, and pregnancy outcome are limited. Thus, I have decided to focus on the impact of CoQ10 on the aforementioned measures in my subsequent studies.

As previous studies of different oral antioxidants in IMI have provided inconsistent results, in P3 I have compared the effect of CoQ10 (200 mg/day) and selenium (200 µg/day) for three months on semen parameters and seminal (TAC, SOD, CAT) in patients with idiopathic OAT. There was a significant improvement in sperm concentration, motility, and seminal antioxidant capacity with CoQ10 providing the higher improvement. Semen parameters also correlated with antioxidant markers. Therefore, the study confirmed a beneficial effect for CoQ10 in IMI. As previous studies have tried different doses of CoQ10 in IMI, in P4 I have explored the impact of two doses of CoQ10 (200 and 400 mg/day) for three months on semen parameters and seminal (TAC, SOD, and CAT) in patients with idiopathic OAT. The study findings were consistent with those of P3 and demonstrated increased sperm concentration, progressive and total sperm motility but not

sperm morphology as well as higher levels of antioxidant markers following CoQ10 therapy of 400 mg/day. Semen parameters also correlated directly with (TAC, SOD, and CAT). Our findings were consistent with previous studies but few studies demonstrated the lack of beneficial effects for CoQ10 on semen parameters (table 4). As such, we were in need to explore the pooled evidence of the impact of CoQ10 in IMI from the previous RCT.

Therefore, in P5 we have conducted a meta-analysis for three RCT that investigated the effects of CoQ10 on semen parameters. The meta-analysis confirmed improvement of semen parameters, mainly sperm motility, following CoQ10 therapy. The meta-analysis also showed that higher improvement can be achieved with a longer duration of CoQ10 therapy of six months. The literature search for P5 also highlighted the impact of CoQ10 on pregnancy and ART outcomes as well as the role of SDF as a potential mechanism for IMI. Therefore, in P6 I have undertaken a systematic review of the effects of CoQ10 on semen parameters, OS markers, SDF, pregnancy, and ART outcomes. The systematic review illustrated that antioxidant properties of CoQ10 and its vital role in mitochondrial bioenergetics form the basis of the ameliorative effects of seminal CoQ10 on semen parameters, antioxidant markers, SDF, male fertility potential, and pregnancy outcome. The findings of P5 and P6 also highlighted the role of SDF as another potential mechanism for IMI. SDF also has detrimental effects on male fertility potential, fertilization, abortion, pregnancy, and ART outcomes. Thus in the subsequent papers, I have explored the seminal level of CoQ10, SDF, and hormonal profile in men with IMI along with semen parameters and antioxidant markers.

In P7, we have investigated the effects of CoQ10 (200 mg/day) for three months on seminal parameters, CoQ10 level, ROS, TAC, CAT, GPx, and SDF in patients with idiopathic OA. The study illustrated significant improvement in sperm concentration, motility, TAC, and GPx levels as well as a reduction in ROS and SDF post-therapy. Seminal CoQ10 level and SDF also correlated significantly with sperm concentration and motility. Next, In P8 I have explored the effect of CoQ10 (200 mg/day) for three months on seminal parameters, CoQ10 level, ROS, TAC, CAT, and

SDF. In addition, we have also estimated serum hormones (FSH, LH, testosterone, and prolactin) in patients with idiopathic OAT. A three months therapy with CoQ10 resulted in increased sperm concentration, progressive and total sperm motility, seminal CoQ10 level, TAC, CAT, and serum LH as well as a reduction in seminal SDF and ROS levels in the patients group. Further, total sperm motility correlated significantly with seminal CoQ10 level and SDF. Both P7 and P8 results confirmed that CoQ10 therapy may improve not only semen parameters and antioxidant status in men with IMI, but also may increase seminal CoQ10 level and reduce SDF level.

Another issue with oral antioxidant therapy in IMI was the lack of agreement on the use of individual or combination antioxidants. As such, I have compared CoQ10 and Centrum multivitamin effects on seminal antioxidant markers and SDF in men with idiopathic OA in P9. The patients were divided randomly into two groups: the first group received Coenzyme Q10 (200 mg/day orally) and the second group received Centrum multivitamin (one tablet/day) for 3 months. Semen parameters, CoQ10 level, ROS, TAC, CAT, SDF, and serum hormones (FSH, LH, testosterone, and prolactin) were compared at the baseline and after 3 months. The study demonstrated higher improvement with Centrum therapy of sperm concentration, motility, ROS, and SDF whereas CoQ10 therapy was more effective in increasing TAC and CAT levels in infertile men. In addition, both CoQ10 and Centrum therapy were associated with reduced serum testosterone level and significant correlations between seminal CoQ10 and SDF levels with sperm motility were also observed. The findings of P9 were consistent with those of P3,4,7 and 8.

Our studies' findings were; however, limited by the small number of participants, short duration of treatment and follow-up, and the lack of data on pregnancy rate and predictors of pregnancy outcome. Therefore, in our main study P10, I have included the largest number of patients and controls (178 patients and 84 controls) in an extended period of CoQ10 therapy of six months with subsequent follow-up of 18 months in a total of 24 months follow-up study. This study aimed to determine the clinical and biochemical predictors of pregnancy outcome and TTP in infertile men with idiopathic OA pre- and post-CoQ10 therapy. Several parameters were explored

including demographics, semen parameters, seminal CoQ10 levels, ROS, TAC, CAT, GPx, SDF, and BMI, and were compared at baseline and the end of six months of the study. All participants were followed up for another 18 months for pregnancy outcome and TTP and their predictors. The study illustrated that 6 months of CoQ10 therapy significantly increases CoQ10 levels in seminal plasma and improves semen parameters, antioxidant capacity, and SDF with a pregnancy rate of 24.2% in men with idiopathic OA. After CoQ10 therapy, CoQ10 level, sperm concentration, motility, and ROS were independent predictors of pregnancy outcome and CoQ10 level, male age, sperm concentration, motility, ROS, and GPx were independent predictors of TTP in patients.

5. Discussion

Data on the impact of CoQ10 on seminal antioxidant markers, SDF, pregnancy rate, and the predictors of pregnancy and TTP in men with idiopathic OA are limited (P1, 2, 5, and 6). Therefore, we have undertaken several prospective controlled clinical studies and systematic reviews to explore the impact of CoQ10 therapy on these measures. To our knowledge, the submitted original studies are the first studies to explore these parameters in men with IMI before and after CoQ10 therapy.

Our studies demonstrated a beneficial effect for CoQ10 therapy of 3-6 months on improving semen parameters and antioxidant capacity in men with IMI as compared to fertile controls (P3,4, 7-10).

The use of WHO reference values of seminal fluid analysis has long been debated as no individual or set of semen measures is highly predictive of male fertility potential(59). Studies have highlighted the need for establishing new biomarkers and predictive tools for male fertility such as sperm function tests, OS, and SDF. The main improvement in our studies was in sperm concentration, progressive and total motility, normal morphology, and markers of antioxidant capacity (ROS, TAC, SOD, CAT, and GPx) in patients following CoQ10 treatment (P3,4, 7-10). Body of literature demonstrated increased OS and ROS and reduced antioxidant capacity in infertile men as compared to fertile controls(40). Lower CoQ10 levels have been also reported in these men(60). Further, studies have shown that treatment with oral antioxidants including CoQ10 ameliorates these markers and was associated with improved semen parameters, antioxidant status, and male fertility potential(13,26,41,61–63). Our previous studies have also demonstrated that CoQ10 therapy improves sperm concentration, motility as well as antioxidant capacity in IMI (P3,4, 7-10). Further, our recent systematic review and meta-analysis (P5) and another two systematic reviews of RCT also confirmed a beneficial role for CoQ10 therapy in IMI (15,28). The main beneficial effect was on sperm concentration and motility. However, in one RCT in men with idiopathic OAT who received CoQ10, there was no improvement in semen

parameters following CoQ10 treatment (27). Additionally, antioxidant markers correlated with semen parameters (mainly sperm concentration and motility) (P3,4, 7-10). Similar correlations have been also reported in previous studies(3,26). Some studies reported the lack of association of sperm motility with fecundity perhaps because of random classification in our semiquantitative scoring system.

The control group also demonstrated mild improvement in semen parameters and antioxidant status and correlations between semen parameters and other study variables but these correlations were weak (P3,4, 7-10). Our comparative studies have also shown that CoQ10 therapy could be more effective than selenium (P3), CoQ10 400 mg/day more effective than 200 mg/day (P4) and both CoQ10 and Centrum multivitamin have selective beneficial effects on antioxidant markers in patients with IMI (P9). The enhancement of semen parameters and antioxidant capacity observed in our study could be attributed to higher CoQ10 level, the antioxidant properties of CoQ10 and its role in mitochondrial chain reaction kinetic, higher levels of seminal antioxidant which counteract OS as well as the longer duration of treatment as compared to shorter periods in other studies. Further, combined antioxidants may be more effective in improving specific seminal antioxidant measures possibly due to the synergistic action of these antioxidants.

CoQ10 therapy increased seminal CoQ10 level and reduced SDF in men with IMI. There were also correlations between CoQ10 and SDF with sperm motility(P7-10).

At baseline, infertile men had higher SDF levels as compared to controls. Treatment with CoQ10 for 3-6 months significantly increased seminal CoQ10 level and reduced SDF in men with IMI as compared to fertile controls (P7-10). Our results are in agreement with previous studies that detected lower CoQ10 levels in infertile men and treatment with CoQ10 was associated with increased seminal CoQ10 levels(60,61). In addition, studies showed increased SDF levels in men

with IMI, and treatment with antioxidants including CoQ10 reduced SDF in these men(7,39,40,64–67). Our studies have also demonstrated that both individual antioxidants such as CoQ10 and combined antioxidants such as Centrum multivitamin may be associated with the reduction of seminal SDF in patients with IMI (P7-10). The correlations between semen parameters and antioxidant capacity and SDF may establish the foundation for the use of oral antioxidants including CoQ10 in the treatment of infertile men with IMI and idiopathic OA to enhance their pregnancy outcomes. Further, these measures could be also used as diagnostic biomarkers for male fertility and pregnancy outcome.

The observed reduction in SDF following CoQ10 treatment in our studies could be due to the associated reduction in OS and improvement in seminal antioxidants levels. In addition, recent studies have also found that some of these effects of exogenously administered CoQ10 on SDF could be attributed to the modulation of gene expression (34). Thus, the results of the present study may indicate that at the molecular level, CoQ10 acts to ameliorate sperm DNA damage and mitigates OS, thereby leading to improvements in sperm parameters, fertilization, male fertility potential, and pregnancy outcome. Further, the observed reduction in SDF could be translated to a lower incidence of genetic diseases, neuropsychiatric diseases, and childhood malignancies in the offspring.

The pregnancy rate in patients in the current study was 24.2% and TTP was 20.52±6.72 months following 6 months of CoQ10 therapy and additional 18 months of follow-up in our main study. We have also identified many independent predictors of pregnancy and TTP (P10).

Our results (P10) are consistent with a study of men with idiopathic OAT treated with CoQ10 that reported a pregnancy rate of 34.1% and time to pregnancy of 8.4±4.7 years (39). Another RCT in men with IMI reported a pregnancy rate of 10% in patients following CoQ10 therapy (13). However, these studies did not explore antioxidant markers, SDF, or TTP. Data on the impact of

CoQ10 on SDF, pregnancy outcome, and TTP in men with IMI are limited (Table 5). A study that compared infertile men treated with combined antioxidants or placebo reported a spontaneous pregnancy rate of 29% versus 17% respectively(68). In fertile men, a study compared TTP in 4 countries and reported a pregnancy rate after 12 months of 77.5-79.6% (69). In contrast, a systematic review and meta-analysis that looked at several studies that supplemented infertile men with CoQ10 did not observe an increase in pregnancy rates (15). Although the findings of this meta-analysis are in contrast to our study and others, the number of events included in the meta-analysis is relatively small, and both live births and pregnancy rates were not the primary outcomes of the included trials. In a study that explored treatment-independent pregnancy rates in patients with severe reproductive disorders, the twelve-month cumulative spontaneous pregnancy rate in infertile men without treatment was 6.4% (70). We could not find previous studies that have explored the impact of CoQ10 on TTP in men with IMI. Ruder *et al.* reported a shorter TTP in couples with idiopathic infertility who received β-carotene, vitamin C, or vitamin E in dietary sources (Hazard Ratio 1.29, 1.09, and 1.07 respectively)(71). A recent review has also shown beneficial effects for preconceptional multiple-micronutrient supplementation on reducing TTP(72). The high pregnancy rate in men with idiopathic OA after CoQ10 therapy could be attributed to increased seminal CoQ10 level, improvement in semen parameters, antioxidant markers, and reduction in OS and SDF and therefore enhanced fertility potential in these patients. Further, CoQ10 has a central role in mitochondrial bioenergetics and antioxidant properties.

In multivariate logistic regression in our main study, factors that independently predicted pregnancy in patients before and after CoQ10 therapy in our study were CoQ10 level and sperm motility. Additional factors that independently predicted pregnancy post CoQ10 therapy were sperm concentration, and ROS (P10).

With the introduction of ICSI, proper interpretation of seminal fluid analysis as well as identifying the clinical and biochemical predictors are becoming even more essential. Improved ability to predict male fertility could reduce the number of patients requiring assisted fertilization and its associated cost and complications. Our results (P10) are in agreement with previous studies

which showed an association between sperm concentration, motility, normal morphology, and pregnancy outcome (73,74). Semen parameters have limitations as WHO reference values of semen analysis were obtained from fertile couples, unequal distribution of population, inability to assess sperm function and fertilization as well as lack of cut-off values that predict pregnancy rate (75). Therefore, additional biomarkers of sperm function and male fertility such as OS markers, antioxidant markers, sperm function tests, SDF, and predictors of pregnancy are essential (76). Some studies reported that sperm concentration and morphology are associated with pregnancy and TTP (77). Patel *et al.* concluded in a recent systematic review that semen parameters alone are poor predictors of male fertility potential except in cases of azoospermia, necrospermia, or globozoospermia(78).

In P10, antioxidant measures also correlated and predicted pregnancy. Studies have reported lower levels of antioxidants in infertile men (40) as well as higher pregnancy rates following oral antioxidant therapy including CoQ10(32,34). Our previous studies have also demonstrated lower antioxidant measures and higher SDF in infertile men with idiopathic OA or OAT and these abnormalities were ameliorated with CoQ10 therapy (P3,4, 7-10). High levels of SDF have been linked to IMI, abnormal semen parameters, pregnancy loss, and poor fertilization(79). Further, different cut-off values from 4 to 56% have been proposed for SDF prediction of pregnancy in infertile men(23). Obesity and high BMI have been associated with IMI, poor semen parameters, OS, and reduced fertilization and pregnancy rates (80). Our findings suggest that CoQ10 level, sperm motility, and ROS could be diagnostic biomarkers for male fertility as well as predictors of pregnancy outcome in men with idiopathic OA with CoQ10 therapy. CoQ10 therapy could be also used as a modulator of OS, antioxidant status, SDF, and male fertility potential.

In multivariate cox regression in our main study, factors that independently predicted TTP in patients before and after CoQ10 treatment were male age, sperm concentration, sperm motility, and CoQ10 level. Additional factors that predicted TTP post-therapy were sperm concentration, ROS, and GPx (P10).

Our results (P10) are consistent with a follow-up study on 501 couples that showed longer TTP and lower fecundability odds ratios were associated with normal sperm morphology, male age, and female BMI (59). Elevated ROS can be associated with a seven-fold decrease in pregnancy rate (81). High SDF levels among infertile men were associated with idiopathic infertility, recurrent intrauterine insemination (IUI) failure, recurrent pregnancy loss, and *in vitro* fertilization (IVF)/ intracytoplasmic sperm injection (ICSI) outcomes (82). The association between obesity and high BMI with longer TTP could be attributed to abnormal semen parameters, OS, low testosterone/estradiol ratio, and increased SDF among infertile men with obesity (83). A lower level of education was a significant factor in the occurrence of infertility in our patient group, which also correlated with pregnancy outcomes. A lower level of education has previously been linked to infertility in males (84). Our results point out that male age, sperm concentration, motility, ROS, and GPx could be used as diagnostic biomarkers as well as independent predictors of TTP in men with idiopathic OA with CoQ10 therapy (P10). Predictors of pregnancy and TTP will probably help to decide whether sufficient exposure to the achieved conception has occurred when to start a routine infertility evaluation, and how to avoid premature treatment with ART with their associated complications. TTP could also be used as an epidemiological tool on the effect of time on semen quality and fertility potential.

The lack of predictive power for some variables in our multivariate models could be explained by the biologically- based organization of male fertility potential to compensate for defects in one or more semen parameters relative to couple fertility. It is essential to mention that comparing the findings of our studies with other studies is challenging. This is due to the heterogeneity in study design, research methodologies, participants, type and dose of antioxidants, and the

primary endpoints. In addition, establishing accurate predictors of male fertility potential, pregnancy and TTP is also not straightforward due to variability and complex fertilizing functions of sperm, as well as the so far poorly understood molecular mechanisms of oocyte and fertilization.

5.1. Limitations

Limitations of my study include a smaller number of controls in comparison with patients and the lack of placebo arm due to ethical considerations although we have used fertile controls as no treatment group. Other limitations include the lack of dietary assessment, heterogeneity of the studies that I have used to compare my research results with, and participants were recruited from one location so our findings may not be generalized as there are genetic, racial, and geographical variations in semen parameters so further multicenter studies are warranted to consolidate the evidence provided in our studies.

5.2. Conclusion

Our findings demonstrated that 3-6 months of CoQ10 therapy (200 mg/day) significantly increased CoQ10 levels in seminal, semen parameters, antioxidant capacity, and reduced SDF and ROS with a pregnancy rate of 24.2% in men with idiopathic OA after 24 months of follow-up (P3,4, 7-10). CoQ10 level, sperm concentration, motility, and ROS could be independent predictors of pregnancy outcome and CoQ10 level, male age, sperm concentration, motility, ROS, and GPx could be independent predictors of TTP in these patients (P10). Further, CoQ10 therapy for 6 months could be a potential therapy for the challenging group of men with IMI and may enhance their fertility and pregnancy outcomes (P3,4, 7-10).

5.3. Future prospective

Future research should be focused on identifying the molecular genetic, epigenetic, and biochemical determinants of IMI. Limitations of seminal fluid analysis motivate the search for additional markers such as OS, SDF, sperm function test, and cytogenetic testing. Identification of novel biomarkers for male infertility such as seminal oxidation-reduction potential (ORP), microRNAs, cell-free nucleic acids, lipocalin-type prostaglandin D synthase (L-PGDS), acrosomal vesicle protein 1 (AVP1), extracellular matrix protein 1(ECM1), transcription/export 1(TEX1) and mitochondrial function is also essential. While a link between OS and SDF with IMI has been established, there is a requirement for developing new measurement techniques such as MiOXSYS, standardization of the established tests, identifying reference and cutoff values, target patients' group and comparing the sensitivity and specificity of different tests. Establishing OS testing and treatment guidelines, individualized antioxidant therapy as well as conducting further double-blind RCT are recommended to consolidate the evidence provided in the current work. Finally, the impact of the SARSCoV2 pandemic on OS and male fertility along with other systemic effects is under current investigations.

Table 4: Summary of the clinical studies on the effects of CoQ10 in men with idiopathic male infertility.

Author	Study design	Participants	Intervention	Measures	Main results
Balercia *et. al.* 2004 (61)	Open, uncontrolled	Idiopathic AS (n=22)	CoQ10 200 mg/day for 6 months.	Semen kinetic parameters, including computer-assisted sperm data and CoQ10 and phosphatidylcholine levels.	Higher forward sperm motility and sperm kinetics and seminal CoQ10 level.
Balercia *et. al.* 2009 (13)	RCT (double blind)	Idiopathic AS (n=60)	CoQ10 200 mg/day for 6 months.	Semen parameters, seminal CoQ10, and ubiquinol levels.	Higher forward and progressive sperm motility and increment in seminal CoQ10 and ubiquinol levels.
Safarinejad 2009 (41)	RCT (double blind)	Idiopathic OAT (n=212)	CoQ10 300 mg/day for 26 weeks.	Semen parameters, acrosome reaction test, immunobead test, FSH, LH, prolactin, testosterone, and inhibin levels.	Improvement in sperm concentration and motility post-therapy, correlations between duration of treatment and semen parameters, reduced FSH and LH, and increase in inhibin level.

Nadjarzadeh *et al*. 2011 (27)	RCT (double blind)	Idiopathic OAT (n=60)	CoQ10 200 mg/day for 3 months.	Semen parameters, serum malondialdehyde, TAC.	No significant improvement in semen parameters after CoQ10 therapy. Increase in seminal plasma TAC.
Safarinejad *et al*. 2012 (85)	RCT (double blind)	Idiopathic OAT (n=228)	CoQ10 200 mg/day for 26 weeks	Semen parameters	Improvement in all semen parameters, correlations between duration of treatment, seminal plasma TAC and semen measures, reduction in FSH and LH and increase in inhibin level.
Safarinejad *et al*. 2012 (86)	Open, uncontrolled	Idiopathic OAT (n=287)	CoQ10 300 mg/day for 12 months	Semen parameters, FSH, LH, TSH, prolactin, and inhibin.	Improvement in all semen parameters, correlations between duration of treatment, seminal plasma TAC and semen measures, reduction in FSH and LH and increase in inhibin level,

					improvement in pregnancy rate.
Nadjarzadeh *et al*. 2014 (26)	RCT (double blind)	Idiopathic OAT (n=287)	CoQ10 200 mg/day for 3 months	Semen parameters, diet and physical activity, seminal CoQ10, SOD, CAT, and 8-isoprostane levels.	Higher forward and progressive sperm motility and seminal CoQ10 level, correlations between CAT, SOD, and sperm morphology, increased CAT, SOD, and lower seminal 8-isoprostane concentration post-therapy.
Alahmar and Sengupta 2021 (3)	Randomised prospective	Idiopathic OAT (n=70)	CoQ10 200 mg/day or selenium 200 µg/day for 3 months	Semen parameters, TAC, CAT and SOD.	Improvement in sperm concentration, progressive and total sperm motility, increase in TAC, SOD, and CAT, correlations between semen parameters and antioxidant measures, CoQ10

					provided higher improvement.
Alahmar *et al*. 2019 (4)	Randomised prospective	Idiopathic OAT (n=65)	CoQ10 200 or 400 mg/day for 3 months	Semen parameters, TAC, CAT and SOD.	Higher sperm concentration and motility increased TAC, SOD, and CAT, correlations between semen parameters and antioxidant measures, CoQ10 400 mg/day provided higher improvement.
Alahmar *et al*. 2021 (8)	Prospective controlled	Idiopathic OA (n=65) and fertile controls (n=40)	CoQ10 200 mg/day for 3 months.	Semen parameters, seminal CoQ10, TAC, ROS, GPx, and SDF.	Improvement in sperm concentration, motility, seminal CoQ10, TAC, and GPx and reduction in SDF and ROS in patients post-therapy. Correlations between seminal CoQ10 level and SDF with sperm motility and concentration.

Alahmar *et al*. 2021 (7)	Prospective controlled	Idiopathic OA (n=50) and fertile controls (n=50)	CoQ10 200 mg/day for 3 months.	Semen parameters, seminal CoQ10, TAC, ROS, CAT, SDF, serum FSH, LH, testosterone, and prolactin.	Improvement in sperm concentration, progressive and total motility, seminal CoQ10, TAC and CAT, and reduction in SDF and ROS in patients post-therapy. Correlations between seminal CoQ10 level and SDF with sperm total motility.
Alahmar and Singh 2022 (9)	Randomised prospective	Idiopathic OA (n=130) and fertile controls (n=58)	CoQ10 200 mg/day or Centrum (one tablet/day) for 3 months.	Semen parameters, seminal CoQ10, TAC, ROS, CAT, SDF, serum FSH, LH, testosterone, and prolactin.	Both CoQ10 and Centrum improved sperm concentration and motility but the improvement was higher with Centrum therapy, CoQ10 was more effective in increasing TAC and CAT, Centrum was more effective in reducing ROS and

					SDF. Both therapies reduced serum testosterone levels.
Alahmar and Naemi 2022 (10)	Controlled prospective	Idiopathic OA (n=178) and fertile controls (n=84)	CoQ10 200 mg/day for 6 months in patients and additional 18 months of follow-up.	Semen parameters, seminal CoQ10, TAC, ROS, CAT, SDF BMI, pregnancy rate, and TTP and their predictors.	Significant improvement in semen parameters, antioxidant measures, and reduced SDF in patients post-therapy, correlations between semen parameters and other study variables, the Pregnancy rate was 24.2% and TTP was 20.52±6.72 months in patients, After CoQ10 therapy, CoQ10 level, sperm concentration, motility, and ROS were independent predictors of pregnancy outcome and CoQ10 level, male age, sperm

					concentration, motility, ROS, and GPx were independent predictors of TTP in patients.
Cakiroglu *et al*. 2014 (87)	Retrospective	Idiopathic AT (n=62)	CoQ10 100 mg twice daily for 6 months.	Semen parameters	Improved sperm motility and morphology.

AT, Asthenoteratospermia; OA, Oligoasthenospermia; OAT, Oligoasthenoteratospermia; CoQ10, Coenzyme Q10; TAC, Total Antioxidant Capacity; SOD, Superoxide Dismutase, CAT, Catalase; GPx, Glutathione Peroxidase; SDF, Sperm DNA Fragmentation; ROS, Reactive Oxygen Species; TTP, Time To Pregnancy; FSH, Follicle-Stimulating Hormone; LH, Luteinizing hormone; TSH, Thyroid-stimulating hormone; RCT, Randomized Controlled Trial.

Table 5: Summary of the clinical studies on the effects of antioxidants on pregnancy outcome in infertile men

Author	Participants	Intervention	Pregnancy outcome
Tremellen *et al*. 2007 (88)	Infertile men (n=60)	Menevit multivitamin, 1 tablet/day for 3 months.	No difference in fertilization, implantation, and pregnancy rate, higher live pregnancy rate post-therapy with Menevit.
Salehi *et al*. 2019 (89)	Infertile men (n=1654)	Vitamin E (50 mg), C (500 mg) and CoQ10 (100 mg) for 3 months.	Pregnancy rate 16.8%
Schisterman *et al*. 2020 (90)	Infertile men (n=2370)	Folic acid (5 mg) and zinc (30 mg) for 6 months.	No significant differences in clinical pregnancy, ectopic pregnancy, or live birth.
Vicari and Calogero 2001 (91)	Infertile men (n=54)	Carnitine (1g/day) for 3 months.	Increased pregnancy rate.
Balercia *et al*. 2004 (61)	Idiopathic AS (n=22)	CoQ10 (200 mg/day) for 6 months.	Pregnancy rate 2.4%
Ghanem *et al*. 2010 (92)	Idiopathic OA (n=60)	Clomiphene citrate (25 mg/day) and vitamin E (400	Increased pregnancy rate (36.7%).

		mg/day) for 6 months.	
Moslemi and Tavanbakhsh 2011(45)	Idiopathic AT (n=690)	Selenium (200 µg/day) and vitamin E (400 unit/days) for 100 days.	Increased spontaneous pregnancy rate (10.8%).
Safarinejad 2012 (85)	Idiopathic OAT (n=287)	CoQ10 (300 mg/day) for 12 months.	No difference in pregnancy and abortion rate.
Abad *et al*. 2013 (93)	Infertile men with AT (n=20)	Androferti multivitamin (1 capsule/day) for 3 months.	Pregnancy rate 5%
Kobori *et al*. 2014 (65)	Infertile men with OA (n=169)	CoQ10 (120 mg), vitamin C (80 mg) and vitamin E (40 mg) /day for 6 months.	Pregnancy rate 28.4%
Busetto *et al*. 2020 (94)	Infertile men with abnormal semen parameters (n=104)	Multivitamin (1 capsule/day) for 6 months.	Increased pregnancy rate.
Steiner *et al*. 2020 (95)	Infertile men with OAT (n=174)	Multivitamin (one tablet/day) for 6 months.	No difference in pregnancy and live birth rates.

AT, Asthenoteratospermia; OA, Oligoasthenospermia; OAT, Oligoasthenoteratospermia; CoQ10, Coenzyme Q10; AS: Asthenospermia.

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Review Article

Role of Oxidative Stress in Male Infertility: An Updated Review

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ABSTRACT

Current evidence links oxidative stress (OS) to male infertility, reduced sperm motility, sperm DNA damage and increased risk of recurrent abortions and genetic diseases. A review of PubMed, Medline, Google Scholar, and Cochrane review databases of published articles from years 2000–2018 was performed focusing on physiological and pathological consequences of reactive oxygen species (ROS), sperm DNA damage, OS tests, and the association between OS and male infertility, pregnancy and assisted reproductive techniques outcomes. Generation of ROS is essential for reproductive function, but OS is detrimental to fertility, pregnancy, and genetic status of the newborns. Further, there is a lack of consensus on selecting OS test, type, and duration of antioxidants treatment as well as on the target patients group. Developing advanced diagnostic and therapeutic options for OS is essential to improve fertility potential and limit genetic diseases transmitted to offspring.

KEYWORDS: *Antioxidants, male infertility, oxidative stress, sperm DNA damage*

INTRODUCTION

Infertility is defined as the inability to achieve pregnancy after 1 year of regular unprotected sexual intercourse.[1] The global prevalence of infertility varies between 2.5%–15%, correlating to at least 30 million infertile men worldwide.[2] Infertility has been linked to several emotional, physical, and sociocultural problems.[3] One of the mechanisms proposed for idiopathic male infertility is oxidative stress (OS). Male infertility accounts for about 40% of all cases and it is known that some conditions such as varicocele, cryptorchidism, hypogonadism, and genetic factors can cause infertility. However, no underlying cause can be identified for primary or secondary infertility in approximately 25% of couples which is termed idiopathic infertility.[4] One of the proposed mechanisms for idiopathic infertility is OS and reactive oxygen species (ROS).

Increased ROS along with decreased antioxidant defense result in redox imbalance, reduced sperm motility and sperm DNA damage. Spermatozoa are highly susceptible to the deleterious effects of ROS due to the large amounts of unsaturated fatty acids found in their cell membranes. Reactive oxygen species promote peroxidation of lipids, resulting in intracellular oxidative burden. The sequence of events involves lipid peroxidation, loss of membrane integrity with increased permeability, reduced sperm motility, structural DNA damage, and apoptosis.[5-7] Several intrinsic and extrinsic factors have been associated with increased OS in the male reproductive system.

The World Health Organization (WHO) has published references values for seminal fluid analysis parameters.[8] Decreased sperm concentration – defined as $< 15 \times 10^6$ sperm/ml – is termed oligozoospermia; whereas asthenozoospermia corresponds to progressive sperm motility of <32% or total sperm mobility under 40%. Teratospermia is defined as normal sperm motility of <4% using Kruger's strict criteria.[9] The combination of all these abnormalities is termed oligoasthenoteratozoospermia.

Currently, there is a lack of agreement on which patients should be tested for OS, as well as which test to perform. There are also controversies on types, dose, and duration of antioxidants treatment of in patients with excessive ROS levels.[10] Therefore, the aim of this review is

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to provide an update on current evidence regarding ROS production, tests and the association between OS and male infertility as well as pregnancy and assisted reproductive techniques (ART) outcomes.

Methods

This review of literature included a systematic search strategy performed in electronic scientific databases PUBMED, Medline, Google Scholar, and Cochrane review to include published articles from years 2000 to 2018. The search involved keywords including combinations of search terms "oxidative stress," "reactive oxygen species," "semen parameters," "male infertility," "sperm function," "antioxidants," "semen analysis," "oxidative stress tests," "pregnancy outcomes," and "assisted reproductive techniques." Articles were perused, and their reference lists were checked for relevant publications. We included articles published in English only.

Oxidative Stress Definition

OS is defined as an imbalance between the production of reactive oxygen species (ROS) and the scavenging capacity of available antioxidants resulting in redox paradox.[11] Sperm cells are vulnerable to ROS because of the abundance of polyunsaturated fatty acids in their plasma membrane and cytoplasm[12] and limited antioxidant capacity and DNA repair system.[13] Certain levels of ROS are required for maturation of spermatozoa, acrosome reaction, capacitation, hyperactivation, and sperm-oocyte fusion.[14] Excessive ROS production, however, overwhelms the neutralizing capability of antioxidants (enzymatic and nonenzymatic) in the seminal plasma. ROS are formed as natural byproducts of oxygen during metabolism and have important roles in cell signaling and homeostasis.[15] Sources of ROS can be endogenous or exogenous [Figure 1] and body antioxidant defense mechanism aims to neutralize the harmful effects of these pro-oxidants molecules.

Sources of Reactive Oxygen Species

Intrinsic

Redox reactions in aerobic metabolism yield ROS as byproducts. In mitochondria, these reactions require nicotinamide adenine dinucleotide (NADH) as an electron donor and acceptor in the electron transport chain, which allows synthesis of adenosine triphosphate (ATP)[16] [Figure 1]. Seminal fluid ROS can also originate from cytoplasmic glucose-6-phosphate dehydrogenase.[17] Varicocele, the most common etiology of male infertility, has been linked with the increased oxidative burden and ROS-induced sperm DNA damage;[14] as well as with increased scrotal temperature.[18] These findings have been corroborated with evidence describing reduced levels of seminal lipid peroxidation and sperm DNA damage after varicocelectomy.[18] Moreover, increased temperature has also been related to increased ROS

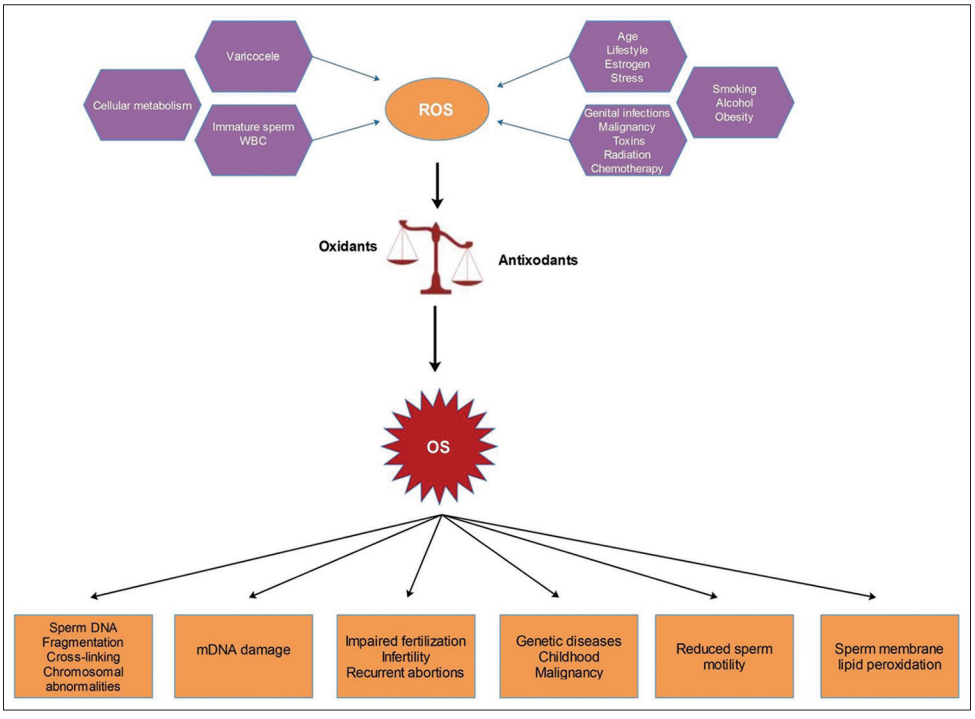

Figure 1: Sources of reactive oxygen species in the body and their pathological consequences on semen, fertility and health. ROS = Reactive oxygen species, OS = Oxidative stress

production and negative effects on other seminal fluid parameters.[19,20] In particular, elevated ROS have been associated with male accessory gland infection, including the urethra, prostate, deferent ducts, seminal vesicles, epididymis, or testes.[21] This has been attributed to the capacity of seminal leukocytes to produce 1000-fold more ROS and free radicals than any other cell with aerobic metabolism.[22] Infections have also been independently associated with increased ROS.[23,24] Furthermore, hyperglycemia is another important factor for male infertility, as strong correlations have been identified between prediabetes and diabetes mellitus with increased OS and altered sperm parameters.[25]

Extrinsic

Extrinsic factors such as smoking, alcohol intake, and exposure to radiation and industrial heavy metals have been associated with increased ROS and male infertility [Figure 1]. Smoking has been associated with reduced sperm concentration, motility, and altered morphology.[26] Smoking also elicits a chronic inflammatory response which recruits leukocytes to the genital tract and causes a substantial increase in seminal ROS levels,[27] as well as increased sperm DNA damage.[13]

Seminal fluid abnormalities have been associated with excessive alcohol intake, including decreased spermatogenesis, abnormal sperm morphology, decreased seminal fluid volume, low levels of testosterone, and increased OS.[28] Indeed, alcohol abuse results in increased production of acetaldehyde which promotes the generation of ROS due to its interactions with proteins and lipids.[11]

Altered sperm function and increased DNA damage have been associated with industrial exposure to heavy metals such as lead, cadmium, iron, and copper, as well as exposure to phthalates, pesticides, and pollution.[20,29] Malignancies are another important extrinsic source of ROS, along with the accompanying exposure to radiation and chemotherapy. Men treated with chemotherapy medications such as cisplatin, doxorubicin, or cyclophosphamide have been linked to increased OS.[30] Radiotherapy has also been associated with increased OS, while low-level radiation therapy appears to modulate NADH oxidase activity promoting sperm death.[31,32]

Antioxidants in Semen

Both enzymatic and nonenzymatic antioxidant protective systems have been identified in semen, which positively interact with each other to counteract the adverse effects of ROS.

Enzymatic antioxidants

Superoxide dismutase, catalase, and glutathione (GSH) peroxidase form the main antioxidant system in semen.[33] These metalloenzymes are present in both the intracellular and extracellular space. Superoxide dismutase catalyzes the dismutation of the superoxide anion, utilizing the copper and zinc molecules in its active center.[34] There are two main isoforms of superoxide dismutase (SOD) enzyme: SOD-1, with about 75% of the antioxidant, and SOD-3, with the remaining 25%.[35] Seminal SOD activity has been positively correlated with sperm concentration and motility.[36]

Catalase acts on hydrogen peroxide, resulting in its decomposition to water and molecular oxygen. The heme system with an iron atom in the center is the prominent characteristic of this enzyme, which can be found in the cytoplasm, endoplasmic reticulum, and various other organelles.[37] This enzyme is synthesized in the prostrate and catalase activity involves capacitation of nitric oxide and involvement of hydrogen peroxide.[33,38] Catalase level has been positively correlated with progressive sperm motility in normospermic individuals.[39] GSH peroxidase is responsible for the catalytic reduction of hydrogen peroxide and organic peroxides, including the peroxides of phospholipids.[34] This enzyme contains selenium in its active site and is primarily located in the mitochondrial matrix of spermatozoa. A specific isoform protects sperm DNA from oxidative damage and chromatin condensation.[40] GSH peroxidase activity was reduced in individuals diagnosed with severe asthenozoospermia, oligozoospermia, and teratozoospermia.[41]

Nonenzymatic antioxidants

Numerous nonenzymatic antioxidants are present in semen, including Vitamins A, E, C, and B complex, GSH, coenzyme Q10, carnitine, and minerals such as zinc, copper, selenium, and chromium.[42] GSH is a tripeptide thiol with a wide array of biological functions, including the preservation of the intracellular redox status and detoxification of exogenous and endogenous compounds. Structurally, it stems from a combination of three amino acids; cysteine, glycine, and glutamine. GSH possesses an innate reducing power that protects cells against OS, in particular, due to its sulfhydryl group (SH). GSH is found in both its oxidized and reduced forms. The antioxidant mechanisms of GSH are mediated by associated enzymes such as GSH peroxidase and GSH reductase.[43]

Vitamin E or alpha-tocopherol is a fat-soluble molecule found in almonds, avocados, spinach, and sweet potatoes. It has potent antioxidant properties, by neutralizing free radicals and inhibiting ROS damage to cell membranes;

resulting in prevention of lipid peroxidation and enhancement of other antioxidants. According to the European Commission Directive 2008/100/EC, the recommended daily intake of Vitamin E is 12 mg.[44] In an interventional placebo-controlled trial on infertile men conducted by Greco *et al.*,[45] minimal sperm DNA damage was observed after 2-month supplementation of Vitamin E (1 g/day) and Vitamin C (1 g/day).

Vitamin C is a water-soluble vitamin with antioxidant properties found in citric fruits and fresh berries. Numerous studies have evaluated the effect of Vitamin C supplementation on sperm function. A study performed on male rats showed ascorbic acid could revert testicular OS induced by cyclophosphamide.[46] In another study, reduced levels of Vitamin C and increased ROS levels were detected in the seminal fluid of men with asthenozoospermia.[47] Carotenoids are a group of organic compounds found in orange, red, yellow, and pink vegetable dyes, which act as precursors for Vitamin A, whose integral component is retinol. These are naturally occurring antioxidants, necessary for maintaining the integrity of cell membranes. They are also involved in the regulation of spermatogenesis. Carotenoid deficiency can lead to decreased sperm motility and male infertility.[48]

Zinc is the second-most abundant metal in the human body and a cofactor for various enzymes involved in DNA transcription and protein synthesis, playing a pivotal role in reproduction. Zinc participates in various reproductive processes such as steroidogenesis, testicular development, gonadal differentiation, production of luteinizing hormone and follicle stimulating hormone, formation and maturation of spermatozoa, acrosome reaction, and fertilization.[49,50] The WHO currently estimates zinc deficiency to affect one-third of the population worldwide. Zinc, along with various antioxidant enzymes, may improve sperm parameters and increase the likelihood of pregnancy for men with oligoasthenoteratozoospermia.[51] Selenium is another essential trace element, which intervenes in sperm formation and testosterone synthesis.[52] At least 25 selenoproteins have been identified in humans and animals, involved in the maintenance of sperm structural integrity. Several randomized clinical trials have tried selenium in combination with other antioxidants with promising results.[53,54]

Physiological Role of Reactive Oxygen Species in Male Fertility

The development of male germ cells yields significant amounts of ROS which constitutes a principal source of OS in spermatozoa.[55] Reactive oxygen species modulate sperm chromatin condensation by adjusting the number of germ cells and inducing apoptosis or proliferation of spermatozoa.[56] ROS are also involved in the processes of capacitation, acrosome reaction, mitochondrial stability, and sperm motility in mature sperm. ROS can function as messengers, by modulating the NADPH oxidase enzyme complex in the cell membrane, and intervening in the respiratory chain within mitochondria. In spermatozoa, superoxide anion metabolism is regulated by the NADH oxidoreductase enzyme, which works in close conjunction with the mitochondrial respiratory chain and xanthine oxidase found in sperm and seminal plasma.[33] Immature spermatozoa with cytoplasmic residues show increased production of ROS when compared to sperm with normal morphology.[17,57]

Seminal leukocytes are another source of ROS, producing 1000 times more of these molecules than sperm cells under physiological conditions. This is because seminal leukocytes represent the first line of defense against offending infectious agents, using primarily oxidative and inflammatory mechanisms.[22] However, this can become a double-edged sword, as an imbalance between oxidants and antioxidants could result in cellular injury. Indeed, ROS generated to counteract infectious agents can also damage host cells, which can result in the disintegration of the cell membrane or sperm DNA damage.

Pathological Effects of Reactive Oxygen Species on Male Fertility

The rationale behind the use of antioxidants for the treatment of male infertility relies on excessive levels of ROS and free radicals cause altered sperm function and sperm DNA damage. A study Desai *et al.* found that sperm characteristics were significantly lower in infertile men with high levels of ROS in semen as assessed through chemiluminescence.[32] Reactive oxygen species alter DNA integrity in the sperm nucleus by inducing breakage of DNA strands, base modifications, and chromatin cross-linking [Figure 1].[58] Moreover, spermatozoa have limited defense mechanisms against ROS-induced DNA damage.

Human ejaculate contains sperm cells with various degrees of maturity, along with leukocytes, epithelial cells and round cells from different stages spermatogenesis. Among these cells, peroxidase-positive leukocytes and immature spermatozoa produce significant amount of free radicals.[59] Spermatozoa are especially susceptible to oxidative damage due to the presence of abundant polyunsaturated fatty acids in their plasma membrane.

These fatty acids are important as they provide membrane fluidity, a key feature for several membrane fusion events such as acrosome reaction and sperm-egg interactions. However, these unsaturated fatty acids render them vulnerable to free radical attacks and ongoing lipid peroxidation.[60]

Nevertheless, in around 85% of cases, the sperm genome is protected from free radical damage as it is bound to central nucleoprotamines.[13] Deficient protamination has been observed in infertile men, representing yet another source of ROS-induced DNA damage[61] which is compounded by the limited capacity for sperm DNA repair seen during spermatogenesis.[62] ROS-mediate disruption of mitochondrial membranes leads to caspase activation, resulting in apoptosis. The apoptotic pathways involve cytochrome c release, which augments the levels of ROS, DNA damage, and apoptosis.[63]

DNA bases are also prone to OS-induced damage with base modifications, strand-breaks, and chromatin cross-linking. Indeed, OS and apoptosis are key events involved in causing DNA damage in the germ line.[64] The major role of ROS in the etiology of sperm DNA damage in infertile men has been corroborated in multiple studies.[65-68]

Spermatozoa carry a complete haploid genome to the ovum to form a new individual. Condensation of the nuclear material in the sperm nucleus is essential for this process to be successful. This condensation is promoted by the unique process of protamination, which involves the replacement of histones by positively charged protamines, which in turn form tight toroidal complexes. This is essential, as chromatin organization is necessary for fertilization and early embryonic development.[69] However, normal sperm appears to possess varying degrees of fragmented DNA; although, infertile men appear to have larger proportions of fragmented DNA.[70]

Both extrinsic and intrinsic factors are involved in the pathogenesis of fragmented DNA. The latter include poor chromatin structure and limited repair capacity. Intrinsic factors include abortive apoptosis and defective maturation.[71,72] Accumulating evidence suggests extrinsic factors are responsible for the increased DNA fragmentation found in the epididymis and ejaculated sperm in comparison to testicular sperm.[73] Recent research posits OS as another extrinsic cause of sperm DNA fragmentation (SDF),[74] as ROS can surpass the limited antioxidant mechanisms of sperm and damage polyunsaturated fatty acids in membranes, resulting in SDF.[75,76]

Sperm DNA Damage Induced by Reactive Oxygen Species

Although ROS seem to play a physiological role in the acrosome reaction, normal sperm function, activation, motility, and capacitation;[77] their potentially deleterious effects cannot be overlooked. Spermatozoa are especially vulnerable to ROS as they contain large amounts of polyunsaturated fatty acids in their plasma membrane and cytoplasm. OS could induce a rapid loss of intracellular ATP, resulting in axonemal damage with decreased sperm viability and mobility and increased mid-piece structural defects, with deleterious effects on sperm capacitation and the acrosome reaction. Lipid peroxidation of the sperm membrane is a key mediator of ROS-induced sperm damage, leading to infertility [Figure 1].[78,79]

Hydrogen peroxide is the principal ROS in human spermatozoa, while excessive production of ROS by abnormal spermatozoa or leukocytes appears to be associated with male infertility.[75] Moderately elevated concentrations of hydrogen peroxide cause sperm immobilization, mostly through depletion of intracellular ATP and reduced phosphorylation of axonemal proteins, with no impact on viability. In contrast, higher concentrations of hydrogen peroxide promote lipid peroxidation and cell death.[80,81]

In a study by Pasqualotto *et al.*,[82] the levels of antioxidants in seminal plasma from infertile men were significantly lower than in fertile controls, and the levels of ROS produced by spermatozoa were negatively correlated with sperm quality. In semen of infertile men, pathological levels of ROS are likely to be the result of increased ROS production and impaired antioxidant capacity.[83]

Exogenous or endogenous sources of ROS can induce sperm DNA damage that in turn may cause childhood diseases such as autosomal dominant disorders, neuropsychiatric disorders, and childhood cancers like retinoblastoma.[84,85] OS tends to target on telomeres, which are key genome protectors. Telomeres erode faster when exposed to OS, resulting in telomere dysfunction, chromosome instability, and apoptosis; all of which have been related to aging and carcinogenesis.[86]

This form of DNA damage could be particularly important in recurrent spontaneous abortion (RSA). Various paternal factors have been linked to RSA, including ROS-induced sperm DNA damage. In a study on 25 couples with idiopathic RSA and 25 proven fertile controls, ROS levels and DNA damage were significantly higher among the men in the RSA group.[87] Mitochondrial dysfunction and OS have been associated

with cancer, cellular senescence, apoptosis and aging; as well as with isolated cases of asthenozoospermia.[88] Antioxidants may prevent telomere loss and promote genomic stability in cells with mitochondrial dysfunction, corroborating the association with OS. Furthermore, nuclear transfer protected the genomes from telomere dysfunction and reconstitution of the mitochondria, thereby promoting cell survival.[89]

Lipid peroxidation cascade contributes to the production of free radicals and induces the production of lipid aldehydes such as acrolein, 4-hydorxynonenal (4-HNE), and malondialdehyde (MDA).[12] These have been linked with OS and damage to nuclear and mitochondrial DNA, with shorter telomeres, formation of the base product 8-hydroxy-deoxyguanine (8-OHdG), and fragmentation of mitochondrial DNA. They can also affect sperm plasma membranes, thus affecting their motility and ability to fuse with the oocyte. Production of 8-OHdG facilitates DNA damage by limiting the repairing capacity of spermatozoa.[90] Because fragmented DNA carries a high mutagenic potential, the oocyte may skip the base-excision repair and correction of 8-OHdG-associated changes, resulting in genomic hypermutability and instability, as well as infertility.[91] A high incidence of genetic aberrations in embryos have been attributed to ROS-induced OS in the male germ line; in association with conditions such as childhood cancers, neuropsychiatric disorders such as autism and schizophrenia, and dominant gene mutations such as Apert syndrome and achondroplasia.[13]

Antioxidant Therapy in Male Infertility

The rationale for oral antioxidant therapy is because seminal OS is due to increased ROS production and/or decreased levels of seminal antioxidants.[60] The different oral antioxidants available belong to the exogenous antioxidant category and they include Vitamin C, Vitamin E, coenzyme Q10, N-acetyl cysteine, carnitines, trace elements such as zinc, selenium, pentoxifylline, and a combination of these oral antioxidants. Numerous studies have been conducted to assess the effectiveness of oral antioxidant supplementation for the treatment of male infertility. Most of the studies showed an improvement in one or more of seminal fluid parameters,[92,93] whereas some studies reported no positive effect [Table 1].[94-96]

Tests to Measure Reactive Oxygen Species in Semen

Assessment of sperm ROS levels among infertile men can aid in determining which individuals who may benefit from antioxidant therapy. Various tests have been developed to detect seminal ROS levels which can be classified into direct and indirect assays [Tables 2 and 3]. Currently, there are no infertility guidelines that recommend routine ROS measurement and there is still an ongoing debate on which type of patients have to be tested for the oxidative burden. Asthenozoospermia in a semen sample is probably a marker of ROS.[11] Hyperviscosity has also been suggestive of increased OS because it is attributed to increased malondialdehyde levels. Increased leukocytes or round cells which is one of the principal sources of ROS may suggest further testing for OS. Abnormal sperm morphology due to cytoplasmic residues also correlates with high levels of ROS.[10] The hypo-osmotic swelling test suggests membrane damage in the sperm due to lipid peroxidation and this might imply higher levels of ROS in semen. Besides, some studies recommend ROS testing in individuals with idiopathic infertility.[110]

Reactive Oxygen Species Effects on Pregnancy Loss and Outcomes

The effects of SDF on natural pregnancy and pregnancy outcome have been recognized.[111] The Danish First Pregnancy Planner study illustrated a correlation between infertility and an SDF index of >30%. There was a significant association between a high sperm SDF and increased time to conceive naturally and lower fertility potential.[112] Similarly, in 500 couples with no infertility history who discontinued contraception for the purpose of pregnancy and they were enrolled in the Longitudinal Investigation of Fertility and the Environment study, SDF was associated with low fecundity.[113] A meta-analysis included 616 couples demonstrated that three studies showed an odds ratio of 7.01 suggestive of an association between high SDF and failure to achieve natural pregnancy.[114]

OS has become a growing concern for researchers and clinicians because of association with decreased fertilization, poor embryonic development, pregnancy loss, potential birth defects such as autism and cancers.[11] The chromatin in the sperm DNA is vulnerable to OS and there are base-pair modifications along with DNA fragmentation. Sperm and oocyte DNA damage may interfere with implantation and ultimately result in abortion. Evidence suggests that about 80% of the chromosomal aberrations are of paternal origin in humans.[115] Further, sperm DNA damage has been implicated in apoptosis, poor fertilization rate, higher frequency of miscarriage, and morbidity in offspring.[11]

Emerging evidence suggests that in ART, there is a correlation between high SDF and an increased risk of miscarriage. In a systematic review reported by Rilcheva

Table 1: Antioxidant therapy in male infertility

Author, years	Groups/number of participants	Controlled	Type of antioxidant and dose	Intervention period	Results
Safarinejad, 2011[97]	Idiopathic oligoasthenoteratozoospermia/211	Yes	EPA and DHA acids, 1.84 g/day versus placebo	32 weeks	Increase in total sperm count and concentration. Both EPA and DHA positively correlated with plasma superoxide dismutase and catalase activity
Wirleitner *et al*., 2012[98]	Oligoasthenoteratozoospermia and nonoligoasthenoteratozoospermia/147	Yes	Fertilovit M-Plus Vitamin C-100 mg Vitamin E-100 mg Folic acid-500 µg Zinc-25 mg Selenium-100 µg N-acetyl L-cysteine 50 mg L-carnitine 300 mg Citrulline 300 mg GSH reductase 50 mg Lycopene 4 mg Co-enzyme Q10 15 mg twice daily	2 months	Increased sperm concentration and motility. No significant improvement in morphology
Safarinejad *et al*., 2012[99]	Idiopathic oligoasthenoteratozoospermia/228	Yes	Coenzyme Q10-200 mg per day	26 weeks	Increased sperm density, motility and morphology. Decreased follicle stimulating hormone activity
Safarinejad, 2012[100]	Idiopathic oligoasthenoteratozoospermia/287	No	Coenzyme Q10-300 mg twice daily	12 months	Increased mean sperm concentration, progressive motility and normal morphology
Busetto *et al*., 2012[101]	Idiopathic asthenoteratozoospermia/114	No	L-carnitine-145 mg Acetyl L carnitine-64 mg, fructose 250 mg, citric acid 50 mg Selenium 50 µg CoQ10 20 mg Zinc 10 mg Ascorbic acid 90 mg Cyanocobalamine 1.5 µg Folic acid 200 mcg/once a day	4 months	Increased progressive sperm motility, and no significant improvement in sperm concentration and morphology
Chen *et al*., 2012[102]	Oligozoospermia and asthenozoospermia/64 and 42	Yes	Oligospermia Tamoxifen 10 mg bid Tamoxifen 10 mg + Vitamin E 100 mg tid Asthenospermia Levocarnitine 1 bottle bid Levocarnitine 1 bottle bid, Vitamin E 100 mg tid	3 months	Progressive increase in sperm motility in oligospermic men. There was nonsignificant improvement in sperm motility in asthenozoospermic individuals

Contd...

Table 1: Contd...

Author, years	Groups/number of participants	Controlled	Type of antioxidant and dose	Intervention period	Results
Cavallini, 2006[8]	Idiopathic oligoasthenoteratozoospermia/55	Yes	L-carnitine 1 g bid L-carnitine 500 mg bid +30 mg cinnoxicam every 4 days	3 months	Improvement in morphology and number of spermatozoa. Increased percentage of pregnancy following ICSI. Nonsignificant improvement in the number of fertilised oocytes and embryos transferred
Abad *et al*., 2013[103]	Asthenoteratozoospermia/20	Yes	L-carnitine-1500 mg Vitamin C-60 mg Coenzyme Q10-20 mg Vitamin E-10 mg Zinc-10 mg Vitamin B9-200 µg Vitamin B12-1 µg Selenium-50 µg	0 h, 2 h, 6 h, 8 h and 24 h	Increase in sperm concentration, motility, vitality and morhological parameters
Nadjarzadeh *et al*., 2014[104]	Idiopathic oligoasthenoteratozoospermia/60	Yes	200 mg/day CoQ10 or placebo	3 months	Improved semen parameters
Raigani *et al*., 2014[105]	Oligoasthenoteratozoospermia/83	Yes	Folic acid (5 mg/day) Zinc sulfate (220 mg/day) or placebo	16 weeks	Increased sperm concentration with combined treatment
Hadwan *et al*., 2014[106]	Asthenozoospermia/60	Yes	Zinc sulfate (220 mg/day) bid	3 months	Increased in semen volume, sperm count and forward motility
Cyrus *et al*., 2015[107]	Clinical varicocele/115	Yes	Vitamin C (250 mg) Bid or placebo	3 months	No effect on sperm count but improved sperm motility and morphology
Haghighian *et al*., 2015[108]	Idiopathic asthenozoospermia/44	Yes	Alpha lipoic acid (600 mg) or placebo	12 weeks	Sperm count, concentration, and motility were significantly improved
ElSheikh *et al*., 2015[94]	Idiopathic oligoasthenozoospermia/90	Yes	I: Vitamin E (400 mg/day) II. Clomiphene citrate (25 mg/day) III: Combination of drugs with same dosage	6 months	There was no significant increase in sperm concentration but only in the Vitamin E group. Combination therapy showed increased sperm concentration and motility
Bozhedomov *et al*., 2017[109]	Oligo or astheno or teratozoospermia/173	Yes	L-carnitine fumarate (1 g), acetyl L-carnitine (0.5 g) twice daily, combination of Vitamins A, E, C, selenium, zinc, clomiphene (25 mg) bid	3-4 months	Improve in the concentration of spermatozoa but no effect on sperm morphology, motility and pregnancy rates

EPA=Eicosapentaenoic acid, DHA=Docosahexaenoic acid, ICSI=Intracytoplasmic sperm injection, GSH=Glutathione

et al., it was found that after *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI), there were increased chances of pregnancy loss due to high SDF with a combined odds ratio of 2.48.[116] Another systematic review with 16 individual cohorts and 2969 couples corroborated the above results and it observed a

Table 2: Direct assays of oxidative stress

Test	Method of measurement	Function	Advantages	Disadvantages
Chemilum-inescence assay	Charged or uncharged probes undergo oxidation/reduction with generation of light as by-product	Deduce oxidation or reduction through the generation of light	High sensitivity and specificity. Robust test	Large and expensive equipment Time consuming Requires higher sample volume Interfering variables like semen age, volume, and temperature control
Flow cytometry	When excited by light of differing wavelengths, the incubation with the dye emits fluorescence	Measurement of ROS	It requires a small number of spermatozoa - patients with low sperm count- and can measure multiple markers simultaneously	Expensive tool that is not practical for widespread clinical use
Electron spin resonance	Obtains the absorption spectra of spin energy among unpaired electrons in an applied magnetic field	Detection of free radicals	Broad usage covering various parameters like: Observation of free radicals Analysis of free radicals characteristics Quantitative analysis of free radicals Kinetic analysis Good for high levels of ROS production	Free radicals can react with another molecule other than spin-trapping agent Interference factors like neutralization
Cytochrome c reduction	Superoxide radicals and reduced ferricytochrome c are identified	Evaluation of ROS on the cell membrane	Good in detecting high levels of ROS Quantifies superoxide released during respiratory burst of neutrophils or enzymes	If enzyme activity is less, relative insensitivity to the detection of NADPH oxidase activity
Nitroblue tetrazolium test	Nitroblue tetrazolium turns yellow to purple/blue when exposed to ROS	Localization of reaction between leukocytes or sperm cells and superoxide ions	Detects neutrophils at a concentration of 0.5×10^6/mL or higher Easy and cost-effective	Subjective interpretation of a positive or negative neutrophil
Thiobarbituric acid assay	Based on the reaction of a chromogenic agent, 2-thiobarbituric acid with MDA	Used to evaluate the resistance of sperm to oxidative stress	Can assess sperm MDA levels Needs expensive microplate readers	Expensive equipment is required
Xylenol orange-based assay	Oxidants in semen samples oxidise the ferrous ion-o-dianisidine complex to ferric ion. Ferric ion forms a colored compound with xylenol that can be detected using a spectrophotometer	Colorimetric automated assay	Measures the net oxidative imbalance between ROS production and antioxidant concentration	Limited widespread utility due to cost
FITC-labelled lectins	Used to detect the sperm acrosome status	Used to detect sperm peroxidase using plant lectins labeled with a fluorescent agent (FITC) to detect a group of sperm peroxidases	Detects sperm acrosome status	Difficult to detect true and false acrosome reaction Cannot detect sperm viability and acrosomal reaction status in one picture Fluorescent signal can fade sometimes

ROS=Reactive oxygen species, MDA=Malondialdehyde, NADPH=Nicotinamide adenine dinucleotide phosphate, FITC=Fluorescein isothiocyanate

Table 3: Indirect assays of oxidative stress

Test	Method of measurement	Function	Advantages	Disadvantages
Myeloperoxidase or Endtz test	Peroxidase positivity is assessed through staining using benzidine as a buffer	Detection of granulocytes in semen	Specifically distinguishes WBC's especially producing granulocytes from immature germ cells in semen	Cannot be used to detect ROS production in spermatozoa
Lipid peroxidation levels	Detection of MDA and toxic 4-HNE through colorimetric and thiobarbituric acid assays	Identification of by-products of lipid peroxidation	MDA is a colored substance that can be measured by fluorometry or spectrophotometry. Low sperm concentration of MDA can be measured through sensitive HPLC equipment or spectrofluorometric measurement of iron-based promoters	Not a widely-used test in clinic practice
MiOXSYs	Assessment of electron transfer in millivolts from a reducing agent to the oxidant using a galvanostat-based system	Measurement of oxidation-reduction potential	Easy to employ in a clinical setting Can be used in patients with low semen volume	Larger cohort studies to establish the reference value are needed
Total antioxidant capacity	Evaluates the reductive ability of the antioxidants within the semen against an oxidative agent such as hydrogen peroxide and measures the effect on the substrate	Assesses the cumulative effect of antioxidants within the semen	Rapid colorimetric method Total antioxidants in seminal plasma can be measured	Does not measure individual or enzymatic antioxidants. Requires expensive assay kit and microplate reader
Gpx activity	The activity of Gpx is measured by the decrease in GSH content after incubating the sample in the presence of H_2O_2 and NaN_3	Based on the principle that Gpx catalyzes the reaction between hydrogen peroxide and reduced GSH	Gpx protects the sperm from lipid peroxidation and the DNA damage can be significant if the Gpx levels are lower	Some studies show that Gpx activity does not correlate with sperm motility or concentration
Comet assay	Allows DNA migration in an agarose gel under an electric field. The loose DNA forms a pattern of migration that resembles a comet	Single-cell gel electrophoresis assay to assess DNA damage	Can detect extent of DNA damage equivalent to 50-single strand breaks per cell Can be employed in men with low sperm concentrations (requires only 100 cells for analysis)	No consensus reached on the standardization protocol

GSH=Glutathione, GPx=GSH peroxidase, 4-HNE=4-hydorxynonenal, MDA=Malondialdehyde, WBC's=White blood cells, ROS=Reactive oxygen species, HPLC=High-performance liquid chromatography

2.16-fold increase in the risk of pregnancy loss after IVF and ICSI with semen specimens with high SDF.[117] Both systematic reviews stated that the significant correlations between miscarriage rates and high SDF were independent of the method of fertilization used. A study involving 25 fertile sperm donors and 20 recurrent pregnancy loss (RPL) couples showed double-stranded DNA breaks analyzed by Comet assay among RPL sperm donors without any female factor.[118] A more recent study showed that there was a positive association between RSA and high SDF.[119]

Reactive Oxygen Species, Fertility Capacity and Assisted Reproduction Techniques Outcomes

Reactive oxygen species and oxidation-reduction potential (ORP) play significant roles in the fertility process and in the outcome of ART. In a prospective case-control study carried out in 1168 infertile and 100 fertile men, semen analysis parameters, the ORP and SDF were compared. The study concluded that infertile men had significant lower semen parameters and higher ORP and SDF levels, and that a significant positive correlation exists between ORP, SDF, and sperm head defects.[120] In a meta-analysis by Van Waart *et al.*, pregnancy rates achieved with intrauterine insemination (IUI) were correlated with normal sperm morphology and it was found a significant improvement in the pregnancy rate associated with >4% sperm morphology, and hence, they concluded that sperm morphology assessment by strict criteria is a good predictor of IUI outcome.[121] However, more recent studies failed to show an association of ART outcomes in groups with or without isolated teratozoospermia is semen.[122] Hotaling *et al.*, reported that in four retrospective studies, isolated teratozoospermia was not associated with lower pregnancy rates following IVF or

ICSI. Hence, the predictive power of sperm morphology on ART outcomes is still debatable.[123,124]

The existing literature about the consequences of sperm DNA damage on ART outcomes is still controversial. Li *et al.* performed a meta-analysis and found that sperm DNA damage had a negative impact on IVF clinical pregnancy rates but did not affect the IVF and ICSI fertilization and the ICSI clinical pregnancy.[125] Another meta-analysis by Zini *et al.*, evaluating the influence of sperm DNA damage on spontaneous pregnancy loss after IVF and ICSI, showed the detrimental effect of sperm DNA damage on ART outcomes and suggested a clinical test to evaluate the DNA damage before IVF or ICSI procedures.[126] However, the Practice Committee of the American Society for Reproductive Medicine concluded that the data in the existing literature does not support the adverse consequences of sperm DNA damage on ART and natural pregnancy outcomes, but recommends future research to validate the clinical utility of sperm integrity tests.[127] A more recent meta-analysis by Simon *et al.* including 41 studies showed that DNA damage in the sperm significantly correlated with adverse ART outcomes with IVF or ICSI techniques. The sperm DNA damage was evaluated using various tests such as Comet assay, sperm chromatin structure assay, terminal deoxyuridine nick end labeling assay, and sperm chromatin dispersion assay.[128] Therefore, despite that studies demonstrated correlation between sperm DNA damage and adverse ART events, the evidence is inconclusive as there is no standardization of the tests that has been used to detect sperm DNA damage.

Conclusion

Although reactive oxygen species are essential for some reproductive processes such as capacitation and acrosome reaction, increased ROS along with decreased antioxidant defense result in OS status which ultimately leads to sperm membrane lipid peroxidation, reduced motility, sperm DNA damage, poor pregnancy, and ART outcomes and increased risk of genetic diseases in offspring. Various diagnostic and therapeutic options have been developed for OS. However, there is lack of agreement on selecting OS test, type, and duration of antioxidants treatment as well as on defining the target patients group. Further studies are warranted to overcome these limitations, improve fertility potential and reduce the risk of genetic diseases and malignant tumors in newborns.

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Conflicts of interest

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CERM

The effects of oral antioxidants on the semen of men with idiopathic oligoasthenoteratozoospermia

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It has been estimated that approximately 15% of reproductive-age couples suffer from infertility. Male factors contribute to almost half of infertility cases, and in many patients the underlying cause of oligoasthenoteratozoospermia is unknown. Accumulating evidence suggests that oxidative stress plays a role as a contributing factor to male infertility, and reactive oxygen species have been shown to impair sperm function and motility and to damage sperm membrane and DNA. Therefore, this review explored the evidence provided by studies published from 2002 to 2017 on the impact of oral antioxidants (vitamin C, vitamin E, L-carnitine, coenzyme Q10, zinc, selenium, and pentoxifylline) on seminal fluid parameters in men with idiopathic oligoasthenoteratozoospermia. Most of the studies were randomized controlled studies that investigated the effect of single or combined antioxidants and reported improvements in at least one semen parameter. The most noteworthy effect that was found was that the use of multiple antioxidants increased sperm motility and concentration. Nonetheless, there is a lack of agreement on the dose, the duration of treatment, and whether individual or combined oral antioxidants should be used. Therefore, the current review provides evidence supporting the use of oral antioxidants in the treatment of infertile men with idiopathic oligoasthenoteratozoospermia.

Keywords: Antioxidants; Idiopathic; Male infertility; Oligoasthenoteratozoospermia; Oxidative stress

Introduction

Infertility is defined as the inability to achieve successful pregnancy after 12 months of regular unprotected sex. It has been estimated that approximately 15% of reproductive-age couples suffer from infertility, which has become a global concern [1]. Roughly 70 million couples worldwide are affected by infertility and seek treatment for this condition, and male factors contribute to almost half of cases [2]. Male infertility can be attributed to several conditions, including varicocele, hypogonadism, cryptorchidism, infection, autoimmune diseases, systemic diseases, testicular cancer, and genetic abnormalities. Nevertheless, in around 30%–40% of cases, no known cause is identified, and this condition has been termed as idiopathic oligoasthenoteratozoospermia (OAT). Idiopathic OAT includes a combination of low sperm concentration ($< 15 \times 10^6$/mL), reduced motility (progressive motility $< 32\%$ and total motility $< 40\%$) and abnormally shaped spermatozoa ($< 30\%$ normal morphology by the 2010 World Health Organization criteria or $< 4\%$ by the Kruger strict criteria) in men who do not have any disease that could affect their fertility [3].

Oxidative stress has been implicated in the development of many diseases, such as cancer, diabetes, cardiovascular disease, rheumatoid arthritis, liver disease, AIDS (acquired immune deficiency syndrome), Parkinson disease, and motor neuron disease [4]. Reactive oxygen species (ROS) levels have been also linked to male infertility, and studies have reported higher levels of ROS and suppressed antioxidant capacity in the semen of infertile men in comparison to their fertile counterparts [5-7]. ROS are normally present to some extent in seminal plasma as they are required for capacitation, the acrosome reaction, and fertilization, but the excessive production of ROS triggered by inflammatory cells has detrimental effects on sperm [8]. The adverse effects of ROS on sperm encompass reduced sperm motility, DNA fragmentation, impaired hyperactivation and oocyte fusion, damage to the sperm membrane due to lipid peroxidation, and

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poor chromatin packing [9-11]. The DNA damage due to ROS can be in the form of base modifications, attacks on phosphodiester bonds, point mutations, deletions, and frameshift mutations [12,13]. The resultant sperm damage can decrease the fertilization rate, reduce implantation, hinder embryonic development, and increase the risk of miscarriage and birth defects [14-18]. In addition, increased oxidative stress can adversely affect the proportions of polyunsaturated fatty acids, which are important for providing fluidity during membrane fusion events such as the acrosome reaction, sperm-egg interactions, and sperm motility [19].

Maintenance of redox homeostasis is governed by a balance between ROS production and antioxidants in the body. Antioxidants can be endogenous or exogenous. Endogenous antioxidants are classified into enzymatic antioxidants, such as catalase, superoxide dismutase, glutathione peroxidase, and non-enzymatic antioxidants such as glutathione, vitamin E, vitamin A, vitamin C, pyruvate, taurine, urate, coenzyme Q10 (CoQ10), and L-carnitine (LC) [20]. Exogenous antioxidants, such as vitamin E, vitamin C, and carotenoids, are mainly obtained from dietary sources [21]. In addition, semen also contains a recently recognized family of antioxidant enzymes known as peroxidoxins [12].

There is accumulating evidence that supports the use of oral antioxidants for the treatment of men with idiopathic OAT. The rationale is that seminal oxidative stress is a potential contributing factor to infertility due to decreased levels of seminal antioxidants [18]. Oral antioxidant therapy helps to scavenge seminal ROS and restore the redox balance [18]. The various available oral antioxidants encompass vitamin A; vitamin C; vitamin E; LC; CoQ10; N-acetyl cysteine; micronutrients such as zinc, selenium, and pentoxifylline (PTX); and combinations of these oral antioxidants. Therefore, this review aimed to explore the evidence provided by studies published from 2002 to 2017 on the impact of oral antioxidants (vitamin C, vitamin E, LC, CoQ10, zinc, selenium, and PTX) on seminal fluid parameters in men with idiopathic OAT.

Vitamin C

Vitamin C, also known as ascorbic acid, is a water-soluble vitamin freely available in citrus fruits and fresh berries that is known to have many beneficial effects through its antioxidant properties. Various studies have been carried out on the effects of ascorbic acid supplementation on sperm function. A prospective study conducted by Akmal et al. [22] observed that vitamin C supplementation (2 g/day) in infertile men with idiopathic oligozoospermia induced a significant increase in sperm motility and sperm count, as well as an increase in the percentage of normal spermatozoa, but there was no placebo group in that study. Eslamian et al. [23] performed a case-control study to assess associations between dietary patterns and asthenozoospermia in 107 patients and 235 age-matched controls. It was observed that a diet rich in vitamin C, vitamin E, vitamin D, zinc, folate, total fiber, polyunsaturated fatty acids, and selenium was significantly associated with a lower risk of asthenozoospermia. Vitamin C supplementation (250 mg twice daily) improved sperm motility and normal morphology, but not sperm count, in 115 infertile men following varicocele surgery in a double-blind randomized controlled trial [24]. Kobori et al. [25] investigated the effect of administration of vitamin C (80 mg), vitamin E (40 mg), and CoQ10 (120 mg) in 169 men with oligoasthenozoospermia and observed improvements in sperm concentration and motility after 3 and 6 months of treatment, as well as a pregnancy rate of 28.4% (seven after 3 months, eight after 6 months of treatment). A study of male rats demonstrated that ascorbic acid could reverse cyclophosphamide-induced testicular oxidative stress and testicular androgenic disorders [26]. Another study explored the effects of astaxanthin (100 mg/kg), vitamin E (100 mg/kg), vitamin C (100 mg/kg), and calorie restriction in male rats for 86 days and reported that antioxidant supplementation with or without calorie restriction did not have a significant influence on isoprostane, which is an oxidative stress marker. The antioxidant combination partially improved male infertility [27]. Another study reported that vitamin C mitigated the cyclophosphamide-induced reduction in seminal fluid parameters in rats attributed to oxidative stress [28].

Vitamin E

Vitamin E, also known as alpha-tocopherol, is a fat-soluble antioxidant vitamin found in almonds, avocados, spinach, and sweet potatoes that neutralizes free radicals and protects the cellular membrane against damage from ROS. It prevents lipid peroxidation and enhances the function of other antioxidants. The recommended daily allowance of vitamin E is 12 mg according to the European Commission Directive 2008/100/EC [29]. An interventional, placebo-controlled study of infertile men by Greco et al. [30] showed that 2-month supplementation of vitamin E (1 g/day) and vitamin C (1 g/day) resulted in a significant reduction of sperm DNA damage. However, there was no significant association between vitamin C and E intake and sperm parameters such as motility and concentration. In a prospective study of 690 men suffering from idiopathic OAT, selenium supplements (200 μg) in combination with vitamin E (400 IU) were administered for a period of 100 days, and in 52.6% of cases, there was an overall improvement in sperm motility, morphology, or both. Moreover, spontaneous pregnancy occurred in 10.8% of cases in the treatment group [31]. Another study by Greco and colleagues [32] showed that treatment with 1 g of vitamin E and vitamin C resulted in improved success rates of intracytoplasmic sperm injection (ICSI)

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and decreased sperm DNA damage.

In a randomized controlled trial conducted by ElSheikh et al. [33], 90 men with idiopathic OAT were divided into three groups that received vitamin E (400 mg/day), clomiphene citrate (25 mg/day), or a combination of vitamin E and clomiphene citrate for 6 months. Semen examinations post-therapy revealed that the sperm concentration and motility improved in all three groups, but to a greater extent in the combination group. It was found that CoQ10 and vitamin E plasma concentrations were reduced in 40 infertile men diagnosed with OAT. Accordingly, CoQ10 and vitamin E can be used as potential metabolic biomarkers for the diagnosis and treatment of male infertility [34].

L-carnitine

LC, or 3-aminobutyric acid, is a naturally occurring substance found in red meat and dairy products that is required for human metabolism. This molecule is involved as an intermediate in bioenergetic processes, where it plays an important role in the formation of acyl carnitine esters of long-chain fatty acids [35]. The concentration of LC is 2,000-fold greater in the epididymis than in the blood plasma, which is due to the active secretory process in the epididymis [35,36]. In a randomized controlled trial performed by Moslemi Mehni et al. [37] on 212 infertile men with idiopathic OAT, the combination of LC (500 mg) and PTX (400 mg) twice daily for a 3-month duration resulted in increases in all sperm parameters and improved outcomes of assisted reproductive technologies. An observational study of infertile men in Pakistan showed that seminal free LC levels were lower in infertile men than in fertile controls. Furthermore, strong positive correlations were found between seminal LC levels and sperm count, motility, and normal morphology [38]. Another study showed that seminal free LC levels were lower in infertile men than in fertile controls, and the lowest concentration was observed in the azoospermic group [39].

Lenzi et al. [40] carried out a double-blind placebo-controlled trial to assess the effect of LC supplements in 56 men with idiopathic OAT. The intervention group received 2 g/day of LC and 1 g/day of L-acetyl carnitine (LAC) for a duration of 6 months. Significant relationships between LC/LAC and improvements in all semen parameters were reported, and this trend was more prominent in infertile men with lower sperm motility at baseline. Another double-blind trial was conducted by Garolla et al. [41], examining the effects of LC therapy on phospholipid hydroperoxide glutathione peroxidase (PHGPX) levels in men suffering from idiopathic OAT. PHGPX levels were assessed at baseline and after receiving LC (2 g/day) for a period of 3 months. Semen analysis showed that LC therapy significantly improved sperm motility, and that the PHGPX levels were restored to normal. Balercia et al. [42] also carried out a randomized, placebo-controlled, double-blind trial of 59 men with idiopathic OAT, and assessed total oxygen radical scavenging capacity as well as sperm motion kinetics. A combination therapy utilizing LC and LAC (3 g/day) or placebo for a period of 6 months significantly increased sperm motility and total oxygen radical scavenging capacity. Nine pregnancies were achieved during this period, and five of them were in couples where the male partner was receiving combination therapy. Another study randomized 135 patients with asthenozoospermia to receive either LC (2 g/day) with vitamin E or vitamin E only for 3 months. The first group showed an increase in sperm motility, but no improvements were found in sperm density or normal morphology [43].

Coenzyme Q10

CoQ10 is a nonenzymatic antioxidant that is responsible for the protection of cells against lipid peroxidation-induced damage. It is found in organic meats, beef, soy oil, sardines, and peanuts. Lipid peroxidation is a significant feature of ROS-mediated cellular damage, in which cellular membrane fluidity is altered, membrane potential is reduced, harmful lipid epoxides are generated. CoQ10 protects lipids by scavenging the superoxide anion and peroxides, and it also helps in the mitochondrial electron transport chain, which generates adenosine triphosphate [44-46]. In a study of male Wistar rats with high low-density lipoprotein and oxidized low-density lipoprotein levels, protective effects of CoQ10 and LC were observed. It has been hypothesized that hypercholesterolemia can lead to fertility issues, and the protective effects of LC and CoQ10 supplementation led to significant improvements in sperm parameters, sperm function, and reproductive hormone profiles [4].

A randomized placebo-controlled trial carried out by Safarinejad [47] showed that CoQ10 therapy (300 mg for a 26-week period) in 212 infertile men suffering from idiopathic OAT resulted in a significant improvement in sperm count, motility, and morphology. Another double-blind placebo-controlled clinical trial investigated the effects of the reduced form of CoQ10 on sperm parameters and seminal plasma antioxidant capacity in 228 men with idiopathic OAT. The patients received oral supplementation of CoQ10 (200 mg) for a period of 26 weeks, and they showed improvements in sperm density, motility, and morphology. The study demonstrated that during the off-drug period, the sperm parameters gradually returned to their baseline values, but the observed differences were still significant [48]. In an open-label prospective study by Safarinejad [49], in 287 infertile men with idiopathic OAT, treatment with CoQ10 (300 mg orally, twice daily) for 12 months resulted in improvements in sperm concentration, progressive motility, and normal sperm morphology, and a pregnancy rate of 34.1% was reported. In contrast, in a clinical trial, Nadjarzadeh et al. [50] observed that supplementation with

CoQ10 (200 mg for a 16-week period) produced no significant changes in sperm parameters, but significantly increased the seminal total antioxidant capacity. In another clinical trial performed by Nadjarzadeh and colleagues [51], the effects of CoQ10 supplementation on seminal antioxidants and oxidative stress apart from sperm parameters in men with idiopathic OAT were studied. It was observed that CoQ10 supplementation for a period of 3 months improved the activity of enzymatic antioxidants, such as catalase and superoxide dismutase. It was also shown that there were lower 8-isoprostane (oxidative stress marker) levels in the intervention group that received CoQ10 therapy. The researchers concluded that CoQ10 levels were significantly correlated with key semen parameters such as sperm morphology, motility, and density due to improvements in the total antioxidant capacity. Thakur et al. [52] showed that supplementation with 150 mg of CoQ10 significantly enhanced sperm parameters in infertile men. Finally, a meta-analysis of the effect of CoQ10 supplementation in infertile men showed that global improvements in sperm parameters such as motility, morphology, and concentration accompanied increased concentrations of CoQ10 in semen. These results, however, must be interpreted with caution because they do not necessarily indicate an increased chance of pregnancy or live birth [53].

Zinc

Zinc deficiency has been postulated as a putative contributing factor to male factor infertility [54]. Zinc is the second most abundant trace element found in human tissue, following iron. Food sources of zinc include red and white meat, fish, and milk, and the World Health Organization estimated that about one-third of the global population is deficient in zinc [55]. The trace element zinc serves as a cofactor for numerous enzymes responsible for cellular development, such as DNA transcription and protein synthesis. Zinc plays a pivotal role in testicular development, steroidogenesis, the synthesis and secretion of luteinizing hormone and follicle-stimulating hormone, gonadal differentiation, the formation and maturation of spermatozoa, the acrosome reaction, and fertilization [56,57]. A recent systematic review concluded that seminal zinc levels were lower in infertile men and that zinc supplementation increased semen volume, sperm motility, and normal sperm morphology [5]. Another study of 150 infertile men demonstrated positive correlations between seminal plasma zinc levels and semen parameters and serum free testosterone [58]. In a clinical trial conducted in Iraq, semen samples were acquired from 60 asthenozoospermic infertile men and 60 age-matched fertile men. Zinc sulfate supplementation, with a daily dose of 440 mg, was given to the infertile men for a period of 3 months, and the qualitative and quantitative characteristics of semen, along with peroxynitrite, arginase, and nitric oxide (NO) synthase activity, were assessed. The peroxynitrite levels and NO synthase activity were significantly higher in the infertile men than in the fertile group, whereas arginase activity was higher in the fertile group than in the infertile men. Moreover, peroxynitrite, NO synthase, and arginase levels were restored to normal values following zinc supplementation in infertile men. Semen volume, sperm count, and progressive sperm motility significantly improved following zinc supplementation in asthenozoospermic men [59]. In another study, the same researchers investigated the effect of zinc supplementation on zinc binding protein levels and qualitative and quantitative semen characteristics. Semen samples were collected from 37 fertile men and 37 subfertile men with asthenozoospermia who were given zinc supplementation (440 mg/day) for a 3-month period. The results of the study showed that zinc supplementation significantly increased semen volume, sperm motility, and sperm count in the subfertile men [60]. A combination of zinc and folic acid supplementation was studied in men suffering from OAT. In this randomized, double-blinded, placebo-controlled trial, 83 men with OAT received zinc (220 mg/day) and folic acid (5 mg/day) orally for a period of 16 weeks. The study did not show improvements in sperm quality in subfertile men suffering from OAT, even after adjusting for the placebo effect [61].

Selenium

Selenium is an essential trace element that plays an important role in sperm formation and testosterone synthesis [62]. At least 25 selenoproteins have been identified in humans and animals, and these selenoproteins help to maintain the structural integrity of sperm [37]. A placebo-controlled clinical trial was performed on men with idiopathic OAT who received 200 μg of selenium orally along with 600 mg of N-acetyl-cysteine or a similar regimen of placebo for a period of 26 weeks, followed by a 30-week treatment-free period. Significant positive correlations were found between seminal plasma concentrations of selenium and N-acetyl cysteine and mean sperm concentration, motility, and normal morphology [63]. Another clinical trial was conducted on 54 infertile men and healthy controls who received supplementation with vitamin E (400 mg) and selenium (225 μg) for a 3-month period, while a placebo group received vitamin B (4.5 g/day) for the same duration. In comparison to vitamin B, selenium and vitamin E supplementation resulted in a significant decrease in an oxidative stress marker (malondialdehyde), and sperm motility and viability were inversely correlated with malondialdehyde levels [64]. Selenium-fortified probiotics reduced triglyceride levels and improved sperm count, mobility, and morphology in obese rats [65].

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Pentoxifylline

PTX is a derivative of xanthine that increases local blood flow by increasing the deformability of red blood cells and decreasing blood viscosity [66]. PTX has been recommended as an artificial sperm movement enhancer, and it has been shown to be particularly useful in patients with asthenozoospermia, who exhibit decreased sperm motility in their ejaculate [67]. An *in vitro* study showed that PTX significantly improved sperm movement in asthenozoospermic semen samples without adverse effects on sperm DNA or chromatin integrity during a vitrification program [68]. It was also shown that PTX had a positive effect on ICSI outcomes, including fertilization, embryo quality, and pregnancy rates, in asthenozoospermic patients [67]. Safarinejad [69] performed a randomized controlled trial on men with idiopathic OAT, investigating the response of semen parameters to supplementation with PTX (400 mg twice daily) for a 24-week treatment phase followed by a 12-week treatment-free period. The results of that study showed a significant improvement in seminal parameters such as concentration, motility, and morphology. An increase was also observed in the acrosome reaction in the PTX group. Another study observed that PTX (1,200 mg) in combination with 5 mg of folic acid and 66 mg of zinc sulfate administered orally every day for a period of 12 weeks in patients suffering from varicocele improved sperm morphology starting in the 4th week of treatment [70].

Combination of oral antioxidants

The current trend is to employ multiple antioxidants to treat male infertility in order to achieve synergistic antioxidant effects. Gharagozloo et al. [13] found that a combination of LC (500 mg), folic acid (450 µg), vitamin C (60 mg), lycopene (10 mg), selenium (55 µg), vitamin E (200 mg), and zinc (10 mg) significantly reduced levels of 8-hydroxydeoxyguanosine, a marker of DNA damage, in sperm cells in mice. They also observed that in a scrotal heat stress model, pretreatment with antioxidants led to 74% of female mice becoming pregnant, resulting in 427 fetuses, which constituted an improvement in fertility. The effects of another combination of vitamin C (100 mg), vitamin E (100 mg), folic acid (500 µg), zinc (25 mg), selenium (100 µg), N-acetyl cysteine (50 mg), LC (300 mg), citrulline (300 mg), lycopene (4 mg), and CoQ10 (15 mg) on sperm quality were investigated in 147 patients who underwent *in vitro* fertilization (IVF). The patients were categorized as OAT and non-OAT men, and following the first and second sperm analyses, they received vitamin C (100 mg), vitamin E (100 mg), folic acid (500 µg), zinc (25 mg), selenium (100 µg), N-acetyl-L-cysteine (50 mg), and LC (300 mg). Significant improvements were found in sperm concentration, motility, and normal morphology following micronutrient and vitamin supplementation, and the effects were stronger in men with OAT with restricted sperm parameters. The sperm parameters that improved were motility, sperm count, and nuclear vacuolization [71]. These findings are congruent with those of our recent study that demonstrated that daily supplementation with vitamin C (90 mg/day), vitamin E (15 mg/day), CoQ10 (4 mg/day), selenium (30 µg/day), and zinc (5 mg/day) for 3 months improved sperm concentration, progressive motility, and total motility in men with idiopathic oligoasthenozoospermia [72].

Abad et al. [73] performed a study to assess the effect of oral antioxidant supplementation on the sperm dynamics of DNA fragmentation in a cohort of 20 infertile men with asthenoteratozoospermia. Patients received 1,500 mg of LC, 60 mg of vitamin C, 20 mg of CoQ10, 10 mg of vitamin E, 10 mg of zinc, 200 µg of folic acid, 50 µg of selenium, and 1 µg of vitamin B12 for a duration of 3 months. Semen analysis showed that the proportion of degraded sperm significantly decreased, and significant improvements were found in sperm concentration, motility, and morphology. There was also a significant improvement in DNA integrity, and It was concluded that treatment with multiple antioxidants can open a significant therapeutic window in the treatment of male infertility. Piomboni et al. [74] observed that a combination of antioxidants including beta-glucan (20 mg), fermented papaya (50 mg), lactoferrin (97 mg), vitamin C (30 mg), and vitamin E (5 mg), administered twice daily for a period of 3 months, yielded significant improvements in the percentage of morphologically normal sperm cells and progressive sperm motility, as well as a decrease in leukocyte concentrations in asthenoteratozoospermic patients.

Gopinath et al. [75] conducted a placebo-controlled trial in men suffering from OAT and initiated treatment with the oral administration of a fixed-dose combination (FDC) of multiple antioxidants (50 mg of CoQ10, 500 mg of LC, 2.5 mg of lycopene, and 12.5 mg of zinc). The participants were allocated into three groups, which received two tablets twice daily of FDC, one tablet of FDC and one tablet of placebo, or two tablets of placebo twice daily for 180 days. It was found that FDC administration was safe and effective at improving sperm count and sperm motility in the first interval (90 days), and further improvement was observed in the second interval, at 180 days. Tremellen et al. [76] performed a prospective, randomized, double-blind, placebo-controlled trial in 60 couples with male infertility. The subjects were randomly assigned to one capsule per day containing 400 IU of vitamin E, 50 mg of vitamin C, 6 mg of lycopene, 25 mg of zinc, 25 µg of selenium, 5 mg of folic acid, and 1,000 mg of garlic or placebo for 3 months prior to their partner's IVF or ICSI cycle. The group that received antioxidant supplementation showed a statistically significant improvement in the viable pregnancy rate (38.5%) in comparison to the control group (16%). There were no significant changes in the oocyte fertilization rate or embryo quality between the control group and the group that received antioxidants.

Another study showed that a combination of LC (145 mg), acetyl-LC (64 mg), fructose (250 mg), citric acid (50 mg), selenium (50 µg), CoQ10 (20 mg), zinc (10 mg), ascorbic acid (90 mg), cyanocobalamin (15 µg), and folic acid (200 µg) administered once daily for a duration of 4 months in 96 men with idiopathic asthenoteratozoospermia resulted in a significant increase in progressive sperm motility, and 16 patients achieved pregnancy during the course of the trial [77]. In a study conducted by Ghanem et al. [78], it was found that in men suffering from idiopathic OAT, supplementation with a combination of antioxidants including vitamin E (400 mg/day) and clomiphene citrate (25 mg/day) resulted in a significant increase in sperm concentration and progressive sperm motility. The rate of spontaneous pregnancy was higher (36.7%) in the treatment group than in the placebo group (13.3%). Comhaire et al. [79] showed that the combination of N-acetyl cysteine or vitamins A or E and essential fatty acid supplementation in infertile men was associated with a significant reduction in ROS activity and an increase in the acrosome reaction, but nonsignificant improvement was shown in sperm motility and morphology, with only a slight increase in the sperm concentration in oligozoospermic men.

Discussion

Accumulating evidence suggests that semen quality has considerably declined over the past 20 years. This change in semen quality has been linked to environmental factors, metal toxicity, chemicals, radiation, and heat. Furthermore, factors such as obesity, inflammation, pollutants, cigarette smoking, and ROS are negatively correlated with spermatogenesis and sperm DNA integrity [80]. Elevated ROS levels can result in damage to the proteins, lipid membranes, or DNA integrity of spermatozoa, meaning that sufficient antioxidant release is essential for maintaining normal sperm quality and quantity, which ensure fertility in men [18]. Oxidative stress has emerged as a key player in the pathogenesis of male infertility, which can be attributed to an increase in free radicals and ROS that results in cellular and tissue damage [21,81]. Antioxidants neutralize oxidative stress, and the seminal plasma contains these substances in order to maintain the redox balance. It has been shown that infertile men have high oxidative stress levels in their seminal plasma, and their antioxidant capacity has been found to be insufficient to combat the corresponding damage [9,82].

The current review provides evidence for the use of oral antioxidants in the treatment of male infertility and their effects on seminal parameters. Several studies have explored the actions of vitamin C, vitamin E, LC, CoQ10, zinc, selenium, and PTX, as monotherapy or polytherapy. Most of these studies were randomized placebo-controlled trials or case-control studies. Various doses were used for each antioxidant, and the duration of treatment ranged between 3 and 6 months. Some studies showed positive effects of these antioxidants on sperm concentration, motility, normal morphology, DNA fragmentation, seminal plasma antioxidant capacity, pregnancy rate, and IVF/ICSI outcomes. The most noteworthy effects, however, were on sperm motility and concentration, especially with the use of multiple oral antioxidants. These positive effects can primarily be attributed to the antioxidant properties of the investigated vitamins and micronutrients and the corresponding reduction of ROS and/or increase in seminal plasma antioxidant capacity [13,20,83]. The enhanced effects associated with combinations of antioxidants could be due to their synergistic antioxidant action. Alternatively, the mechanism by which antioxidants increase sperm concentration could be through the suppression of ROS-induced sperm damage or another unidentified mechanism. A small number of other studies, in contrast, demonstrated a lack of impact of these antioxidants on one or more semen parameter. Comparing the outcomes of various studies is challenging for several reasons. While many studies were randomized placebo-controlled trials, others were either open, uncontrolled, prospective studies or case-control studies. The patients included in the studies were also heterogeneous, as some studies included patients with idiopathic OAT and others included patients with idiopathic oligoasthenozoospermia or asthenoteratozoospermia. Furthermore, variability was present in the inclusion and exclusion criteria used to define idiopathic infertility. Further, some trials included a small number of patients, while others had a large number of patients, so the negative results obtained in some studies may be attributed to a small sample size and insufficient power. Finally, there were significant differences in the doses of antioxidants used and the duration of the treatment period; these factors are likely to have affected the improvements in seminal fluid parameters in men with idiopathic OAT.

In the normal physiological state, the seminal plasma contains endogenous antioxidant enzymes that can quench ROS and protect the spermatozoa from damage. However, exogenous antioxidants such as vitamin C, vitamin E, CoQ10, and glutathione have been shown to protect against oxidative stress in men experiencing infertility [84]. A synergy exists between the exogenous and endogenous antioxidants that neutralize the free radicals and ROS in the seminal plasma [85]. The interaction of endogenous and exogenous antioxidants results in redox homeostasis [85,86]. Both the synthetic and natural forms of antioxidants have recently been the focus of attention in the field of reproduction and fertility management. Three months of administration of vitamins A, E, and C and selenium significantly increased sperm motility [87]. However, in contrast, some studies have reported that treatment with oral antioxidants did not result in a significant improvement in sperm parameters [79,88]. Once the positive effects of antioxidant supplementation are con-

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firmed, it remains necessary to optimize the dose and duration of treatment and to identify which seminal parameters benefit the most from certain antioxidants, either as single agents or in combination. In patients experiencing high levels of oxidative stress, doses should be taken for a minimum of 3 months, as the maturation of sperm takes around 72 days [89].

Conclusion

Most of the studies in this review were randomized controlled studies that explored the effects of oral antioxidants in men with idiopathic OAT and reported improvements in at least one semen parameter (motility, concentration, normal morphology, and antioxidant capacity), but the most noteworthy effect was that the use of multiple antioxidants for 3–6 months increased sperm motility and concentration. There is, however, lack of agreement on the dose and duration of treatment and whether individual or combined oral antioxidants should be used. Future directions include identifying the underlying molecular mechanisms that explain the specific effects of some antioxidants on semen parameters, optimizing the dose and duration of therapy and the choice between individual or combined therapy, and measuring ROS and oxidants/antioxidants in seminal plasma. The current review provides cumulative evidence of the positive role of oral antioxidants in the treatment of infertile men with idiopathic OAT. Further large-scale randomized placebo-controlled trials are required to consolidate the evidence provided in the current review.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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Impact of Coenzyme Q10 and Selenium on Seminal Fluid Parameters and Antioxidant Status in Men with Idiopathic Infertility

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Abstract

Oxidative stress (OS) is a key contributing factor in 30–80% of male infertility cases. To date, several antioxidant treatments have been put forth to manage OS-induced male infertility. This study intended to elucidate the impact of coenzyme Q10 (CoQ10) and selenium on seminal fluid parameters and antioxidant status in infertile men with idiopathic oligoasthenoteratospermia (OAT). In this prospective study, 70 patients with idiopathic OAT were randomly allocated to receive CoQ10 (200 mg/day) or selenium (200 μg/day) for 3 months. Semen quality parameters (following WHO guidelines, 5th edition), total antioxidant capacity (TAC), catalase (CAT), and superoxide dismutase (SOD) activities were compared before and after the treatment. The results of the study showed an increase in sperm concentration with CoQ10 treatment ($p < 0.01$) as well as increased progressive sperm motility ($p < 0.01$ and $p < 0.05$) and total sperm motility ($p < 0.01$ and $p < 0.05$) with CoQ10 and selenium treatment respectively. There was also a significant improvement in TAC ($p < 0.01$ and $p < 0.05$) and SOD ($p < 0.01$ and $p < 0.05$) following treatment with CoQ10 and selenium respectively while CAT improved only with CoQ10 therapy ($p < 0.05$). Sperm concentration, motility, and morphology also correlated significantly with TAC, SOD, and CAT ($r = 0.37$–0.76). In conclusion, treatment with CoQ10 (200 mg) or selenium (200 μg) could improve sperm concentration, motility, and antioxidant status in infertile men with idiopathic OAT with CoQ10 providing the higher improvement.

Keywords CoQ10 · Selenium · Semen · Antioxidants

Introduction

Infertility is the inability to achieve pregnancy after 12 months of regular unprotected sexual intercourse, affecting approximately 10 to 15% of couples globally [1, 2]. Male infertility encompasses a complex pathophysiology with multivariate underlying factors, the most common being varicocele, cryptorchidism, hypogonadism, genital tract infection, endocrine abnormalities including hypothalamic, pituitary, thyroid, diabetes mellitus, adrenal gland, testicular cancer, environmental toxins, systemic disease, exogenous drugs, and genetic factors [3]. In most infertility and/or subfertility cases, the underlying causes remain elusive which may be referred to as idiopathic infertility [4].

Oxidative stress (OS) has been implicated in male infertility [5] and contributes 30 to 80% of male infertility cases worldwide [6, 7]. OS results when there is an imbalance of redox status producing high levels of oxidants and/or low levels of antioxidants resulting in cellular damage [8]. Reactive oxygen species (ROS) are highly reactive molecules produced as a byproduct of cellular metabolism and play important roles in cell signaling and homeostasis [5]. ROS are produced in small amounts in sperm cells to accomplish physiological functions such as regulation of sperm maturation, sperm capacitation and hyperactivation, and sperm-oocyte fusion [8–10]. Nevertheless, an imbalance of oxidative/antioxidant factors leads to OS, increased sperm DNA fragmentation, reduced sperm motility and damage to the sperm cell membrane, and reduced fertility [11, 12]. Spermatozoa are particularly susceptible to ROS-mediated damage due to the presence of structurally unstable polyunsaturated fatty acid in their plasma membrane [8, 13, 14].

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Several studies have shown beneficial effects for antioxidant therapy in idiopathic male infertility [15, 16]. Coenzyme Q10 (CoQ10), non-enzymatic antioxidants [17], has been also reported to improve semen parameters in several studies [1, 18]. It is an isoprenylated benzoquinone that transports electrons from complexes I and II to complex III in the mitochondrial respiratory chain–regulating cytoplasmic redox potential-protecting cell membrane against lipid peroxidation-induced damage and regulating the mitochondrial permeability transition pores [19]. CoQ10 has two forms: a reduced form ubiquinol and an oxidized form ubiquinone [20]. Safarinejad and colleagues have reported an increase in sperm concentration and motility after CoQ10 therapy [21]. Observations by Nadjarzadeh et al. also support the above findings which reported a reduction in the sperm OS markers after CoQ10 therapy [20]. Other studies, however, reported no improvement in one or more of seminal fluid parameters following CoQ10 therapy [22, 23].

Selenium is an integral part of selenoproteins and cofactor of the thioredoxin reductase and the antioxidant enzyme glutathione peroxidase that regulate spermatogenesis, chromatin condensation and protection of sperm outer membrane and DNA against oxidative stress [2, 24]. In addition to antioxidant effect, selenium plays essential roles in inflammation, cell growth, cytotoxicity, and transformation, probably via activator protein 1 (AP1) [24]. Deficiencies of serum and seminal plasma selenium have been shown to positively correlate with abnormal semen parameters, due to depletion of glutathione peroxidase or inactivation of AP1 [24]. Several studies have proved the beneficial effect of selenium in improving sperm parameters and reducing OS [25–27]. In contrast, a clinical trial on 42 men demonstrated that administration of selenium (300 μg/day) for 48 weeks increased plasma and seminal fluid selenium concentrations but without improvement in any of semen parameters [28].

Antioxidant treatment including CoQ10 and selenium may improve seminal fluid parameters, live births, and clinical pregnancy rates [29, 30], but there is lack of agreement on the type of antioxidant, dose, and duration of treatment and whether to use monotherapy or combination of antioxidants [18]. Therefore, the present study aims to evaluate the effects of CoQ10 and selenium treatment on seminal fluid parameters and antioxidant status in men with idiopathic oligoasthenoteratospermia.

Methods

Patients

In this prospective randomized study, seventy patients (mean age 25.4 ± 7.71 years) with idiopathic oligoasthenoteratospermia were recruited at Fertility Clinic, Babyl, Iraq (EC/2018/8866) from June to October 2018 and enrolled in the study (four patients did not complete the study). All patients underwent medical assessment including history, physical examination and laboratory and radiological investigations. The study was conducted as a prospective study with 3 months of follow-up. The selected patients who fulfilled the selection criteria were randomly assigned (using simple randomization) to two treatment groups: Group 1 ($n = 35$) received 200 mg of oral CoQ10 (reduced form ubiquinol) (America Medic and Science AMS, WA, USA) single dose daily for 3 months. Group 2 ($n = 35$) received 200 μg of selenium orally (21st Century, AZ, USA) single dose daily for 3 months. The second group served as an active control. We have used doses used in previous studies [26, 31]. Semen analysis, seminal total antioxidant capacity (TAC), catalase (CAT), and superoxide dismutase (SOD) were measured and compared before and after therapy (follow-up period from September 2018 to January 2019). The sample size was calculated to have 80% power and 5% level of significance with (1:1) enrolment ratio and was estimated to be 30 for each group. The study protocol was approved by local ethical committee at the University of Sumer, Iraq (EC/2018/8866) and all participants consented for participation in the study.

Eligibility Criteria

Inclusion criteria comprised a history of infertility of at least 12 months despite regular unprotected intercourse. Oligoasthenoteratospermia was diagnosed according to the WHO guidelines (5th edition) [32] by semen analysis showing abnormal sperm concentration (< 15 million/ml), progressive motility (< 32%), and total motility (< 40%). Abnormal morphology (< 30% normal morphology) was assessed by the WHO guidelines (4th edition) [33]. Exclusion criteria comprised azoospermia, varicocele, genital tract infection, cryptorchidism, testicular trauma or scrotal surgery, endocrine disorders like hypothalamic, pituitary, thyroid, diabetes mellitus, adrenal gland and exogenous medications, systemic illness, recent antioxidants intake, smoking, alcohol, relevant medications, and the presence of female factors. All participants consented for participation in the study.

Semen Analysis

The semen samples were obtained by masturbation after sexual abstinence for 2 to 3 days, collected into a sterile wide-mouth plastic container, and held at 37 °C until liquefied and then analyzed within 1 h of collection. Semen analysis was performed according to the WHO guidelines (5th edition) [32] and morphology was assessed using the WHO guidelines (4th edition) [33] to determine volume, sperm concentration, motility, and morphology. All semen analysis was performed by the same investigator for the sake of data consistency and all

patients underwent two semen analyses before and after therapy and average scores were used.

Seminal Total Antioxidant Capacity

Semen samples were centrifuged at 3000 rpm for 5 min and seminal plasma was aspirated and stored frozen for further biochemical analysis. TAC was measured in seminal plasma by a colorimetric method using the Total Antioxidant Capacity Assay Kit (#E-BC-K136, Elabscience, TX, USA). The test is based on the principle that antioxidants in the body can reduce Fe^{3+} to Fe^{2+} and Fe^{2+} can form stable complexes with phenanthroline substance. TAC was calculated by measuring the absorbance at 520 nm using a standard formula.

Seminal Superoxide Dismutase Activity

SOD activity was determined in seminal plasma by a colorimetric method as described by Magnani [34]. The principle of this method is based on the competition between the pyrogallol autoxidation by $O_2^{\bullet -}$ and the dismutation of this radical by SOD. The activity of SOD was calculated by measuring the absorbance at 420 nm using the standard formula.

Seminal Catalase Activity

Seminal catalase (CAT) activity was determined in seminal plasma by a colorimetric method using Catalase (CAT) Assay Kit (#E-BC-K031, Elabscience, TX, USA). The test is based on the principle that catalase (CAT) decomposes H_2O_2 and the reaction can be stopped by ammonium molybdate. The residual H_2O_2 reacts with ammonium molybdate to generate a yellowish complex. CAT activity was calculated by measuring the absorbance of the yellowish complex at 405 nm using a standard protocol.

Statistical Analysis

Statistical Package for Social Sciences (SPSS, v.24) was used for the statistical analysis. Results were expressed as mean ± SD. The Kolmogorov-Smirnov test was used to assess data normality. Paired *t* test was used to compare means before and after treatments. Pearson correlation coefficient (r) was used for correlations between seminal parameters and TAC, CAT, and SOD. *p* value of less than 0.05 was considered significant.

Results

Following selenium and CoQ10 therapy, there was a significant improvement in sperm concentration in the CoQ10 group ($p < 0.01$) was noticed but not in the selenium group ($p > 0.05$). An increase in progressive motility was observed in both the groups, but higher in the CoQ10-treated subjects than in the selenium group ($p < 0.01$ and $p < 0.05$, respectively) (Table 1). Total motility was also significantly increased with both the treatments, but higher with CoQ10 than selenium ($p < 0.01$ and $p < 0.05$, respectively). There were no significant changes in morphology observed in either of the groups (Table 1).

There was a significant improvement in the TAC level, which was more evident in the COQ10 treated group ($p < 0.01$ and $p < 0.05$, respectively) (Table 2). Seminal SOD activity was higher in the COQ10 group ($p < 0.01$ and $p < 0.05$, respectively), but CAT activity was found to be increased only in the CoQ10 group ($p < 0.01$ and $p > 0.05$, respectively).

There was a significant positive correlation recorded between TAC and sperm concentration ($r = 0.52$, $p = 0.008$), motility ($r = 0.76$, $p = 0.001$), and morphology ($r = 0.37$, $p = 0.04$) following the treatments (Table 3). A significant positive correlation also was found between SOD and sperm concentration ($r = 0.46$, $p = 0.022$), motility ($r = 0.54$, $p = 0.006$),

Table 1 Patients characteristics and seminal fluid parameters before and after coenzyme Q10 and selenium treatment

	CoQ10 treated (200 mg)			Selenium treated (200 μg)		
	Before treatment $n = 35$	After treatment	P value	Before treatment $n = 35$	After treatment	*p* value
Age (years)	26.23 ± 7.22			24.85 ± 8.46		> 0.05
Duration of infertility (years)	4.34 ± 3.64			5.62 ± 3.25		> 0.05
Volume (ml)	2.38 ± 1.30	2.51 ± 1.42		2.11 ± 1.51	2.35 ± 1.86	
Concentration (million/ml)	8.22 ± 6.88	12.53 ± 8.11	< 0.01	6.42 ± 7.18	6.95 ± 8.33	> 0.05
Progressive motility (%)	16.54 ± 9.26	22.58 ± 10.15	< 0.01	14.75 ± 10.95	20.61 ± 11.53	< 0.05
Total motility (%)	25.68 ± 6.41	29.96 ± 8.09	< 0.01	28.68 ± 5.44	31.59 ± 4.85	< 0.05
Normal morphology (%)	22.17 ± 6.08	23.64 ± 7.45	> 0.05	19.52 ± 7.20	17.62 ± 6.47	> 0.05

Results are expressed as mean ± SD

Table 2 Serum total antioxidant capacity, superoxide dismutase, and catalase activity before and after coenzyme Q10 and selenium treatment

	CoQ10 treated (200 mg)			Selenium treated (200 μg)		
	Before treatment $n = 35$	After treatment	p value	Before treatment $n = 35$	After treatment	p value
TAC (mmol/l)	1.1 ± 0.30	1.28 ± 0.26	< 0.01	1.21 ± 0.22	1.32 ± 0.28	< 0.05
SOD (U/ml)	12.6 ± 3.71	15.4 ± 4.31	< 0.01	10.80 ± 4.53	13.50 ± 4.11	< 0.05
CAT (U/ml)	11.3 ± 2.53	12.5 ± 2.24	< 0.05	11.72 ± 3.10	12.14 ± 3.35	> 0.05

Results are expressed as mean ± SD

and morphology ($r = 0.4$, $p = 0.034$). Regarding CAT, it showed a significant positive correlation with sperm concentration ($r = 0.41$, $p = 0.028$) and motility ($r = 0.48$, $p = 0.014$), and a non-significant positive correlation with morphology ($r = 0.34$, $p = 0.08$). The correlations of antioxidants were more pronounced with sperm concentration and motility than with sperm morphology.

Discussion

The present study has shown an improvement in sperm concentration, motility, and semen antioxidant capacity in infertile men with oligoasthenoteratospermia after 3 months of treatment with CoQ10 and selenium treatment. It is noteworthy that greater improvements in semen parameters have been seen with CoQ10 therapy. Similar to several other studies, this study also supports that seminal fluid parameters positively correlate with antioxidant measures. These results suggest a beneficial effect of CoQ10 and selenium therapy in men with idiopathic OAT.

The results of our study on the effects of CoQ10 on improving semen parameters are consistent with previous studies [35, 36]. In a randomized placebo-controlled double-blind clinical trial by Boscaro et al., an oral administration of CoQ10 in idiopathic asthenospermic patients for 6 months resulted in a statistically significant improvement in all kinetics semen parameters in the treatment group as well as increased levels of CoQ10 in seminal plasma [35]. In a randomized placebo-controlled study by Safarinejad et al., treatment with CoQ10 for 26 weeks in infertile men with idiopathic OAT significantly improved the sperm count and motility but did not improve sperm morphology [36]. Furthermore, another study by Safarinejad in infertile men with idiopathic oligoasthenospermia treated with CoQ10 for 12 months demonstrated a significant improvement in concentration, sperm progressive motility, and morphology [1]. Kobori et al. also reported an improvement of semen concentration and motility, but no change in spermatozoa morphology, after 6 months of vitamin C, vitamin E, and CoQ10 treatment [37]. Two recent systematic reviews on the effect of antioxidants in men with idiopathic oligoasthenospermia reported beneficial effects for CoQ10 and other antioxidants on seminal fluid parameters [8, 38]. In another study, however; treatment with CoQ10 100 mg/day for 6 months did not improve sperm concentration in men with idiopathic infertility [22]. Furthermore, a meta-analysis of three randomized controlled trials of CoQ10 in infertile men demonstrated improvement in semen parameters but a lack of positive effect on live birth or pregnancy rates [39].

Sperm motility (total and progressive) showed greater improvement than morphology, probably due to the antioxidant effect on the mitochondrial respiratory chain. The lipophilic antioxidant effect of CoQ10 has been recognized in plasma lipoproteins [31]. Moreover, endogenous CoQ10 is significantly related to sperm concentration and kinetics due to its bioenergetic role in mitochondrial function [40]. Studies have also confirmed that exogenous administration of CoQ10 is associated with higher levels in seminal plasma [41]. Therefore, the increase in semen parameters in our study could be contributed to the role of CoQ10 in mitochondrial bioenergetics and its well-established antioxidant potential.

Selenium therapy also improved seminal fluid parameters in our study and these results were congruent with previous studies. In an experimental study by Ghafarizadeh et al., semen from asthenoteratospermic men were incubated in vitro with selenium, resulting in an improvement of sperm motility, viability, mitochondrial membrane potential, lower levels of malondialdehyde, and reduced DNA fragmentation when compared with semen incubated without selenium [42]. In

Table 3 Correlations between serum total antioxidant capacity, superoxide dismutase, and catalase activity and seminal fluid parameters in patients post-therapy

	Concentration r (p value)	Motility r (p value)	Morphology r (p value)
TAC	0.52 (0.008)	0.76 (0.001)	0.37 (0.04)
SOD	0.46 (0.022)	0.54 (0.006)	0.4 (0.034)
CAT	0.41 (0.028)	0.48 (0.014)	0.34 (0.08)

r, Pearson's correlation coefficient

addition, carnitine and selenium levels in semen correlated with a better hypo-osmotic swelling test (HOS) as a measure of membrane integrity in low-grade varicocele patients [43]. In a longitudinal study on 125 men with infertility, a positive correlation between seminal plasma selenium levels and sperm count and motility were observed and low levels of semen selenium were associated with male infertility [27]. Similarly, a recent study explored the impact of selenium (50 μg/day) for 3 months showed an increment in sperm concentration, motility, and morphology [44]. Administration of selenium to rats with varicocelectomy also improved the activity of antioxidant enzymes and decreased MAD levels [25]. Another study, however, reported that selenium (300 mg/day) administration for 48 weeks was not associated with increased sperm selenium, concentration, or motility [28].

Selenium plays a crucial role in spermatogenesis via two selenoproteins, namely phospholipid hydroperoxide glutathione peroxidase (PHGPx) and selenoprotein P [26]. Sperm capsular selenoprotein is also an integral part of sperm glutathione peroxidase (GSH-Px) and GSH-Px activity increases with selenium supplementation [45]. Therefore, the observed improvement in seminal fluid parameters in our study could be attributed to increment in seminal plasma antioxidant capacity mediated by selenoproteins and GSH-Px activity. Our findings are also corroborated by studies that have reported lower selenium levels [46] and a correlation between seminal plasma selenium levels and sperm concentration and motility in infertile men [47]. A study on 100 infertile men who received Se (200 μg) in combination with vitamin E (400 units) for at least 100 days showed improvement in sperm motility and morphology but not in sperm concentration [26]. Furthermore, other studies have failed to demonstrate improvement in semen parameters following selenium therapy [28, 40]. Once the positive effects of CoQ10 and selenium are confirmed, it remains necessary to optimize the dose and duration of treatment and to identify which seminal parameters benefit the most from certain antioxidants.

The present study showed an increase in TAC, CAT, and SOD following CoQ10 and selenium therapy. Moreover, a positive correlation between semen parameters and these antioxidants was observed. Previous studies have reported lower TAC [48], CAT [49], and SOD [50] activities in infertile men. Our results are consistent with those of Nadjarzadeh et al. [51] who found a significant positive correlation between TAC and sperm total motility after CoQ10 therapy. Eroglu et al. [47] also found a significant positive correlation between sperm parameters and seminal TAC in men with idiopathic infertility who received CoQ10 and selenium treatment. According to Agarwal et al., ROS in semen correlates negatively with all sperm parameters [13] so an increase in TAC and other antioxidant capacity measures may enhance sperm function and fertility potential. In a randomized placebo-controlled study by Nadjarzadeh et al., there was a significant positive correlation between CoQ10 concentration and normal sperm morphology, catalase activity, and SOD after 3 months in infertile men with OAT following CoQ10 supplementation [20].

Reduction of seminal plasma antioxidant capacity is associated with defective structural and functional integrity of spermatozoa [52], sperm DNA damage, and poor reproductive outcomes [53] due to limited intrinsic antioxidant mechanisms of sperm. Hence, our study findings of increased TAC, CAT, and SOD following CoQ10 and selenium treatment explain the observed improvement in seminal fluid parameters in patients due to enhanced seminal plasma antioxidant defense. Our study has some limitations including pregnancy or live birth rates post-therapy that were not measured but these were not the primary outcome measures of the study. Dietary regulation was also not measured in this study. Another limitation is the small sample size and lack of long follow-up, so further large-scale long-term clinical studies are warranted.

Conclusion

In conclusion, treatment with CoQ10 (200 mg/day) or selenium (200 μg) could improve sperm concentration, motility, and antioxidant status in infertile men with idiopathic oligoasthenoteratospermia, while CoQ10 treatments showed higher improvements.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. The study protocol was approved by the local ethical committee at the University of Sumer, Iraq (EC/2018/8866), and all participants consented for participation in the study. The study was registered in clinicaltrials.gov (NCT03834831).

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The impact of two doses of coenzyme Q10 on semen parameters and antioxidant status in men with idiopathic oligoasthenoteratozoospermia

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Objective: Oxidative stress contributes to male infertility, and antioxidants have been recommended for treating idiopathic oligoasthenoteratozoospermia (OAT). There is, however, a lack of agreement on the type, dosing, and use of individual antioxidants or combinations thereof. The purpose of this study was to compare the effects of two doses of coenzyme Q10 (CoQ10) on semen parameters and antioxidant status in men with idiopathic OAT.

Methods: In this prospective study, patients with idiopathic OAT received 200 mg/day (n=35) or 400 mg/day (n=30) of CoQ10 orally for 3 months. All patients underwent semen analysis according to the fifth editions of the World Health Organization criteria. Total antioxidant capacity (TAC), catalase (CAT) activity, and superoxide dismutase (SOD) activity were measured both before and after treatment.

Results: Treatment with CoQ10 (200 mg/day or 400 mg/day) resulted in a significant increase in sperm concentration from baseline (8.22±6.88 to 12.53±8.11 million/mL, $p=0.019$; 7.58±5.41 to 12.33±6.1 million/mL, $p=0.002$, respectively), progressive motility (16.54%±9.26% to 22.58%±10.15%, $p=0.011$; 14.22%±12.85% to 26.1%±14.52%, $p=0.001$, respectively), and total motility (25.68%±6.41% to 29.96%±8.09%, $p=0.016$; 23.46%±12.59% to 34.82%±14.17%, $p=0.001$, respectively). CoQ10 therapy also increased TAC ($p=0.009$, $p=0.001$, respectively), SOD activity ($p=0.004$, $p=0.001$, respectively), and CAT activity ($p=0.039$, $p=0.024$, respectively). Furthermore, antioxidant measures correlated significantly with seminal fluid parameters ($r=0.36$–0.76).

Conclusion: CoQ10 supplementation improved semen parameters and antioxidant status in men with idiopathic OAT, with a greater improvement shown in men who took 400 mg/day than in those who took 200 mg/day.

Keywords: Antioxidants; Coenzyme Q10; Oxidative stress; Semen

Introduction

The prevalence of infertility has increased significantly in recent decades, and it is currently estimated to affect approximately 15% of the population worldwide [1]. Male infertility has been linked to many factors, including varicocele, infections, undescended testis, and autoimmune, endocrine, genetic, and environmental factors [2]. However, no underlying cause for infertility is identified in 30%–40% of patients; such cases are termed idiopathic male infertility (IMI). Potential mechanisms underlying IMI include genetic, biochemical, hormonal, and environmental factors [3].

Oxidative stress (OS) has been proposed as a mechanism underlying IMI. OS is defined as a disruption of the prooxidant-antioxidant balance that leads to DNA damage, peroxidation of plasma membrane lipids, and protein oxidation [4]. OS occurs when reactive oxygen species (ROS) disrupt the equilibrium of reduction and oxidation. ROS are highly reactive molecules produced during normal cellular metabolism and physiology, formed due to the incomplete reduction of oxygen [5]. In seminal plasma, ROS play a fundamental physi-

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ologic role in several sperm functions, such as the development, maturation, and capacitation of spermatozoa, as well as the acrosome reaction and fertilization [6]. Sperm cells and leukocytes are the main sources of ROS in seminal plasma. Although adequate amounts of ROS are crucial for normal sperm function, excess levels trigger OS and negatively affect reproductive outcomes [4,7]. ROS affect sperm motility by altering axonemal protein phosphorylation and sperm membrane fluidity. Furthermore, ROS may induce sperm DNA damage, including DNA deletion, cross-linking, and chromosome rearrangement, leading to impaired fertilization, abnormal embryonic development, and possibly, congenital foetal defects.

Spermatozoa have a limited intrinsic antioxidant capacity and DNA repair system [8]. However, semen contains several endogenous and exogenous antioxidant molecules that allow maintenance of the balance between reduction and oxidation, including catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase, vitamin C, vitamin E, coenzyme Q10 (CoQ10), carnitine, selenium, zinc, copper, and carotenoids [9,10]. Nevertheless, in men with IMI, poor semen quality is associated with decreased antioxidant capacity [11]. Both increased ROS production and reduced seminal plasma antioxidant capacity have been reported in infertile men [12].

CoQ10 is a lipid-soluble compound synthesized de novo, which may be found in a reduced (ubiquinol) or oxidized (ubiquinone) form [1]. Endogenous CoQ10 levels are closely related to overall oxidative activity, as it plays a role in mitochondrial bioenergetics as part of the mitochondrial respiratory chain [13]. CoQ10 also modulates gene expression, cell signalling, transport, and metabolism. Moreover, the reduced form acts as an electron acceptor in plasma and subcellular fractions [14].

Studies have demonstrated low levels of CoQ10 in infertile men [1,3]. Further, CoQ10 supplementation was associated with improvement of 1 or more seminal fluid parameters [15]. A placebo-controlled study found that in infertile men with oligoasthenoteratozoospermia (OAT), CoQ10 treatment improved semen parameters and antioxidant status [16]. A meta-analysis by Lafuente et al. [3] reported improvements in sperm concentration and motility, as well as an increase in seminal CoQ10 levels. Several studies have shown beneficial effects of antioxidant treatment in infertile men [17-19]. However, there is no consensus on the type, dosing, patient selection, and the use of individual or combination therapy [20]. In this context, the objective of this study was to evaluate the effect of oral CoQ10 at doses of 200 mg/day or 400 mg/day for 3 months on sperm parameters and seminal plasma antioxidant status in infertile men with idiopathic OAT.

Methods

1. Patients

Out of 70 patients initially selected for the study, 65 patients (mean age, 27.24 ± 7.81 years) with idiopathic OAT were recruited at the Fertility Clinic, Babil Governorate, Iraq from June to November 2018 and enrolled in the study (five patients did not complete the study). All patients underwent a medical assessment including a history, physical examination, and laboratory and radiological investigations. The study was conducted as a prospective randomized clinical trial with 3 months of follow-up. The selected patients who fulfilled the selection criteria were randomly assigned to two treatment groups. Group 1 (n = 35) received 200 mg (single dose) of oral CoQ10 and group 2 (n = 30) received 400 mg (single dose) of oral CoQ10 (in the reduced form ubiquinol; America Medic and Science, Woodinville, WA, USA) daily for 3 months. The first group served as active control. We chose CoQ10 doses analysed in previous studies [1,21]. Semen analysis was performed, and total antioxidant capacity (TAC), CAT, and SOD activity were measured in seminal plasma samples and compared before and after therapy.

2. Eligibility criteria

The inclusion criteria comprised a history of infertility lasting for at least 12 months despite regular unprotected intercourse. OAT was diagnosed by semen analysis results showing abnormal sperm concentration (< 15 million/mL), progressive motility (< 32%), and total motility (< 40%) as defined by the fifth edition of the World Health Organization (WHO) criteria for semen analysis [22] and abnormal morphology (< 30% normal morphology) as defined by the fourth edition of the WHO criteria [23]. The exclusion criteria comprised azoospermia, varicocele, genital tract infection, cryptorchidism, testicular trauma or scrotal surgery, endocrine disorders, systemic illness including hepatic and renal diseases, smoking, recent intake of antioxidants, and the presence of female factor infertility.

3. Semen analysis

Semen samples were obtained by masturbation after sexual abstinence for 2–3 days, collected in a sterile wide-mouth plastic container, kept at 37°C until liquefaction, and then analysed within 1 hour of production using the manual method according to fifth edition of the WHO criteria [22] and the fourth edition criteria [23] for sperm morphology. Semen volume, sperm concentration, motility, and morphology were measured. All semen analyses were performed by the same investigator for the sake of data consistency, and all patients underwent two semen analyses before and after therapy; the average values were used.

4. Seminal TAC

Semen samples were centrifuged at 3,000 rpm for 5 minutes, and the seminal plasma was aspirated and stored frozen for further biochemical analysis. TAC was measured by a colorimetric method using the total antioxidant capacity assay kit (#E-BC-K136; Elabscience, Houston, TX, USA). The test is based on the principle that antioxidants in the body can reduce Fe^{3+} to Fe^{2+} and that Fe^{2+} can form stable complexes with phenanthroline. The TAC was calculated by measuring the absorbance at 520 nm using a standard protocol.

5. Seminal SOD activity

SOD activity was determined by a colorimetric method as described by Magnani et al. [24]. The principle of this method is based on competition between pyrogallol autoxidation by superoxide anion and scavenging of this radical by SOD. The activity of SOD was calculated by measuring the absorbance at 420 nm using a standard protocol.

6. Seminal CAT activity

Seminal CAT activity was determined by a colorimetric method using the CAT assay kit (#E-BC-K031, Elabscience). The test is based on the principle that the process through which CAT decomposes H_2O_2 can be quickly stopped by ammonium molybdate. The residual H_2O_2 reacts with ammonium molybdate to generate a yellowish complex. CAT activity was calculated by measuring the absorbance of the yellowish complex at 405 nm using a standard protocol.

7. Statistical analysis

IBM SPSS ver. 24.0 (IBM Corp., Armonk, NY, USA) was used for all statistical analyses. The results were expressed as mean ± standard deviation. The Kolmogorov-Smirnov test was used to assess the normality of the data. The paired *t*-test was used to compare mean values before and after treatment. The Pearson correlation coefficient (*r*) was used to evaluate correlations between seminal parameters and TAC, CAT, and SOD. The *p*-values less than 0.05 were considered to indicate statistical significance.

8. Human and animal rights

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. The study protocol was approved by local Ethical Committee at University of Sumer, Iraq and all participants provided informed consent for participation in the study NCT03850561 (https://clinicaltrials.gov/).

Results

A total of 65 patients were included in the study, with 35 patients in group 1 and 30 patients in group 2. The mean age was 26.23 ± 7.22 and 29.45 ± 8.61 years in groups 1 and 2, respectively ($p = 0.094$), while the mean duration of infertility was 4.34 ± 3.64 and 4.91 ± 4.17 years in groups 1 and 2, respectively ($p = 0.54$) (Table 1). Treatment with CoQ10 (200 mg or 400 mg per day) resulted in a significant increase in sperm concentration from baseline (8.22 ± 6.88 to 12.53 ± 8.11 million/mL, $p = 0.019$; 7.58 ± 5.41 to 12.33 ± 6.1 million/mL, $p = 0.002$, respectively), as well as improvements in progressive motility (16.54% ± 9.26% to 22.58% ± 10.15%, $p = 0.011$; 14.22% ± 12.85% to 26.1% ± 14.52%, $p = 0.001$, respectively), and total motility (25.68% ± 6.41% to 29.96% ± 8.09%, $p = 0.016$; 23.46% ± 12.59% to 34.82% ± 14.17%, $p = 0.001$, respectively). These changes were greater in the group treated with 400 mg of CoQ10 (Table 1).

Likewise, treatment with CoQ10 (200 mg/day or 400 mg/day) resulted in significant increases in TAC ($p = 0.009$, $p = 0.001$, respectively), SOD activity ($p = 0.004$, $p = 0.001$, respectively), and CAT activity ($p = 0.039$, $p = 0.024$, respectively), with greater changes in subjects treated with 400 mg of CoQ10 (Table 2).

Furthermore, in group 1 (200 mg/day), significant positive correla-

Table 1. Patient characteristics and semen parameters before and after CoQ10 treatment (200 and 400 mg/day)

Variable	CoQ10 200 mg/day (n = 35)		*p*-value	CoQ10 400 mg/day (n = 30)		*p*-value
	Before	After		Before	After	
Age (yr)	26.23 ± 7.22			29.45 ± 8.61		0.094
Duration of infertility (yr)	4.34 ± 3.64			4.91 ± 4.17		0.54
Volume (mL)	2.38 ± 1.30	2.51 ± 1.42	0.69	2.15 ± 1.68	2.28 ± 1.35	0.74
Concentration (million/mL)	8.22 ± 6.88	12.53 ± 8.11	0.019	7.58 ± 5.41	12.33 ± 6.1	0.002
Progressive motility (%)	16.54 ± 9.26	22.58 ± 10.15	0.011	14.22 ± 12.85	26.1 ± 14.52	0.001
Total motility (%)	25.68 ± 6.41	29.96 ± 8.09	0.016	23.46 ± 12.59	34.82 ± 14.17	0.001
Normal morphology (%)	22.17 ± 6.08	23.64 ± 7.45	0.36	24.64 ± 4.93	27.41 ± 4.58	0.27

Values are presented as mean ± standard deviation.
CoQ10, coenzyme Q10.

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Table 2. TAC, SOD, and CAT activity in seminal plasma before and after CoQ10 treatment

Variable	CoQ10 200 mg/day (n=35)		*p*-value	CoQ10 400 mg/day (n=30)		*p*-value
	Before	After		Before	After	
TAC (mmol/L)	1.1±0.30	1.28±0.26	0.009	0.92±0.6	1.58±0.83	0.001
SOD (U/mL)	12.6±3.71	15.4±4.31	0.004	10.58±4.29	14.26±4.29	0.001
CAT (U/mL)	11.3±2.53	12.5±2.24	0.039	11.8±3.17	13.6±2.88	0.024

Values are presented as mean±standard deviation.
TAC, total antioxidant capacity; SOD, superoxide dismutase; CAT, catalase; CoQ10, coenzyme Q10.

Table 3. Correlations between semen parameters and antioxidant parameters in patients in group 1

Variable	Concentration		Motility		Morphology	
	r[a]	*p*-value	*r*[a]	*p*-value	*r*[a]	*p*-value
TAC	0.52	0.008	0.76	0.001	0.37	0.04
SOD	0.46	0.022	0.54	0.006	0.4	0.034
CAT	0.41	0.028	0.48	0.014	0.34	0.08

TAC, total antioxidant capacity; SOD, superoxide dismutase; CAT, catalase.
[a]Pearson correlation coefficient.

Table 4. Correlations between semen parameters and antioxidant parameters in patients in group 2

Variable	Concentration		Motility		Morphology	
	r[a]	*p*-value	*r*[a]	*p*-value	*r*[a]	*p*-value
TAC	0.55	0.005	0.68	0.002	0.31	0.07
SOD	0.49	0.009	0.61	0.003	0.29	0.09
CAT	0.38	0.02	0.53	0.005	0.36	0.04

TAC, total antioxidant capacity; SOD, superoxide dismutase; CAT, catalase.
[a]Pearson correlation coefficient.

tions were found between sperm concentration and TAC ($r=0.52$, $p=0.008$), SOD activity ($r=0.46$, $p=0.022$), and CAT activity ($r=0.41$, $p=0.028$). Similar correlations were between sperm motility and TAC ($r=0.76$, $p=0.001$), SOD activity ($r=0.54$, $p=0.006$) and CAT activity ($r=0.48$, $p=0.014$), as well as between normal sperm morphology and TAC ($r=0.37$, $p=0.04$) and SOD activity ($r=0.4$, $p=0.034$) (Table 3). The strongest correlations were found between sperm concentration, motility, and antioxidant measures.

Moreover, in group 2 (400 mg/day), significant positive correlations were found between sperm concentration and TAC ($r=0.55$, $p=0.005$), SOD activity ($r=0.49$, $p=0.009$), and CAT activity ($r=0.38$, $p=0.02$); between sperm motility and TAC ($r=0.68$, $p=0.002$), SOD activity ($r=0.61$, $p=0.003$), and CAT activity ($r=0.53$, $p=0.005$); as well as between normal sperm morphology and CAT activity ($r=0.36$, $p=0.04$) (Table 4). The strongest correlations were likewise found between sperm concentration, motility, and antioxidant measures.

Discussion

The present study demonstrated that the reduced form of CoQ10 exerted beneficial effects on sperm concentration, motility, and antioxidant capacity in men with idiopathic OAT. Greater changes were detected in response to a dose of 400 mg/day. To our knowledge, this is the first study to compare the effect of two doses of CoQ10 on seminal plasma antioxidant capacity.

Our findings are consistent with previous reports. Exogenous intake of CoQ10 may increase its levels in seminal plasma and enhance sperm function [1]. Treatment with ubiquinol (100 mg twice a day) for 6 months improved sperm motility and morphology in patients with idiopathic OAT [25]. Further, in a randomized double-blind placebo-controlled clinical trial in men with men with idiopathic asthenospermia who received CoQ10 (200 mg daily) for 6 months, significant improvements were observed in all seminal parameters, along with increased CoQ10 levels in seminal plasma [1]. Similar findings were reported by Safarinejad and colleagues [16,26] after administration of CoQ10 (200 mg) for 26 weeks in men with OAT and 300 mg daily for 26 weeks in men with OAT. Balercia et al. [1,6] reported an increase in CoQ10 levels in seminal plasma and improvements in sperm parameters following administration of CoQ10 (200 mg/day) for 6 months. In contrast, in an open-label prospective study, men

with OAT who were treated with CoQ10 (600 mg) for 12 months showed significant improvements in sperm progressive motility, concentration, and morphology at the 12-month follow-up [27].

Adequate CoQ10 levels are necessary for proper spermatozoa function given the role of CoQ10 in the mitochondrial respiratory chain and its antioxidant properties. In particular, mitochondrial dysfunction in spermatozoa has been associated with reduced sperm motility; and OS has been shown to be related with mitochondrial DNA deletions [28]. OS is detrimental for sperm parameters, DNA integrity, and fertilization [20]. Therefore, the improvements in sperm parameters detected in the present study could be attributed to the antioxidant properties of CoQ10, leading to reduced OS and enhanced mitochondrial reduction-oxidation function. Previous studies have also linked dietary supplementation with CoQ10 to increased levels of CoQ10 in circulating lipoproteins and enhanced resistance of human low-density lipoprotein to lipid peroxidation [29]. Increased sperm concentration and motility may enhance fertility potential and pregnancy outcomes. A body of literature has demonstrated beneficial effects of CoQ10 and other antioxidants on semen parameters. However, there is a lack of agreement on the type and the use of individual or combination antioxidant therapy [18,30]. A recent meta-analysis also showed that doses of CoQ10 varied (200–300 mg/day) [15]. In addition, a head-head comparison is not always possible due to the heterogeneity of studies in terms of methodology, patient selection, controls, and the dosing and duration of CoQ10 therapy.

The observed improvement in seminal fluid parameters in the current study was higher with a CoQ10 dose of 400 mg per day. Indeed, CoQ10 plays a central role both as an antioxidant and as a facilitator of adenosine triphosphate synthesis [8]. Thus, it is likely that a greater dose and length of exposure yield stronger effects [21]. This is supported by the meta-analysis of Lafuente et al. [3], in which pooled data from three trials revealed that CoQ10 treatment significantly enhanced multiple sperm quality parameters. The studies above also used variable doses of CoQ10 for different durations, highlighting the absence of consensus on the optimal dose for CoQ10 in infertile men. If the optimal effective dose is determined, this treatment is inexpensive, safe, and easy to administer. Furthermore, optimisation of CoQ10 dosing is essential, as recent reports indicated that excessive intake of antioxidants may shift the reduction-oxidation balance to reductive stress, which is as harmful as OS [31]. Once the positive effects of antioxidant supplementation are confirmed, it remains necessary to optimize the dose and duration of treatment and to identify which seminal parameters benefit the most from certain antioxidants. In patients experiencing high levels of OS, doses should be taken for a minimum of 3 months, as the maturation of sperm takes around 72 days. Based on this study, a 400 mg dosage of CoQ10 could be suggested as effective dosage for treatment of patients with idiopathic OAT.

In our study, we also observed enhancements of the oxidative environment, as reflected by the improvement of TAC, SOD and CAT levels, as well as the positive correlations between these variables and semen parameters after 3 months of CoQ10 treatment. Previous studies have demonstrated lower TAC, SOD and CAT levels in infertile men than in fertile men [13,32]. In addition, a meta-analysis by Huang et al. [12] reviewed studies on 3,819 male infertility patients and showed lower levels of antioxidant activity, including TAC, SOD, and CAT, in men with infertility. Previous reports have also detected an increase in antioxidant capacity following antioxidant therapy [17,33]. However, studies of the effects of CoQ10 intake on seminal plasma antioxidant levels are limited. Our findings are congruent with those of Nadjarzadeh et al. [13], who showed that CoQ10 administration (200 mg/day for 3 months) increased SOD and CAT activity in the seminal plasma of infertile men. Furthermore, a positive correlation was found between seminal CoQ10 concentrations and semen parameters [26]. In contrast, in a study by Eroglu et al. [34], basal CoQ10 levels in seminal fluid did not appear to be associated with sperm quality parameters or TAC.

The increased TAC, SOD and CAT activities in our study could partially explain the improvement in sperm parameters after treatment with CoQ10. Although CoQ10 has been recognized as a powerful mediator of cell signalling [35], no specific mechanisms have been identified concerning CoQ10-mediated upregulation of TAC and SOD and CAT activity [36]. Interestingly, CoQ10 has been observed to prevent pro-inflammatory signalling by insulin, interleukin-17, and STAT3 [37], as well as tumour necrosis factor alpha and various chemokines [38]. In turn, this may reflect an enhanced antioxidant status. Future investigations are needed to elucidate the mechanisms of the CoQ10-mediated enhancement of antioxidant capacity. Our study has certain limitations. We did not investigate pregnancy or live birth rates post-therapy, as those were not the primary outcome measures of the study. Dietary regulation was also not measured in this study. Other limitations include the small sample size and lack of long-term follow-up, so further large-scale long-term clinical studies are warranted.

In conclusion, treatment with CoQ10 improved sperm motility, concentration, and semen antioxidant status in infertile men with idiopathic OAT, with a greater improvement observed in response to a dose of 400 mg/day than a dose of 200 mg/day. Supplementation with CoQ10 may enhance the fertility potential and reproductive outcomes of men with idiopathic infertility.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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Coenzyme Q10 effect on semen parameters: Profound or meagre?

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Abstract

Coenzyme Q10 has shown promise in treating male infertility; however, there are inconsistencies across the published data. We undertook a quantitative meta-analysis by pooling data from three placebo-controlled randomised clinical trials (RCTs) in order to evaluate the efficacy of CoQ10 in improving semen parameters. Sperm count, sperm motility, sperm forward motility, sperm morphology and CoQ10 level in the seminal plasma were measured and quantitatively correlated with CoQ10 oral administration. Pooled analysis showed a significant impact of CoQ10 in improving sperm motility and forward motility, without a significant impact on sperm count, sperm morphology, ejaculate volume or seminal plasma level of CoQ10. Efficacy assessment suggested that CoQ10 shows better results at higher doses and when administered for a period of more than 3 months but not longer than 6 months. We conclude that CoQ10 has a profound effect on sperm motility and a meagre effect on all other parameters. Therefore, CoQ10 can be used for treating asthenozoospermic infertility with the dosage and duration depending upon the severity of the disorder and the patient's response to the treatment.

KEYWORDS

asthenozoospermia, Coenzyme Q10, CoQ10, male infertility, sperm motility

1 | INTRODUCTION

Semen quality has declined in the last four decades, and the incidence of infertility has increased significantly (Mishra, Negi, Srivastava, Singh, & Rajender, 2018). Infertility is defined as the failure to initiate a pregnancy after unprotected intercourse for one year or more (Zegers-Hochschild et al., 2009). Infertility is conceptually defined as decreased sperm count and/or motility as per the WHO (World Health Organization) 2010 criteria (Cooper et al., 2010). About 15% of couples are affected by this condition around the world (Agarwal, Mulgund, Hamada, & Chyatte, 2015). Male and female factors contribute almost equally to the aetiology, with some couples presenting with compound issues on both the sides (Kumar & Singh, 2015). Among various factors, oxidative stress has been accounted for infertility since the 1940s (Aitken & Baker, 2006; MacLeod, 1943; Saleh et al., 2003; Sharma & Agarwal, 1996; Tremellen, 2008; Wagner, Cheng, & Ko, 2018). Oxidative stress ensues when reactive oxygen radicals, hydrogen peroxide, hydroxyl species and super oxide anions overpower the antioxidant protection in the cells (Combelles, Gupta, & Agarwal, 2009; Sharma & Agarwal, 1996; Tremellen, 2008). High ROS-level in semen may lead to sub-fertility and even sterility (Adewoyin et al., 2017; Agarwal & Said, 2005; Rato et al., 2012). Latest research in this area shows that oxidative stress might contribute to 30%–80% of infertility in men (Bisht, Faiq, Tolahunase, & Dada, 2017). A few antioxidants have already been found effective in male infertility treatment, for example glutathione, vitamin C, vitamin E, astaxanthin and CoQ10, but there is still no well-standardised male infertility treatment convention (Majzoub & Agarwal, 2018; Omar et al., 2019).

CoQ10 or ubiquinone is a significant mitochondrial respiratory chain antioxidant that has shown promise in treating human male infertility (Balercia et al., 2009; Nadjarzadeh et al., 2011; Safarinejad, 2009). For this purpose, CoQ10 is generally prescribed alone or in combination with other antioxidants. Some studies

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claim profound effect of CoQ10 in treating male infertility, while others deny a significant beneficial effect. Recent studies have warned against indiscriminate use of antioxidants without sufficient evidence of their beneficial effects (Gharagozloo & Aitken, 2011; Palani, 2018). To evaluate the beneficial effects of CoQ10, three randomised placebo-controlled trials have been conducted in infertile men. A meta-analysis has also been conducted, but this meta-analysis was conducted with inappropriate statistical measures to conclude that CoQ10 administration improves sperm motility (Lafuente et al., 2013). They concluded this on the basis of relative risk, which is appropriate for the birth occasion, but not for other semen parameters that are not dichotomous. Sperm motility, forward motility, seminal CoQ10 level and sperm morphology are not the occasions, and hence they ought not to be considered for relative risk. Consequently, there is no meta-analysis on the efficacy of CoQ10 in male infertility.

We undertook the present investigation to evaluate the impact of CoQ10 administration on semen parameters. Out of all in vivo studies, we included only placebo-controlled randomised clinical trials (RCTs) to quantitatively assess the efficacy of CoQ10 in improving semen parameters.

2 | MATERIALS AND METHODS

This exploration was excluded from endorsement by the Institutional Review Board since it was an orderly survey and meta-investigation. We received the ideal reporting flow chart from precise surveys and meta-analysis (PRISMA) to report the results of this systematic assessment (Liberati et al., 2009; Moher, Liberati, Tetzlaff, & Altman, 2009).

2.1 | Search strategy

The meta-analysis began with a descriptive record of double-blind, randomised, placebo-controlled trials that evaluated the impact of CoQ10 supplements on the semen parameters of males who had fertility problems not induced by pathological illnesses. The electronic databases like GoogleScholar, EMBASE, MEDLINE (PubMed), Science Citation Index (SCI) were used for literature review until June 2019. The search strategy included the combination of keywords such as "male infertility," "infertility," "Coenzyme Q10," "CoQ10," "ubiquinol" and "antioxidant." We also searched all databases of preliminary clinical trials, such as www.clinicaltrials.gov, www.controlled-trials.com and the WHO International Clinical Trials Registry Platform (www.who.int/trialsearch). We manually reviewed articles for identification of other studies and acquired the full text of all relevant studies.

2.2 | Inclusion and exclusion criteria

The hits obtained through literature search were subjected to well-defined inclusion/exclusion criteria to select the studies for pooled analysis. Inclusion criteria were as follows: (a) placebo-controlled randomised clinical trials (RCTs) that included placebo and CoQ10 treatment to evaluate the effects of CoQ10 administration on semen parameters, (b) inclusion of the patients was carried out according to the standard diagnostic parameters, (c) the purpose of all the studies was similar, (d) standard methods were used to conduct the trial, and (e) sufficient information and data were provided for inclusion of the study. The exclusion criteria consisted of the following: (a) the studies that failed to provide a detailed description of the subjects, raw data and other information required to specifically understand the study design and the data therein, (b) review articles, meta-analyses, case reports and research on males with disorders such as varicocele, cryptorchidism, and (c) the studies that administered CoQ10 along with other vitamins or antioxidants, which would confound the effect of CoQ10.

2.3 | Data extraction

Data were obtained using a spreadsheet to record study design, the number of subjects, dosage and duration, quantitative effects and the primary outcomes. Two authors (RV and SR) extracted the quantitative data, interventions provided and other information. The discrepancies were resolved by discussion, leading to a consensus.

2.4 | Quantitative data analysis

Comprehensive Meta-Analysis Software version 2 was used to perform all statistical analyses for this study (Comprehensive Meta-Analysis Program, version 2). Standard difference in mean (SDM) for each semen parameter was used as the 'effect size' with their respective 95% confidence intervals. We took 0.5 as a pre-and post-correlation value and the effect direction standardised by post-*SD* value.

The heterogeneity between the studies was quantitatively assessed using Q and I^2 statistics, considering *P* value of <.10 as statistically significant. Heterogeneity index (I^2) value < 25% means low heterogeneity, 50% means moderate heterogeneity and 75% corresponds to high heterogeneity (Higgins, Thompson, Deeks, & Altman, 2003; Huedo-Medina, Sánchez-Meca, Marín-Martínez, & Botella, 2006). Pooled effect size value was calculated using both the fixed effect and random effects models and high-resolution plots (forest plot) were generated. For drawing inference, fixed effect model was used when the heterogeneity was not significant, but random effects model was used in the presence of significant heterogeneity.

Treatment protocols for the dose and length of CoQ10 therapy were comparatively heterogeneous across the studies (Table 1). Balercia et al., 2009, Safarinejad, 2009 and Nadjarzadeh et al., 2011 administered oral CoQ10 for durations of 24, 26 and 12 weeks respectively (Table 1). Methodological information and other details for the included studies are given in Table 2. Since intermittent data were not available for any of these studies, base and end point values were considered for meta-analysis, irrespective of the dose and duration of treatment.

TABLE 1 CoQ10 dose and duration across three randomised controlled trials included in the meta-analysis

Study name	Placebo (number of subjects completing the trial)	CoQ10 (number of subjects completing the trial)	CoQ10 dose
Balercia et al. (2009)	27	28	200 mg daily
Safarinejad (2009)	96	98	300 mg daily
Nadjarzadeh et al. (2011)	24	23	200 mg daily

TABLE 2 Detailed description of each study included in the meta-analysis

Study details	Balercia et al. (2009)	Safarinejad (2009)	Nadjarzadeh et al. (2011)
Study type	Double-blind, randomised, placebo-controlled trial	Double-blind, randomised, placebo-controlled trial	Double-blind, randomised, placebo-controlled trial
Patients recruited	*n* = 60	*n* = 212	*n* = 60
Age group	27–39 years, mean = 32 years	21–42 years, mean = 28 years	25–46 years, mean = 34 years
Idiopathic infertility diagnosis	Asthenozoospermia	Oligoasthenoteratozoospermia	Oligoasthenoteratozoospermia
Inclusion criteria	More than two years, regular unprotected sex with a potentially fertile female	No child besides two years, regular sexual intercourse	No pregnancy during unprotected sex whole year
Genital diseases	Absent	Absent	Absent
Medical therapy	No medication for at least for 3 months before the study began	No medical therapy for at least 12 weeks before the study began	No medical therapy for at least 3 months before the study began
Intervention	CoQ10 200 mg, 2 times a day, placebo: 2 times a day, duration of treatment: 6 months, total 9 months study	CoQ10 300 mg, single daily dose, placebo single dose daily, duration of treatment: 26 weeks, total 20 months study	CoQ10 200 mg single daily dose, placebo: single daily dose, duration of treatment: 12 weeks, total 19 months study
Outcomes	Seminal parameters improved	Seminal parameters improved	Seminal parameters improved

The publication bias was investigated using funnel plot, followed by asymmetry assessment of the funnel plot by Egger's regression intercept test. The sensitivity analysis was done by removing one study at a time and calculating the overall effect on the rest of the studies.

3 | RESULTS

After literature screening, we identified 9 studies that evaluated the effect of CoQ10 with or without other supplements on semen parameters. Since the aim of this study was to evaluate the effect of CoQ10 only, we selected randomised placebo-controlled trials on CoQ10 for quantitative data analysis. Three randomised placebo-controlled trials on CoQ10 (Balercia et al., 2009; Nadjarzadeh et al., 2011; Safarinejad, 2009) were subjected to quantitative data analysis, and the remaining studies were used for qualitative analysis (Figure 1).

3.1 | Sperm concentration (million/ml)

All three studies analysed sperm concentration (million/ml). In the original investigations, only one study showed a significant improvement in sperm concentration (Safarinejad, 2009), while the two other studies showed no significant improvement. Heterogeneity test for sperm count showed a significant heterogeneity ($Q = 17.48$, $I^2 = 88.56$, $p = .00001$), suggesting the use of random effects model for inference. Pooled analysis showed no significant improvement in sperm concentration upon CoQ10 administration (SDM = 0.67, 95% CI = −0.10 to 1.45, $p = .089$; Figure 2 and Table 3). Funnel plot showed the lack of publication bias (Figure S1).

3.2 | Total sperm motility (%)

All three studies included in the meta-analysis evaluated sperm motility. In the original investigations, two studies showed a significant improvement in sperm motility (Balercia et al., 2009; Safarinejad, 2009), while the third study showed no significant improvement. Heterogeneity test for sperm motility showed no significant heterogeneity between studies ($Q = 4.48$, $I^2 = 55.43$, $p = .106$), suggesting the use of fixed effect model for inference. Pooled analysis showed a significant improvement in sperm motility upon CoQ10 administration (SDM = 1.47, 95% CI = 1.22–1.73, $p = .000$; Figure 2 and Table 3). Funnel plot showed the lack of publication bias (Figure S1).

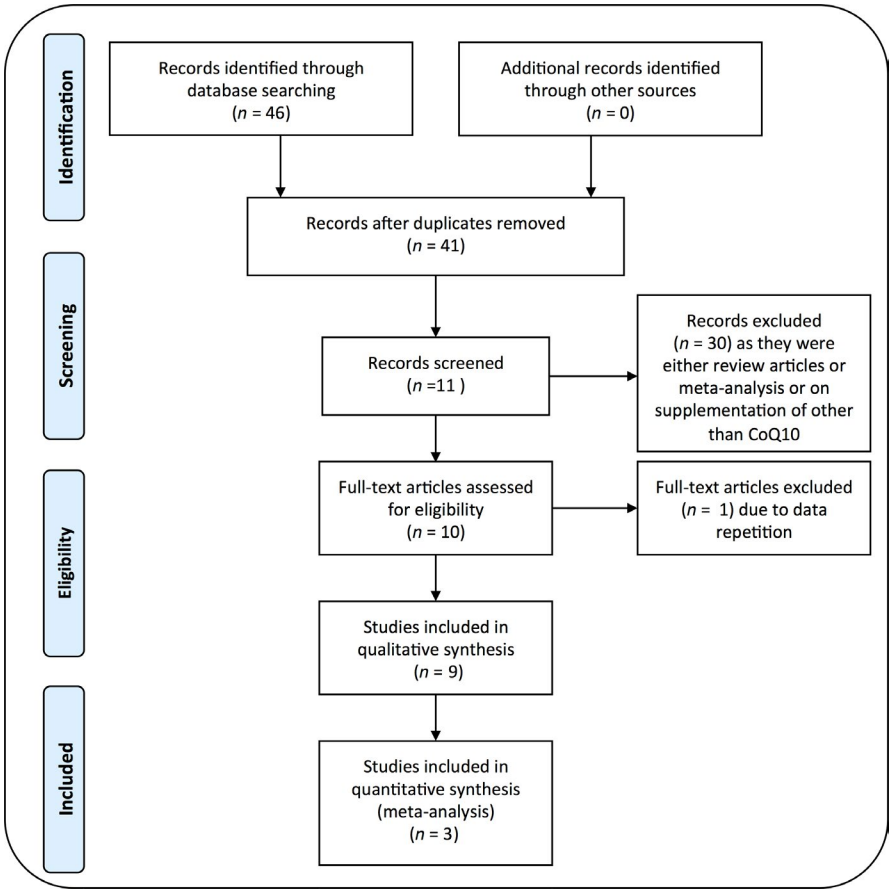

FIGURE 1 PRISMA flow chart for the screening of studies for meta-analysis

3.3 | Forward motility (%)

Only two studies (Balercia et al., 2009; Nadjarzadeh et al., 2011) had analysed forward motility, of which one claimed a significant improvement in forward motility (Balercia et al., 2009), while the other reported no improvement (Nadjarzadeh et al., 2011). Heterogeneity test for sperm forward motility showed no significant heterogeneity between studies (Q = 2.11, I^2 = 52.80, p = .145), suggesting the use of fixed effect model for inference. Pooled analysis showed a significant improvement in sperm forward motility upon CoQ10 administration (SDM = 0.66, 95% CI = 0.27–1.06, p = .001; Figure 2 and Table 3).

3.4 | Sperm morphology (%)

Two studies (Nadjarzadeh et al., 2011; Safarinejad, 2009) evaluated sperm morphology, of which one reported a significant improvement in sperm morphology (Safarinejad, 2009), while the other found no significant improvement (Nadjarzadeh et al., 2011). Heterogeneity test for sperm morphology showed a significant heterogeneity between studies (Q = 3.44, I^2 = 70.93, p = .06), suggesting the use of random effects model for inference. Pooled analysis showed no significant improvement in sperm morphology upon CoQ10 administration (SDM = 1.24, 95% CI = −0.56 to 3.03, p = .176; Figure 3 and Table 3).

3.5 | CoQ10 level in seminal plasma (ng/ml)

Two studies (Balercia et al., 2009; Safarinejad, 2009) had analysed seminal level of CoQ10, but Nadjarzadeh et al., (2011) did not present this data; however, the authors presented these data in their later study on the same patients (Nadjarzadeh et al., 2014). Out of three, two studies reported a significant improvement in seminal plasma CoQ10 level (Balercia et al., 2009; Safarinejad, 2009), while the third reported no significant improvement (Nadjarzadeh et al., 2011). Heterogeneity test for CoQ10 level showed a significant heterogeneity between studies (Q = 107.04, I^2 = 98.13, p = .000), suggesting the use of random effects model for inference. Pooled analysis showed no significant improvement in seminal CoQ10 level upon CoQ10 administration (SDM = 2.11, 95% CI = −0.21 to 4.43, p = .074; Figure 3 and Table 3). Funnel plot showed the lack of publication bias (Figure S1).

3.6 | Ejaculate volume (ml)

Two studies (Nadjarzadeh et al., 2011; Safarinejad, 2009) presented semen volume data, both of which reported no significant improvement in ejaculate volume. Heterogeneity test for ejaculate volume showed no significant heterogeneity between studies (Q = 0.01, I^2 = 0, p = .90), suggesting the use of fixed effect model for inference. Pooled analysis showed no significant improvement in

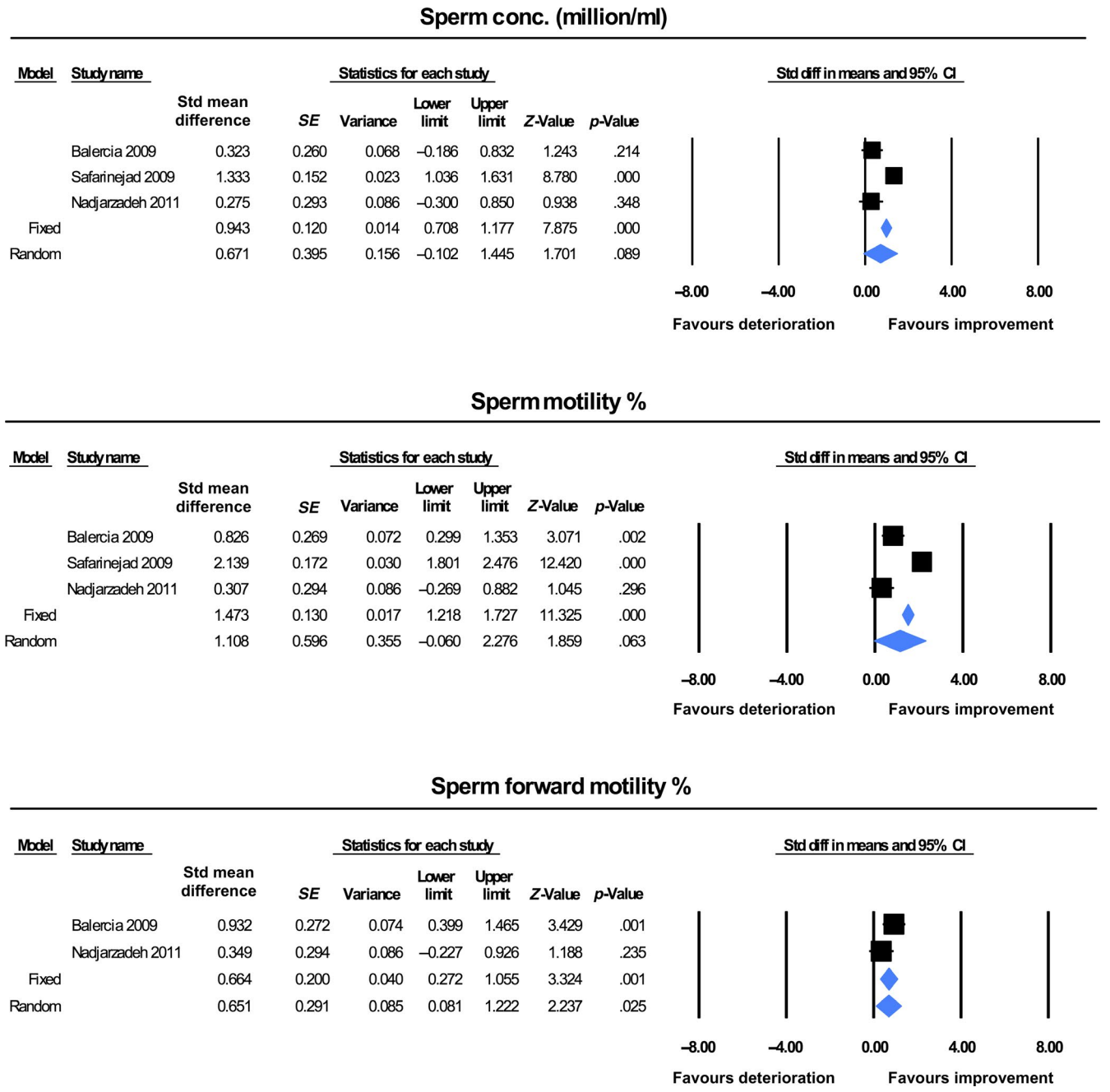

FIGURE 2 Forest plot for meta-analysis on sperm concentration (million/ml), sperm motility (%) and sperm forward motility (%). The *Z* value shows the degree and direction of relationship, whereas the *p* value shows the significance of the relationship. The horizontal bar shows the range of mean with a square in the centre. The direction of the projection of the horizontal bar shows the direction of the association. The diamond-shaped box shows the pooled OR and its width indicates the 95% CI

sperm morphology upon CoQ10 administration (SDM = 0.057, 95% CI = −0.19 to 0.30, p = .647; Figure 3 and Table 3).

4 | DISCUSSION

Various studies on male infertility support the role of CoQ10 in improving sperm motility (Ahmadi, Bashiri, Ghadiri-Anari, & Nadjarzadeh, 2016; Balercia et al., 2004). The purpose of this quantitative assessment was to provide a critical evaluation of the effect of CoQ10 administration on semen parameters in infertile men. To strictly evaluate the impact of CoQ10 on semen parameters, we included only double-blind, placebo-controlled, randomised clinical trials. We found that CoQ10 administration results in significant improvements in sperm motility and forward sperm motility without a significant improvement in sperm count, sperm morphology and ejaculate volume. These results suggest that CoQ10 can be the therapy of choice for asthenozoospermia, but not for oligozoospermia. The improvement in sperm motility upon CoQ10 administration may be explained on the basis of improvement in mitochondrial function,

TABLE 3 Summary of the results of meta-analyses for different semen parameters

Study parameters	Meta-analysis model chosen	SDM (95% CI)	*p* Value	Number of patients
Sperm conc.	Random	0.67 (−0.10 to 1.44)	.089	296
Sperm motility	Fixed	1.47 (1.22–1.73)	.000	296
Forward motility	Fixed	0.66 (0.27–1.06)	.001	102
Sperm morphology	Random	1.24 (−0.56 to 3.03)	.176	241
Seminal CoQ10 level	Random	2.11 (−0.21 to 4.43)	.074	296
Ejaculate volume	Random	0.06 (−0.19 to 0.30)	.647	241

which is supported by in vivo studies in various biological systems (Gvozdjáková et al., 2014), including in ageing (Hernández-Camacho, Bernier, López-Lluch, & Navas, 2018).

Various studies used different doses and durations of CoQ10 administration. There were significant variations in the outcomes of these studies, which in addition to unknown factors can be attributed to the dose and duration. The three RCT studies administered 200–300 mg for a duration of 12–26 weeks. Among the studies included in this meta-analysis, Safarinejad (2009) used the highest dose (300 mg) for the longest duration (26 weeks), which incidentally also showed the most significant improvement in most of the parameters. Recent investigations suggested that a higher daily dose of CoQ10 (400–600 mg) gives better results in comparison with 200 mg dose (Alahmar, 2019). Furthermore, it is seen that the noticeable improvement is seen after a minimum of 3 months of treatment (Alahmar, 2019). Nevertheless, treatment beyond 6 months does not show further improvement (Alahmar, 2019; Safarinejad, 2009). The latter is important as some recent studies have shown that extreme consumption of antioxidants can change the oxidation–reduction equilibrium to reductive stress, which is as damaging as oxidative stress (Henkel, Sandhu, & Agarwal, 2019).

Evidence suggest that semen quality has decreased significantly over the last 40 years (Mishra et al., 2018; Sengupta, Dutta, & Krajewska-Kulak, 2017). Oxidative stress is established as a critical factor in male infertility pathophysiology with approximately 30–80 per cent of male infertility is considered to have contribution from high ROS (Showell et al., 2014). Higher ROS concentrations may damage plasma membrane, proteins and sperm DNA, imposing that suitable antioxidant capacity is critical to ensure male fertility (Darbandi et al., 2018). Previous studies on CoQ10 have established its antioxidant capacity, which improves total antioxidant capacity (TAC), superoxide dismutase (SOD) and catalase (CAT) activity (Alahmar, 2019; Balercia et al., 2009; Safarinejad, 2009). The effect of CoQ10 in improving mitochondrial function and sperm motility may be explained by improvement in the antioxidant capacity, which prevents the loss of cell integrity. Improvement in testosterone level or at least amelioration of the reduction in testosterone could be another mechanism underlying the action of CoQ10 (Banihani, 2018).

This meta-analysis had some limitations. Among the foremost is the less number of studies. Till date, only three placebo-controlled RCTs have been undertaken, most of which were small trials. Despite pooling from the available studies, the maximum sample size for any parameter was 296, which does not provide very high power to statistical analysis. It may be noted that the fixed effect model was used to draw the inferences for sperm motility and forward motility in the absence of heterogeneity. Since fixed effect model is less stringent in comparison with the random effects model, these results need support from more trials. For all other parameters, random effects model was used for drawing inference. Since heterogeneity is a significant player in drawing inference in meta-analysis, further large-scale trials would provide strength to these conclusions. Only one study had scored pregnancy achievement upon CoQ10 administration, limiting the possibility of undertaking a pooled analysis on this parameter.

5 | CONCLUSION

Data analysis across the studies, evidence from all studies and the quantitative synthesis from meta-analysis suggested that CoQ10 appears to have a profound effect on sperm motility, but this is subject to further studies on this. Nevertheless, the effect on other parameters was meagre. Based on this analysis, we conclude that CoQ10 appears to have a significant beneficial effect on sperm motility and forward motility without affecting sperm count. Therefore, CoQ10 can be the therapy of choice for asthenozoospermic infertility with a dose of 200–400 mg for a period of 3–6 months depending upon the severity and patient's response, which should be monitored on a monthly basis. There is evidence that CoQ10 in combination with other vitamins and antioxidants may improve overall semen quality. Certainly, large-scale randomised trials with or without combination with other vitamins and antioxidants are required for unambiguously establishing the importance of CoQ10 in treating male infertility.

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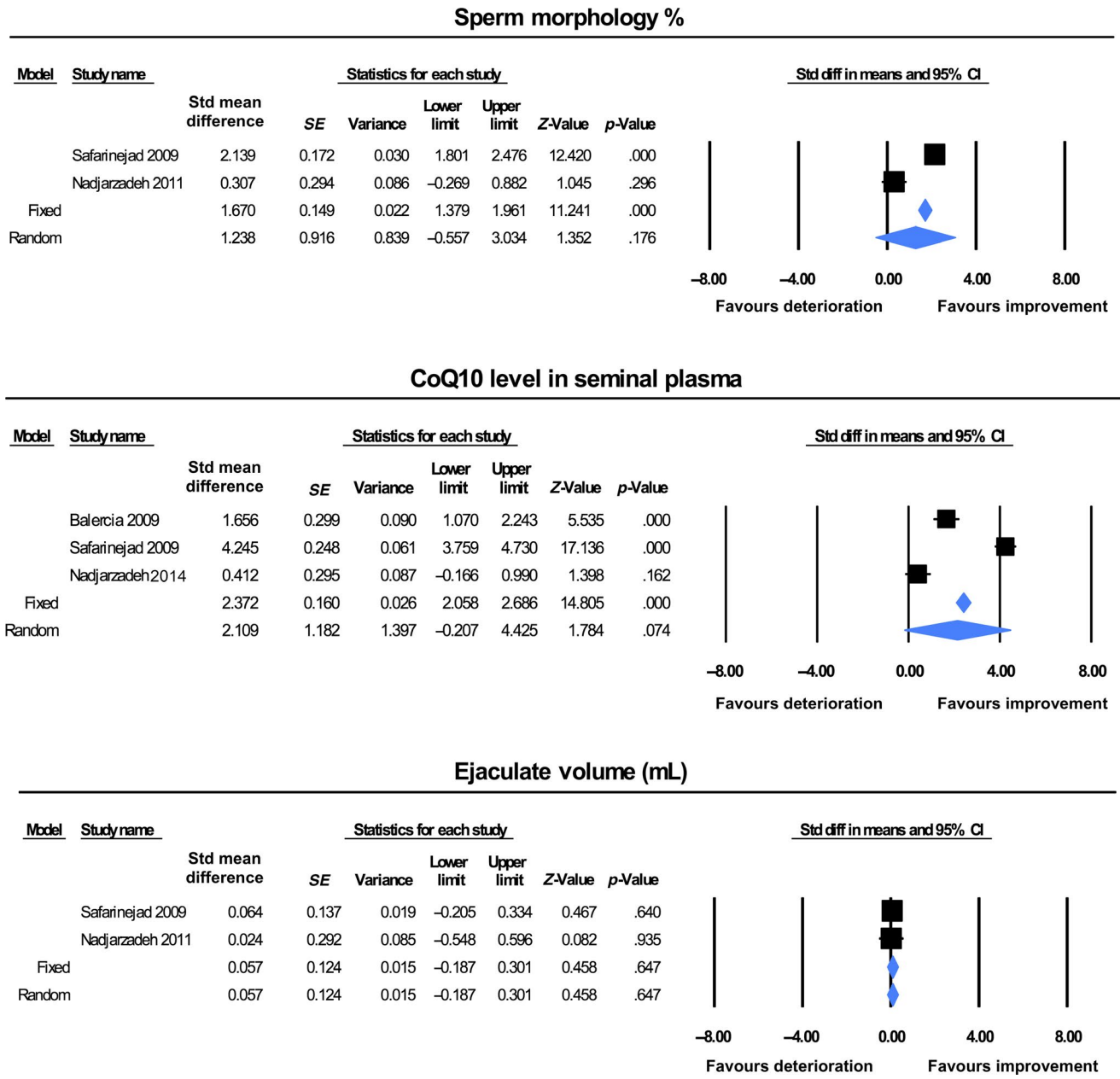

FIGURE 3 Forest plot for meta-analysis on sperm morphology (%), CoQ10 level in seminal plasma (ng/ml) and ejaculate volume (ml). The *Z* value shows the degree and direction of relationship, whereas the *p* value shows the significance of the relationship. The horizontal bar shows the range of mean with a square in the centre. The direction of projection of the horizontal bar shows the direction of association. The diamond-shaped box shows the pooled mean, and its width indicates the 95% CI

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Coenzyme Q10, oxidative stress, and male infertility: A review

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Male infertility has a complex etiopathology, which mostly remains elusive. Although research has claimed that oxidative stress (OS) is the most likely underlying mechanism of idiopathic male infertility, the specific treatment of OS-mediated male infertility requires further investigation. Coenzyme Q10 (CoQ10), a vitamin-like substance, has been found in measurable levels in human semen. It exhibits essential metabolic and antioxidant functions, as well as playing a vital role in mitochondrial bioenergetics. Thus, CoQ10 may be a key player in the maintenance of biological redox balance. CoQ10 concentrations in seminal plasma directly correlate with semen parameters, especially sperm count and sperm motility. Seminal CoQ10 concentrations have been shown to be altered in various male infertility states, such as varicocele, asthenozoospermia, and medical or surgical regimens used to treat male infertility. These observations imply that CoQ10 plays an important physiological role in the maintenance and amelioration of semen quality. The present article thereby aimed to review the possible mechanisms through which CoQ10 plays a role in the regulation of male reproductive function, and to concisely discuss its efficacy as an ameliorative agent in restoring semen parameters in male infertility, as well as its impact on OS markers, sperm DNA fragmentation, pregnancy, and assisted reproductive technology outcomes.

Keywords: Antioxidant; Coenzyme Q10; Male infertility; Oxidative stress

Introduction

Infertility is defined as the failure to successfully achieve pregnancy after 12 months of regular unprotected sexual intercourse [1]. Worldwide, 15% of the world's population is affected by infertility [2]. The factors responsible for infertility have been grouped as male and female factors. Approximate 50% of cases are attributed to combined male and female factors, while 25% are attributed to male factors alone [3]. Infertility in males unambiguously reflects a complex of underlying causes [4,5], and more than 25% of cases of male infertility are idiopathic with no identifiable cause [6]. Oxidative stress (OS) and reactive oxygen species (ROS) are considered damaging to sperm and are responsible for 30%– 80% of cases of subfertility [7]. OS, caused by the disruption of the prooxidant-antioxidant balance [8], affects male fertility and sperm function [9-12].

Although low levels of ROS possess some physiological functions in sperm maturation and capacitation, an imbalance between ROS and seminal antioxidants may disrupt male reproductive function [13]. Similarly, the acrosome reaction and capacitation are boosted by superoxide anion radicals [14]. However, excessive ROS generation leads to OS and diminishes spermatozoa's antioxidant capacity [15-17]. The generation of seminal ROS could be attributed to genital tract infection, genital tract inflammation, varicocele, testicular torsion, and cryptorchidism [18,19]. Other factors include aging and various lifestyle factors, such as exposure to toxic chemicals, cigarette smoking, exposure to radiation, and alcohol abuse [18,20]. Excessive

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ROS generation has been associated with degradation of DNA via the induction of breakage of DNA strands, chromatin cross-linking, and base modifications [21], and lower potential of the mitochondrial membrane [22,23]. The plasma membrane of spermatozoa is composed of lipids, and a high level of polyunsaturated fatty acids and an excessive level of ROS makes the membrane susceptible to damage due to lipid peroxidation [10,24]. In sperm, mobility and decreased membrane fluidity caused by lipid peroxidation have been associated with a lower fertilization capacity [25].

Spermatozoa have a scavenger activity exerted by enzymatic and non-enzymatic antioxidants. The enzymatic antioxidants present in semen include catalase, superoxide dismutase, and glutathione peroxidase, whereas non-enzymatic antioxidants constitute coenzyme Q10 (CoQ10), glutathione peroxidase; vitamins A, B complex, C, and E; carnitines; and minerals (chromium, selenium, zinc, and copper) [21,26]. An imbalance between ROS production and antioxidant capacity results in increased sperm exposure to OS, which plays a critical role in the pathogenesis of male infertility and alters sperm function [9]. Despite the recognition of ROS and OS as a factor contributing to male infertility, antioxidant use for treatment is still debatable. To explore the *in vitro* effective role of different OS in various models, specific differences have been found in the effectiveness exerted by enzymatic and non-enzymatic molecules [27]. Antioxidant therapy has been considered for supplementation and has been introduced into routine clinical practice for the treatment of male infertility [28]. The antioxidants used for male infertility include CoQ10, vitamin A, carnitines, N-acetyl cysteine, vitamin C, vitamin E, pentoxifylline, and micronutrients such as selenium and zinc [28,29]. Antioxidants have been associated with beneficial effects on sperm concentration, motility, morphology, DNA fragmentation, assisted reproductive technology (ART) outcomes (both *in vitro* fertilization [IVF] and intracytoplasmic sperm injection [ICSI]), and seminal plasma antioxidant capacity [30]. However, there is insufficient agreement on the type, dosing, and use of single or combined antioxidants [30,31]. The potential role of CoQ10 in the management of male infertility has been widely investigated. Our attention in this narrative review will be focused on comprehensive and updated evidence on the impact of CoQ10 on the male reproductive system and its efficacy on sperm parameters, sperm DNA fragmentation (SDF), seminal markers of OS, pregnancy, and ART outcomes.

CoQ10: biochemical properties and physiological functions

In humans, CoQ10 is synthesized from tyrosine. CoQ10 is a vital constituent of the inner mitochondrial membrane. It is involved in the inhibition of lipid peroxidation and DNA oxidation [7]. CoQ10 also plays an essential role in electron transport in the mitochondrial respiratory chain and oxidative phosphorylation, and functions as a lipid-soluble antioxidant in cell membranes and lipoproteins [32,33]. CoQ10 also participates in adenosine triphosphate production in aerobic respiration [21]. Moreover, CoQ10 therapy has been applied as a prospective intervention in the management of various pathological dysfunctions such as diabetes, cancer, Parkinson disease, Huntington disease, heart disorders, and infertility [34].

The introduction of CoQ10 therapy started in patients with heart failure; subsequently, it has been more widely recognized as a way to slow down age-related pathologies, improve bioenergetics in the cell, and counteract OS. Various studies have proven the effectiveness of CoQ10 supplementation in enhancing male fertility and cardiovascular function [9,34,35]. CoQ10 functions as an antioxidant by inhibiting lipid peroxidation of the sperm membrane [35]. There are three redox states of CoQ10 in the Q-cycle in organisms. These are ubiquinol (CoQ10-H2-reduced form), ubiquinone (oxidized form), and semiquinone (a radical) [36]. CoQ10 is concentrated in the mitochondria-containing midpiece of the sperm, where it takes part in all energy-regulated processes [35].

Effects of CoQ10 on sperm parameters

Several clinical studies have reported beneficial effects of CoQ10 supplementation on sperm parameters of infertile patients [21,37,38]. In 287 patients with idiopathic oligoasthenoteratozoospermia (OAT), CoQ10 supplementation (600 mg/day) for 12 months significantly increased sperm concentration (+113.7%), sperm progressive motility (+104.8%) and normal sperm morphology (+78.9%) [39]. A systematic review and meta-analysis evaluating the effects of CoQ10 oral administration on CoQ10 seminal concentration, sperm concentration, and sperm motility was conducted on three trials with a total of 149 patients receiving CoQ10 and 147 control men. The results showed that CoQ10 supplementation led to a significant increase in all three endpoints taken into consideration (namely seminal concentration of CoQ10, sperm concentration, and sperm motility) [40]. Furthermore, our recent meta-analysis of three placebo-controlled randomized clinical trials (RCTs) involving 296 participants demonstrated a significant impact of CoQ10 on improving sperm total and progressive motility [32].

Gvozdjakova et al. [36] showed that the administration of CoQ10 (30 mg/day), L-carnitine fumarate (440 mg/day), vitamin E (75 IU/day), and vitamin C (12 mg/day) to infertile male patients improved sperm concentration and pregnancy rates. In one of our RCTs, 35 men with idiopathic OAT were treated for 3 months with CoQ10 at the dose of 200 mg/day and 30 patients with 400 mg/day. The results showed greater improvement in semen parameters in men

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who took 400 mg/day [38]. In another study of 70 men with idiopathic OAT, we also demonstrated that CoQ10 therapy (200 mg/day) was associated with improved sperm concentration and motility, as well as a reduction in OS markers [38].

In another study, patients with idiopathic infertility were supplemented with CoQ10 (200 mg/day) and D-Asp (2,660 mg/day) for 12 weeks. The concentrations of CoQ10 and D-Asp increased significantly in the spermatozoa and seminal plasma. In addition, sperm motility improved, whereas no effect was found on sperm concentration and morphology [36]. Similarly, the efficacy of CoQ10 supplementation has also been evaluated in infertile patients with varicocele. A significant improvement of sperm parameters and total antioxidant capacity (TAC) was reported in men treated with CoQ10 at a dose of 100 mg/day for 3 months [41]. Furthermore, in a study comparing the effects of two doses of CoQ10 on sperm parameters and TAC in patients with idiopathic OAT for 3 months, it was found that CoQ10 significantly increased sperm concentration, total motility, and progressive motility [3]. CoQ10 also increased TAC, superoxide dismutase, and catalase activities, with a stronger improvement found in patients taking the highest dose [21].

Overall, these studies show that supplementation with CoQ10 enhances sperm parameters such as sperm concentration, motility, and morphology, and improves OS markers in men with idiopathic infertility. However, there is no consensus on the dosage to prescribe. In an attempt to answer this question, evidence from clinical trials and meta-analyses on the impact of CoQ10 treatment in male infertility revealed that oral supplementation with CoQ10 raised seminal CoQ10 levels, sperm motility, and spermatozoa concentration [32,42].

CoQ10 effects on OS markers

As previously discussed, ROS affect sperm quality, leading to DNA, protein, and lipid oxidation, and are involved in the pathogenesis of male infertility [43]. However, there is no general agreement on the validation, reproducibility, and standardization for the measurement of ROS-induced changes in DNA, lipids, and proteins; TAC in human body fluids; or enzymatic players involved in redox status [44,45].

There is evidence supporting the protective role of CoQ10 against ROS-induced sperm damage [37,38]. CoQ10 is known to inhibit superoxide production [46], and a strong negative association has been observed between CoQ10 levels and hydrogen peroxide [46]. In patients with idiopathic OAT, a significant increase in superoxide dismutase, TAC, and catalase activity after CoQ10 treatment has been reported [30]. We demonstrated that CoQ10 treatment (200 mg/day) could reduce or ameliorate OS markers such as ROS, TAC, catalase, superoxide dismutase, and glutathione peroxidase in infertile men with idiopathic OAT [21,38] and idiopathic oligoasthenozoospermia (OA) [30,37]. Overall, these studies have demonstrated beneficial effects of CoQ10 on improving both enzymatic and non-enzymatic antioxidant capacity among men with idiopathic infertility (Table 1).

CoQ10 and SDF

SDF is one of the main underlying molecular-level disruptions that may explain idiopathic male infertility. OS is considered to be the key mechanism causing SDF [47]. An excess of ROS causes nicks and breaks in DNA, which need to be repaired [48]. Most DNA in human spermatozoa is transported in a condensed form of chromatin in the sperm head, making sperm DNA more resistant to injury during transit in both the male and female reproductive tracts; however, SDF may result from exposure to seminal OS in the epididymis or abnormal chromatin packaging [49]. Spermatozoa are susceptible to ROS due to their composition of high levels of polyunsaturated fatty acids in their cytoplasm and plasma membrane. DNA damage can be the result of decreased protamination, replication errors, environmental toxins, ultraviolet rays, endogenous endonuclease activation, and ionizing rays [47]. DNA fragmentation can lead to infertility by altering sperm function [50]. Males with a high SDF rate have a substantially lower likelihood of conceiving naturally or via ART procedures [51]. Accordingly, patients with a high percentage of spermatozoa affected by DNA fragmentation have high levels of seminal ROS and decreased antioxidant capacity [26].

Both *in vivo* and *in vitro* studies have shown that increased SDF could impair male reproductive functions via its impacts on fertilization, implantation, early embryo development, and pregnancy [49]. Studies have reported that deficiency of CoQ10 is associated with high sperm DNA damage and low sperm count and motility [32,40,52,53]. This may be explained by the fact that seminal CoQ10, with antioxidant and metabolic properties, plays a major role in mitochondrial bioenergetics and maintenance of seminal redox status [33].

Evidence has shown that antioxidant treatment reduces the prevalence of SDF in semen samples and seems to improve the outcomes of ICSI in patients with elevated SDF levels [49]. Gual-Frau et al. [54] administered antioxidants containing CoQ10 to 20 infertile patients with low-grade varicocele and high SDF levels. A significant decrease in SDF levels and a substantial rise in sperm concentration were observed following treatment . These findings are consistent with our recent randomized controlled study on 65 infertile men with idiopathic OA and 40 fertile men, which illustrated an improvement in semen parameters and a reduction in OS markers and SDF in infertile patients following CoQ10 therapy (200 mg/day for 3 months) (Table 1) [37].

Table 1. Effects of CoQ10 on male infertility, pregnancy outcomes, and assisted reproductive techniques

Study	Participan	RCT	Intervention	Intervention period	Outcome
Alahmar et al. (2021) [37]	Infertile patients with idiopathic oligoasthenozoospermia; 65 patients	Yes	CoQ10 200 mg/day orally	3 mo	Improved sperm concentration, progressive motility, total motility, seminal fluid CoQ10 concentration, TAC, ROS levels and SDF percentage, and glutathione peroxidase levels.
Alahmar and Sengupta (2021) [38]	Men with OAT; 70 patients	Yes	CoQ10 200 mg/day	3 mo	Improved sperm concentration, motility, and antioxidant status.
Alahmar (2019) [21]	Men with idiopathic OAT 35 subjects treated with CoQ10 at the dose of 200 mg/day and 30 patients with 400 mg/day	Yes	CoQ10 200 mg/day, 400 mg/day	3 mo	Idiopathic OAT with a greater improvement shown in men who took 400 mg/day than in those who took 200 mg/day
Cheng et al. (2018) [55]	Idiopathic oligoasthenozoospermia; 262 patients	Yes	L-carnitine 10 mg twice daily and CoQ10 20 mg thrice daily	3 mo	Combination of L-carnitine and CoQ10 can improve the sperm motility and outcome of clinical pregnancy in idiopathic OAT patients. Pretreatment with CoQ10 improves ovarian response to stimulation and embryological parameters in young women with poor ovarian reserve in IVF-ICSI cycles.
Tiseo et al. (2017) [35]	Subfertile couples; 211 subjects	No	CoQ10 19.2 mg/day (2.4–247.2 mg/day)	Not specified	Mean dietary intake of CoQ10 in this study was 10-fold lower than the supplemental dose used in clinical trials, showing improved sperm motility.
Giacone et al. (2017) [56]	12 Normozoospermic men and 12 asthenozoospermic patients	No	Zinc, D-aspartic acid, CoQ10 12 mg	Not specified	Improved sperm motility and increased fertilization rate in IVF/ICSI.
Nadjarzadeh et al. (2014) [51]	Idiopathic OAT; 60 patients	Yes	CoQ10 200 mg/day or placebo	3 mo	Enhanced semen quality and motility.
Gaby et al. (2013) [57]	Idiopathic OAT; 228 patients	Yes	CoQ10/200 mg/day	26 wk	Increased sperm concentration and morphology. Decreased motility and follicle stimulating hormone activity.
Abad et al. (2013) [58]	Asthenteratozoospermic patients; 20 subjects	No	L-carnitine 1,500 mg, CoQ10 20 mg, vitamin C 60 mg, vitamin E 10 mg, vitamin B 9200 µg, vitamin B12 1 µg, zinc 10 mg, selenium 50 µg	3 mo	DNA damage reduced from 28.5% to 20.12%.
Safarinejad (2012) [39]	Idiopathic OAT; 287 patients	No	CoQ10 300 mg twice daily	12 mo	Increased sperm concentration, progressive motility, and normal morphology.
Nadjarzadeh et al. (2011) [49]	Infertile men with idiopathic OAT; 60 patients	Yes	CoQ10 200 mg once daily	19 mo	Improved seminal parameters, lipid peroxidation.
Safarinejad et al. (2009) [59,60]	Infertile men with idiopathic OAT; 212 patients	Yes	CoQ10 300 mg once daily	26 wk	Improved seminal parameters and testicular volume.
Balercia et al. (2009) [61]	Idiopathic asthenozoospermia; 60 patients	No	CoQ10 200 mg/day	3 mo	Administration of CoQ10 increased CoQ10 levels in semen. It could be effective in enhancing sperm kinetic features in idiopathic asthenozoospermic patients.

CoQ10, coenzyme Q10; RCT, randomized clinical trial; TAC, total antioxidant capacity; ROS, reactive oxygen species; SDF, sperm DNA fragmentation; OAT, oligoasthenoteratozoospermia; IVF, *in vitro* fertilization; ICSI, intracytoplasmic sperm injection.

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In a study of 29 known asthenozoospermic males, a substantial decrease of SDF from 28.5% to 20.12% was reported after 3 months of CoQ10 plus L-carnitine administration [62]. In another study, Ghanbarzadeh et al. [33] showed that pretreatment with CoQ10 and L-carnitine significantly improved sperm parameters, sperm function, and reproductive hormone profile in male Wistar rats with high low-density lipoprotein (LDL) and oxidized LDL serum levels. In a recent study, our research group also observed that when idiopathic OA patients were received 3 months of CoQ10 supplementation, their semen parameters significantly improved, along with a significant reduction in seminal OS markers and SDF compared to the baseline [37]. These data suggest that CoQ10 plays a positive role in the amelioration of SDF, although limited studies are available so far. More unbiased and well-performed RCTs are needed to better clarify this issue.

CoQ10 and pregnancy outcomes

Several studies have demonstrated improved pregnancy rates after CoQ10 administration [63,64]. Some studies suggested that the increased pregnancy rate is due to the beneficial effects of CoQ10 on sperm concentration and motility. In line with such findings, Gvozdjakova et al. [36] showed a significant improvement in the pregnancy rate after the administration of CoQ10 at a daily dose of 90 mg for 3 to 9 months in 40 infertile men with OA. The administration of Carni-Q-Nol (each soft gel containing 440 mg L-carnitine fumarate, 30 mg CoQ10, 75 IU vitamin E, and 12 mg vitamin C) was effective for improving the pregnancy rate, as 45% of the female partners of these patients achieved pregnancy. In the same group, three males (7.5%) achieved fatherhood after undergoing ART, and the other 12 women (30%) became pregnant 5–6 months after their partners began therapy [36].

Safarinejad [39] also reported an increase in the pregnancy rate after treatment with CoQ10 in 287 patients with idiopathic OAT who received supplementation of 300 mg of CoQ10 twice daily for 12 months. After treatment, the participants showed improved sperm quality. A positive impact was found on pregnancy rates, as 34.1% of couples achieved spontaneous clinical pregnancy after 9–12 months of treatment [40]. In a study aiming to assess the effects of CoQ10 administered in combination with L-carnitine in idiopathic OAT patients, sperm parameters were found to be improved, with a lower percentage of SDF and consequently a higher clinical pregnancy rate [45], showing that the combination of CoQ10 and L-carnitine improved pregnancy outcomes in patients with idiopathic male infertility.

CoQ10 and ART outcomes

OS significantly impacts the success rate of ART. Spermatozoa and oocytes, once removed from their microenvironments, can be exposed to excessive levels of ROS as a consequence of the lack of scavenger system systems present in the reproductive tract. For this reason, pretreatment with antioxidants could be useful to improve the quality of gametes [30]. According to Arhin et al. [65], evidence from many RCTs has shown that oral antioxidant supplementation leads to a significant increase in the pregnancy rate in couples undergoing ART cycles by enhancing male fertility. However, the results of some of these studies must be interpreted with the utmost care due to discrepancies in the treatment regimens. Thanks to its ability to improve sperm quality, CoQ10 could play a role in improving ART outcomes. An *in vitro* study showed that incubation of spermatozoa for 3 hours with an antioxidant formula containing zinc, D-Asp, and Co-Q10 had a beneficial effect on sperm motility, recovery of spermatozoa by swim-up, and lipid peroxidation. This suggests that these molecules may have a place in sperm preparation before ART [63]. In another study carried by Lewin and Lavon [66], the effects of oral CoQ10 administration on the outcomes of ICSI were investigated in seven patients with low fertilization rates after ICSI at a dose of 60 mg/day for an average of 103 days before undergoing subsequent ICSI cycles. The treatment significantly increased the fertilization rate, from 10.3% ± 10.5% in ICSI cycles without treatment to 26.3% ± 22.8% after CoQ10 intake. Lewin and Lavon [66] also examined the seminal fluid of 38 subjects (normozoospermic and asthenozoospermic) and noted that in patients with asthenozoospermia, there was an increase in motility after incubation with 50 μM CoQ10 for 24 hours. However, they did not test the increase in the fertility rate in ICSI.

In another retrospective study of 797 intrauterine insemination and 253 IVF cycles, women who received supplementation with 600 mg of CoQ10 along with dehydroepiandrosterone (DHEA) for over a month were found to have reduced levels of gonadotropins upon stimulation and a higher number of mature follicles than women taking DHEA alone [50]. In a recent meta-analysis of 61 RCTs including 6,264 infertile patients, antioxidant treatment was found to be correlated with an increase in clinical pregnancy rate and live birth rate [67].

Conclusion

The present review shows that the antioxidant properties of CoQ10 and its vital role in mitochondrial bioenergetics form the basis of the ameliorative role of seminal CoQ10 in male fertility parameters. Evidence reveals that CoQ10 mainly improves sperm count and motility in infertile men, with most studies emphasizing its role in as-

thenozoospermia. It appears that CoQ10 also protects sperm from oxidative damage, thereby improving OS markers and SDF. Moreover, CoQ10 administration in couples resulted in improved ART outcomes, such as increased fertilization rates in IVF/ICSI. Further in-depth interventions are needed to reveal the exact mode of action of CoQ10 and to determine the appropriate standardized dose and duration of CoQ10 supplementation in the treatment of specific male infertility cases.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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Coenzyme Q10 Improves Sperm Parameters, Oxidative Stress Markers and Sperm DNA Fragmentation in Infertile Patients with Idiopathic Oligoasthenozoospermia

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Purpose: Oxidative stress and sperm DNA fragmentation (SDF) are potential contributing factors for idiopathic male infertility. Coenzyme Q10 (CoQ10) have been reported to be effective in the treatment of idiopathic male infertility, in general, owing to its antioxidant properties. Thus, the present study intends to investigate the effects of CoQ10 therapy on semen parameters, oxidative stress markers and SDF in infertile men, specifically with idiopathic oligoasthenozoospermia (OA).

Materials and Methods: In this case-control study, sixty-five infertile patients with idiopathic OA and forty fertile men (control) were included. All participants underwent semen analysis based on the World Health Organization guidelines (5th edition, 2010). Patients received CoQ10 at the dose of 200 mg/d orally for three months. Seminal plasma CoQ10, total antioxidant capacity (TAC), total reactive oxygen species (ROS), glutathione peroxidase (GPx), and SDF levels were measured in controls (baseline) and infertile patients pre- and post-CoQ10 treatment.

Results: CoQ10 treatment for three months significantly improved sperm concentration ($p<0.05$), progressive motility ($p<0.05$), total motility ($p<0.01$), seminal fluid CoQ10 concentration ($p<0.001$), TAC ($p<0.001$), and GPx ($p<0.001$) levels in infertile men with OA. Further, ROS level ($p<0.05$) and SDF percentage ($p<0.001$) were reduced in OA patients as compared to the baseline. CoQ10 levels also correlated positively with sperm concentration ($r=0.48$, $p=0.01$) and total motility ($r=0.59$, $p=0.003$) while a negative correlation was recorded between SDF and sperm motility ($r=-0.54$, $p=0.006$).

Conclusions: CoQ10 supplementation for three months could improve semen parameters, oxidative stress markers and reduce SDF in infertile men with idiopathic OA.

Keywords: Coenzyme Q10; DNA fragmentation; Male infertility; Oxidative stress; Sperm

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INTRODUCTION

World Health Organization (WHO) defines infertility as the inability to conceive after at least 12 months of regular unprotected sexual intercourse [1]. According to WHO, about 60 to 80 million couples suffer from infer-

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tility globally [2]. Men's are found to be solely responsible for 20% to 30% of infertility cases and contribute to 50% of cases overall [3]. Male infertility could be attributed to varicocele, genital infections, developmental abnormalities, endocrine disruptions, genetic, immunological, and systemic diseases as well as environmental factors [4].

In approximately 30% of male infertility cases, the underlying causes are unknown (idiopathic infertility) [5]. One of the factors that have been proven to cause idiopathic male infertility is oxidative stress (OS) which is a key responsible factor for causing about 30% to 80% of men subfertility [6]. OS occurs due to the exhaustion of seminal antioxidant capacity caused by imbalance between pro-oxidants and antioxidants in the seminal plasma [7]. Reactive oxygen species (ROS) are required for certain physiological processes such as sperm capacitation, acrosome reaction and fertilization [8,9]. However, excessive generation of ROS can damage sperm plasma membrane by lipid peroxidation and can also cause sperm DNA fragmentation (SDF). This may decrease sperm membrane fluidity, affecting sperm motility, vitality and ultimately resulting in altered fertilizing potential of sperm [9].

Oligoasthenozoospermia (OA) is defined as sperm concentration lower than 15 million per mL and sperm progressive motility and total motility lower than 32% and 40% respectively according to WHO 2010 guidelines [10]. It has been reported that, in approximately one third of infertile patients the cause of OA is unknown and, therefore, it is called idiopathic OA [5].

One of the mechanisms proposed for idiopathic OA is SDF. The increased OS causes DNA nicks and breaks that need repair [11]. The faulty repair of the DNA owing to decreased protamination can result in DNA damage. Other factors that can lead to sperm DNA damage are replication errors, ionized radiations, activation of endogenous endonucleases and caspases, exposure to environmental toxins and ultraviolet (UV) rays [12]. DNA fragmentation is an irreversible process that can alter sperm function leading to infertility and several tests have been established for detecting SDF [13].

Coenzyme Q10 (CoQ10), also termed as ubiquinone, is a respiratory chain component and acts as an antioxidant molecule. It is present in the human seminal fluid where it is involved in several antioxidant, mitochondrial bioenergetics and metabolic processes [14]. CoQ10 deficiency can lead to sperm damage, lower sperm motility and sperm count. Studies have shown that the supplementation with CoQ10 can improve the reproductive outcomes in men with fertility problems [15]. In addition, seminal fluid CoQ10 concentrations correlate positively with sperm motility and count. Accordingly, patients who have been treated with CoQ10 have a significant higher sperm count and a better sperm morphology compared with those who did not receive CoQ10 [16].

On the other hand, endogenous antioxidants, such as catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD), normally maintain the scavenging potential in the seminal fluid and gonads [17]. However, studies reporting the impacts of CoQ10 in the treatment of idiopathic OA are scanty. Therefore, the aim of this study was to evaluate the effects of CoQ10 (200 mg/d) therapy on conventional sperm parameters, OS markers and SDF in infertile patients with idiopathic OA.

MATERIALS AND METHODS

1. Patients

Sixty-five infertile patients with idiopathic OA (mean age, 29.1±10.2 years; mean duration of infertility 6.3±4.1 years) and forty fertile healthy men who served as controls (mean age, 31.4±11.3 years) were enrolled in the study. The patients were recruited from the Fertility Clinic, Babyl, Iraq, during the period from May to September 2018. Data were collected using a questionnaire designed for this study. Medical history, physical examination as well as laboratory and radiological tests were performed to ascertain the presence of a known cause of OA. Patients with infertility of 12 months or more despite regular unprotected intercourse were included in the study. Patients were considered to have OA when their semen analysis showed abnormal sperm concentration (<15 million/mL), progressive motility (<32%), and total motility (<40%) as defined by the WHO 2010 criteria for semen analysis [10]. Patients with azoospermia, anatomical abnormalities of men genital tract, varicocele, genital infection, scrotal surgery, systemic diseases, smoking, women factor, and recent (within the last six months) antioxidant and selective serotonin reuptake inhibitors intake were excluded from the study. All the participants underwent baseline seminal fluid analysis (WHO 2010 guidelines),

seminal antioxidant measurement (total antioxidant capacity [TAC], GPx, and ROS), seminal fluid CoQ10 concentration and evaluation of the percentage of spermatozoa with DNA fragmentation. Each patient received CoQ10 200 mg/d orally (one a day) for three months and their conventional sperm parameters, TAC measurements, seminal fluid CoQ10 level and SDF were compared before and after CoQ10 treatment.

2. Ethics statement

The study design was approved by the University of Sumer local research ethical committee (EC/2018/8876) and adhered to the Declaration of Helsinki. An informed consent for participation in the study was obtained from each patient and control enrolled in this study.

3. Semen analysis

Semen was collected by masturbation after an abstinence of 2 to 3 days. It was collected into a sterile wide mouth plastic container, held at 37°C until liquefaction was achieved and then analyzed within one hour from ejaculation using the methods suggested in the WHO manual WHO guidelines (5th edition) [10] for all semen parameters but sperm morphology which was evaluated according to the WHO guidelines (4th edition) [18]. Semen analysis was performed by the same researcher to ensure data consistency. Two semen analyses were performed before and after CoQ10 administration and the mean values were recorded and used for statistical evaluation.

4. Seminal total antioxidant capacity

Semen samples were centrifuged at 3,000 rpm for 5 minutes and seminal plasma was collected and stored at -20°C until further testing. A colorimetric method was applied to measure TAC using the total antioxidant capacity assay kit (#E-BC-K136; Elabscience, Houston, TX, USA). Antioxidants can reduce Fe^{3+} to Fe^{2+} and Fe^{2+} can form stable complexes with phenanthroline. TAC was measured using an absorbance at 520 nm as suggested by the manufacturer protocol.

5. Glutathione peroxidase activity

GPx was measured by a colorimetric method using the GPx assay kit (#E-BC-K096; Elabscience). The test is based on the principle that the enzyme promotes the reaction of hydrogen peroxide (H_2O_2) and reduced glutathione (GSH) to produce H_2O and oxidized GSH. The activity of GSH can be calculated by measuring the consumption of reduced GSH. H_2O_2 and reduced GSH can react without catalysis of GPx, so the portion of GSH reduction by non-enzymatic reaction should be subtracted. GSH can react with dinitrobenzoic acid to produce 5-thio-dinitrobenzoic acid anion, which shows a stable yellow color. TAC was calculated by measuring the absorbance at 412 nm as suggested by the manufacturer protocol.

6. Seminal reactive oxygen species measurement

ROS were measured manually using the method previously described by Venkatesh et al [19]. To 400 μL of liquefied neat semen, 10 μL of luminol (5-amino-2,3,-dihydro-1,4-phthalazinedione; Sigma-Aldrich, St. Louis, MO, USA), prepared as 5 mM stock in dimethyl sulfoxide (DMSO), was added. Ten microlitres of 5 mM luminol in DMSO served as blank. Twenty-five microlitres H_2O_2 with 10 μL luminol was used as a positive control. The levels of ROS were assessed by measuring the luminol-dependent chemiluminescence.

7. Measurement of seminal coenzyme Q10 concentrations

The seminal plasma concentrations of CoQ10 were measured by high-performance liquid chromatography (HPLC) method using a UV detector at 275 nm and calculated using the method described previously by Li et al [20]. The principle of this method is based on reversed-phase HPLC method with UV detection using coenzyme Q9 as the internal standard.

8. Sperm chromatin dispersion test

SCD test was performed using the Halosperm kit (Halotech DNA, S.L., Madrid, Spain). The test is based on the principle that spermatozoa with fragmented DNA fail to produce the characteristic halo of dispersed DNA loops that is observed in spermatozoa with non-fragmented DNA, following acid denaturation and removal of nuclear proteins. Intact non-fixed spermatozoa were immersed in an inert agarose microgel on pre-treated slide. Initial acid treatment denatured DNA molecules in those spermatozoa with fragmented DNA. Following this, the lysis solution removed most of the nuclear proteins, and in the absence of massive DNA breakage produced nucleoids with large halos of

spreading DNA loops, emerging from a central core. The nucleoids from spermatozoa with fragmented DNA either did not show a dispersion halo or the halo was minimal. Bright field microscopy with Diff-Quik staining was used to assess the halos. SDF, defined as the percentage ratio of fragmented *versus* total spermatozoa, was calculated for each sample and recorded [21].

9. Statistical analysis

Statistical analysis was performed using IBM SPSS software ver. 24 (IBM Corp., Armonk, NY, USA). The results are shown as mean±standard deviation. Normality of data was assessed by Kolmogorov–Smirnov test. Paired Student's t-test was used to compare means before and after CoQ10 administration. Correlations between sperm parameters and TAC, CAT, and SOD were analyzed using the Pearson correlation coefficient (r). A p-value lower than 0.05 was considered statistically significant.

RESULTS

CoQ10 treatment for three-months improved sperm concentration (p<0.05), progressive motility (p<0.05), and total motility (p<0.01) compared to the baseline (Table 1).

The seminal antioxidant status in the infertile patients were significantly lower (CoQ10 [p<0.05], TAC [p<0.01], and GPx [p<0.001]), while total ROS level was significantly higher (p<0.001) as compared to the controls (Table 2). CoQ10 therapy significantly improved seminal CoQ10 level (p<0.001), TAC (p<0.01), and GPx (p<0.001) levels, whereas it descreased the total ROS levels (p<0.05) in patients with idiopathic OA, as compared with pre-treatment values. SDF was significantly higher (p<0.01) in patients with idiopathic OA compared to the fertile controls, and treatment with CoQ10 significantly decreased the percentage of SDF (p<0.01). CoQ10 levels have been found to be positively correlated with sperm concentration (r=0.48, p=0.01) and motility (r=0.59, p=0.003) (Table 3). Moreover, SDF correlated negatively with sperm motility (r=-0.54, p=0.006). Seminal fluid CoQ10 levels did not show and correlation with sperm concentration or morphology.

Table 1. Semen parameters in fertile and infertile men before and after administration of CoQ10

Semen parameter	Fertile men (n=40)	Infertile patient (n=65)	
		Before CoQ10	After CoQ10
Age (y)	31.4±11.3	29.1±10.2	-
Infertility duration (y)	-	6.3±4.1	-
Volume (mL)	2.9±0.8	2.88±1.4	3.1±1.2
Concentration (million/mL)	51.1±29.4	9.4±5.4[c]	11.5±5.3[a,c]
Progressive motility (%)	47.8±10.8	22.3±9.3[c]	27.1±13.6[a,c]
Total motility (%)	66.4±13.4	30.1±9.8[c]	37.1±15.1[b,c]
Normal morphology (%)	43.4±9.1	42.3±8.8	40.5±9.6

Values are presented as mean±standard deviation.
CoQ10: coenzyme Q10.
[a]*vs.* patients baseline, p<0.05; [b]*vs.* patients baseline, p<0.01; [c]*vs.* fertile men, p<0.001.

DISCUSSION

CoQ10 follows *de-novo* pathway for its synthesis during normal physiological processes in most of the tis-

Table 2. Seminal plasma CoQ10, oxidative stress markers and SDF levels in fertile and infertile men before and after administration of CoQ10

	Fertile men (n=40)	Infertile patient (n=65)	
		Before CoQ10	After CoQ10
CoQ10 level (ng/mL)	63.8±42.38	46.2±33.8[a]	85.8±29.9***[,b]
ROS (×10^4 RLU/min/20 million spermatozoa)	0.11±0.08	4.6±1.95[c]	3.8±1.6*[,c]
TAC (mmol/L)	1.87±0.26	1.03±0.65[c]	1.32±0.59**[,c]
GPx (U/mL)	0.67±0.06	0.22±0.03[c]	0.39±0.05***[,c]
SDF (%)	15.8±4.5	35.6±7.1[c]	30.9±8.3**[,c]

Values are presented as mean±standard deviation.
CoQ10: coenzyme Q10, SDF: sperm DNA fragmentation, ROS: reactive oxygen species, RLU: relative light unit, TAC: total antioxidant capacity, GPx: glutathione peroxidase.
*Significant difference from patients baseline, p<0.05; **Significant difference from patients baseline, p<0.01; ***Significant difference from patients baseline, p<0.001.
[a]Significant difference from control, p<0.05; [b]Significant difference from control, p<0.01; [c]Significant difference from control, p<0.001.

Table 3. Correlation between SDF, CoQ10 level and semen parameters in infertile men post-CoQ10 treatment

	Concentration	Total motility	Normal morphology
SDF	-0.14 (0.28)	-0.54 (0.006)	-0.27 (0.09)
CoQ10	0.48 (0.01)	0.59 (0.003)	0.2 (0.15)

Values are presented as r (p-value).
SDF: sperm DNA fragmentation, CoQ10: coenzyme Q10.

sues [22]. Thus, the exogenous supply of this coenzyme is not required in those tissues. However, in conditions of elevated OS, the endogenous supply might not be enough to fulfil the demands for CoQ10 [23].

It has been reported to be increased in blood and seminal plasma following its supplementation and is well proven to improve semen quality. Balercia et al [16] evaluated the concentration of CoQ10 in the seminal fluid and found increased CoQ10 levels in the patients after supplementation. A study also concluded that CoQ10 levels in the blood as well as in the seminal fluid increases after three or six months of supplementation [24].

An *in-vitro* study with CoQ10 supplementation has shown that it increases sperm motility significantly in asthenozoospermic patients [15]. A placebo-controlled, randomized study reported a significant improvement of sperm concentration, total sperm count, and sperm motility after CoQ10 supplementation to infertile men [25]. Mancini et al [26] have also reported similar observations in idiopathic OA men. By quantifying CoQ10 levels in the seminal plasma from 77 patients, they have recorded that reduced CoQ10 levels are associated with abnormal sperm morphology and motility. Based on their findings, they have concluded that patients with idiopathic OA can benefit from CoQ10 supplementation [26]. Recent studies have found that some of these effects of exogenously administered CoQ10 are due to the modulation of gene expression [22]. A randomized, placebo-controlled study, performed in patients with idiopathic OA showed a reduction in the OS after a three-month long CoQ10 supplementation, which has reported similar findings to the present results [27].

In the state of increased OS, endogenous enzymatic antioxidants also alter their activities to cope with the condition. The present study also showed a significant increase in TAC and GPx after CoQ10 administration. GSH has been found to be associated with men fertility due to its scavenger activity. The seminal TAC plays a significant role in the protection of spermatozoa when there is an elevation of seminal OS. Our study reported an improvement in seminal TAC after the administration of oral antioxidants. In contrast, few studies have reported no relationship between CoQ10 and TAC levels [28].

Studies have shown that men fertility can be compromised by excessive SDF [29]. The present study also focusses on the decrease of SDF index after CoQ10 administration in the infertile patients with idiopathic OA. A significant positive difference in sperm morphology following CoQ10 treatment also indicates that CoQ10 improves sperm structure. Results resembling our studies were obtained thus strengthening the fact that CoQ10 has positive effect on the sperm damage [30].

CONCLUSIONS

Seminal fluid of infertile patients with idiopathic OA is accompanied with high levels of ROS, which induces OS mediated disruptions of sperm functions and sperm DNA integrity. CoQ10 can attenuate ROS effects and enhance sperm functions owing to its antioxidant activity. The study affirms that supplementation with CoQ10 for the duration of least three months decreases SDF and improves semen quality and seminal antioxidant capacity in OA men. Thus, CoQ10 finds potential to be screened further for its application in the management of OA male infertility patients.

Conflict of Interest

The authors have nothing to disclose.

Author Contribution

Conceptualization: ATA. Data curation: ATA. Formal analysis: ATA. Investigation: ATA. Methodology: ATA. Validation: ATA. Writing – original draft: ATA. Writing – review & editing: AEC, PS, SD.

Data Sharing Statement

The data required to reproduce these findings cannot be shared at this time due to legal and ethical reasons.

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CERM

Coenzyme Q10, oxidative stress markers, and sperm DNA damage in men with idiopathic oligoasthenoteratospermia

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Objective: Oxidative stress (OS) plays a key role in the etiology of unexplained male infertility. Coenzyme Q10 (CoQ10) is a potent antioxidant that may improve semen quality and OS in infertile men with idiopathic oligoasthenoteratospermia (OAT), but the underlying mechanism is unknown. Therefore, the present study was undertaken to investigate the effect of CoQ10 on OS markers and sperm DNA damage in infertile patients with idiopathic OAT.
Methods: This prospective controlled study included 50 patients with idiopathic OAT and 50 fertile men who served as controls. All patients underwent a comprehensive medical assessment. Patients and controls received 200 mg of oral CoQ10 once daily for 3 months. Semen and blood were collected and analyzed for sperm parameters, seminal CoQ10 levels, reactive oxygen species (ROS) levels, total antioxidant capacity, catalase, sperm DNA fragmentation (SDF), and serum hormonal profile.
Results: The administration of CoQ10 to patients with idiopathic OAT significantly improved sperm quality and seminal antioxidant status and significantly reduced total ROS and SDF levels compared to pretreatment values.
Conclusion: CoQ10, at a dose of 200 mg/day for 3 months, may be a potential therapy for infertile patients with idiopathic OAT, as it improved sperm parameters and reduced OS and SDF in these patients.

Keywords: Coenzyme Q10; Male infertility; Oxidative stress; Sperm DNA fragmentation

Introduction

Infertility is defined as the failure of conception after at least 1 year of regular unprotected sexual intercourse [1]. The prevalence of infertility among reproductive-aged couples is approximately 8%–15% [2], with male factor infertility accounting for 50% of cases [3]. Infertility in men has been linked to endocrine disorders, developmental anomalies, systemic diseases, and environmental, immunological, and genetic factors [4-6]. However, in approximately 25% of infertile men, no cause can be identified for semen abnormalities; this condition is referred to as idiopathic infertility [7].

Oxidative stress (OS) has been reported as a key factor contributing to idiopathic male infertility [8]. OS may occur as a consequence of higher ratios of oxidants (free radicals and/or reactive oxygen species [ROS]) to antioxidants in the seminal plasma [9]. The etiology of OS can be attributed to several intrinsic or extrinsic factors, including ageing, varicocele, infection, cryptorchidism, testicular torsion, radiotherapy, chemotherapy, and toxins [10,11]. Specific physiological functions, such as sperm capacitation, the acrosome reaction, and fertilization require ROS [6,12]. Nonetheless, overproduction of ROS may impair sperm membrane and DNA integrity, resulting in de-

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creased sperm membrane fluidity and changes in the fertilizing capability of sperm [13]. ROS-induced sperm DNA damage may affect sperm motility and the ability to fertilize an oocyte [14]. Sperm DNA fragmentation (SDF) is irreversible and leads to alteration of sperm function, resulting in infertility [15].

Coenzyme Q10 (CoQ10) is a potent antioxidant that protects sperm against ROS-induced damage [16]. It is quite ubiquitous, with a high amount in sperm mitochondria [17], and has been reported to enhance sperm motility and concentration [8,11]. Furthermore, insufficient CoQ10 levels have been linked to low sperm count and motility, as well as elevated sperm DNA damage [18]. Our recent study conducted on patients with idiopathic oligoasthenospermia (OA) for 12 weeks showed that CoQ10 therapy substantially enhanced progressive motility, sperm concentration, and seminal fluid CoQ10 concentrations [1]. It also led to an increase in glutathione peroxidase (GPx) levels and total antioxidant capacity (TAC) [1]. Moreover, our recent meta-analysis of three randomized clinical trials on the effect of CoQ10 on semen quality demonstrated beneficial effects of CoQ10 on improving sperm motility, but not on sperm concentration or morphology [18]. The maintenance of testicular scavenging function is normally exerted by intrinsic antioxidants such as GPx, catalase (CAT), and superoxide dismutase (SOD) [19,20].

The present study aimed to investigate the impact of CoQ10 on OS markers and sperm DNA damage in infertile men with idiopathic oligoasthenoteratospermia (OAT) in an attempt to better understand its mechanism of action.

Methods

1. Patients

Fifty patients with idiopathic OAT and 50 fertile men (controls) were recruited at the Fertility Clinic, Hillah, Babyl, Iraq, from July 2018 to January 2019. All patients and controls underwent a comprehensive medical assessment. A prospective controlled study was conducted with a 3-month follow-up. Seven patients dropped out of the study and were therefore excluded. The patients received a daily dose of 200 mg of CoQ10 (in its reduced form as ubiquinol) (America Medic and Science AMS, Woodinville, WA, USA) as a single oral dose for 3 months [21]. Semen analysis, seminal CoQ10 levels, ROS, TAC, CAT, SDF as well as serum hormonal profile (follicle-stimulating hormone [FSH], luteinizing hormone [LH], testosterone, and prolactin levels) in patients after therapy were compared with the baseline values for patients and controls (patients were followed-up from September 2018 to February 2019). Sample size calculation was performed using 80% power and a 5% level of significance and was 42 for each group. Study approval was obtained from the University of Sumer Local Research Ethical Committee (EC/2018/8879). All participants consented to the study prior to enrollment in the study.

2. Eligibility criteria

The fertile controls enrolled in the study had fathered a child in the last 24 months and had normal semen analysis results. The patients had a history of infertility of at least 1 year despite regular unprotected intercourse. OAT was defined according to the World Health Organization (WHO) 2010 criteria. Men with varicocele; genital infection; azoospermia; anatomical abnormalities; testicular injury or surgery; endocrine, renal, hepatic, or other systemic illnesses; smoking; alcohol intake; and recent antioxidant intake were excluded, as were those taking relevant medications and male partners in couples affected by female factor infertility.

3. Semen analysis

Semen samples were collected by masturbation following abstinence for 2–3 days. A special wide-mouth container was used to collect semen and incubated at 37°C until the semen was liquefied. Semen analysis was then performed within 1 hour following the WHO manual criteria (5th edition, 2010) [22]. Duplicate semen analyses were performed at the beginning and at the end of the study and the average of the two values was used for analysis. The same investigator performed all semen analyses to optimize repeatability.

4. Measurement of seminal CoQ10 concentrations

Seminal CoQ10 levels were measured using reverse-phase high-performance liquid chromatography utilizing an ultraviolet light detector at 275 nm, with coenzyme Q9 as an internal standard, and calculated using a published method [23].

5. Seminal ROS measurement

Semen samples were centrifuged at 3,000 rpm for 5 minutes to obtain seminal plasma and then were stored at –20°C. A manual method was used for ROS measurement as previously described by Venkatesh et al. [24]. To 400 µL of liquefied neat semen, 10 µL of luminol (5-amino-2,3,-dihydro-1,4-phthalazinedione; Sigma, St. Louis, MO, USA), prepared as 5 mM stock in dimethyl sulfoxide (DMSO), was added. Ten microliters of 5 mM luminol in DMSO served as a blank, while 25 µL of H_2O_2 with 10 µL of luminol was used as a positive control. Luminol-dependent chemiluminescence served as indicator of ROS levels.

6. Measurement of seminal TAC and CAT

TAC was estimated with a colorimetric method using a total antioxidant capacity assay kit (#E-BC-K136; Elabscience, Houston, TX, USA). Seminal plasma CAT activity was assessed using a CAT assay kit (#E-BC-K031, Elabscience), following the protocol recommended by

the manufacturer.

7. Sperm chromatin dispersion test

Sperm chromatin dispersion was tested using the Halosperm kit (Halotech DNA, Madrid, Spain). The principle of the test is that, after acid denaturation and removal of nuclear proteins, sperm with SDF do not exhibit the halo of dispersed DNA loops that is observed in sperm without SDF. The nucleoids from spermatozoa with SDF show no dispersion halo or a minimal halo. Bright-field microscopy with Diff-Quik staining was utilized to examine the halos. SDF, defined as the percentage ratio of sperm with SDF to the total spermatozoa, was calculated using previously published methods [1,25].

8. Hormonal assays

Blood samples (5 mL) were collected using venipuncture in clean plain labeled tubes, allowed to clot, and centrifuged at 3,000 rpm for 10 minutes for analysis of hormones. Serum FSH, LH, testosterone, and prolactin levels were measured using enzyme-linked fluorescent assays (mini-VIDAS; Biomerieux, Lyon, France).

9. Statistical analysis

IBM SPSS ver. 24 (IBM Corp., Armonk, NY, USA) was used for data analysis. The results were expressed as mean ± standard deviation. The normality of the data distribution was assessed using the Kolmogorov-Smirnov test. One-way analysis of variance was used to compare mean values between subgroups. Pearson correlation coefficients were applied to assess the correlations of seminal fluid parameters with CoQ10 levels and SDF. For all tests, a *p*-value lower than 0.05 was considered to indicate statistical significance.

Results

The mean age of the control participants and patients was 34.2 ± 13.4 and 31.3 ± 12.6 years, respectively, and the mean duration of infertility in the patients was 7.1 ± 4.8 years. CoQ10 therapy in patients with idiopathic OAT significantly increased the total motility ($p < 0.01$), progressive motility ($p < 0.05$), and sperm concentration ($p < 0.05$) compared with the baseline (Table 1). Treatment with CoQ10 increased progressive motility from 26.5% ± 10.8% to 32.6% ± 15.1%. Sperm concentration also increased from 11.2 ± 6.4 to 13.3 ± 8.6 million/mL following treatment with CoQ10, and total motility also rose from 36.1% ± 10.8% to 44.2% ± 18.5%, with a high level of significance when compared with other sperm parameters.

The results also showed that the seminal antioxidant status was significantly lower in infertile patients than in controls (CoQ10, $p < 0.05$; CAT, $p < 0.001$; and TAC, $p < 0.01$), but ROS levels were significantly higher than in controls ($p < 0.001$) (Table 2). Moreover, treatment with CoQ10 substantially enhanced CAT ($p < 0.001$), TAC ($p < 0.01$), and seminal CoQ10 ($p < 0.001$) levels, while decreasing total ROS levels ($p < 0.05$) in patients with idiopathic OAT. The comparison between fertile controls and idiopathic OAT patients showed that SDF was significantly higher in patients ($p < 0.001$), and was significantly reduced by CoQ10 therapy ($p < 0.001$) (Table 2).

FSH ($p < 0.001$), LH ($p < 0.001$), and prolactin levels ($p < 0.01$) were significantly higher in infertile patients than in controls. Following CoQ10 treatment, LH levels increased significantly in infertile patients compared with their baseline values ($p < 0.05$) (Figure 1). The SDF of infertile patients was negatively correlated with total sperm motility ($r = -0.62$, $p = 0.001$). Furthermore, total sperm motility ($r = 0.56$, $p = 0.005$) was positively associated with CoQ10 levels ($r = 0.23$, $p = 0.12$) in infertile patients (Table 3).

Discussion

CoQ10 is an essential antioxidant present nearly in all body tissues. It is also present in sperm mitochondria, where it plays a critical role in cellular respiration and energy generation [26]. CoQ10 is also involved in the inhibition of superoxide formation, protecting against

Table 1. Comparison of semen parameters in fertile men and infertile patients before and after treatment with CoQ10

Demographic characteristics and semen parameter	Fertile men (n = 50)	Infertile patients (n = 50)	
		Before CoQ10	After CoQ10
Age (yr)	34.2 ± 13.4	31.3 ± 12.6	-
Infertility duration (yr)	-	7.1 ± 4.8	-
Volume (mL)	3.5 ± 1.4	3.48 ± 1.7	3.7 ± 1.8
Concentration (million/mL)	61.3 ± 34.6	11.2 ± 6.4[a)]	13.3 ± 8.6[c)]
Progressive motility (%)	57.8 ± 12.2	26.5 ± 10.8[a)]	32.6 ± 15.1[a),c)]
Total motility (%)	79.1 ± 15.0	36.1 ± 10.8[a)]	44.2 ± 18.5[b),c)]
Normal morphology (%)	8.2 ± 3.6	5.4 ± 3.2[a)]	5.7 ± 2.9[c)]

Values are presented as mean±standard deviation.
CoQ10, coenzyme Q10.
[a)] $p<0.05$, vs. baseline; [b)] $p<0.01$, vs. baseline; [c)] $p<0.001$, vs. fertile men.

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Table 2. Seminal plasma CoQ10 levels, oxidative stress markers, and sperm DNA fragmentation in fertile controls and infertile patients before and after the administration of CoQ10

Variable	Fertile men (n = 50)	Infertile patients (n = 50)	
		Before CoQ10	After CoQ10
CoQ10 level (ng/mL)	56.7 ± 37.4	41.4 ± 29.3[c)]	76.2 ± 26.7[b),d)]
ROS (× 10^4 RLU/min/20 million spermatozoa)	0.08 ± 0.06	4.3 ± 1.6[d)]	3.3 ± 1.5[a),d)]
TAC (mmol/L)	1.4 ± 0.25	0.9 ± 0.44[d)]	1.2 ± 0.51[b),c)]
Catalase (U/mL)	15.54 ± 3.12	10.6 ± 2.8[d)]	12.4 ± 2.61[a),d)]
Sperm DNA fragmentation (%)	16.4 ± 4.7	38.6 ± 7.9[d)]	34.5 ± 9.3[a),d)]

Values are presented as mean±standard deviation.
CoQ10, coenzyme Q10; ROS, reactive oxygen species; TAC, total antioxidant capacity.
[a)]Significant difference from baseline, $p<0.01$; [b)]Significant difference from baseline, $p<0.001$; [c)]Significant difference from control, $p<0.05$; [d)]Significant difference from control $p<0.001$.

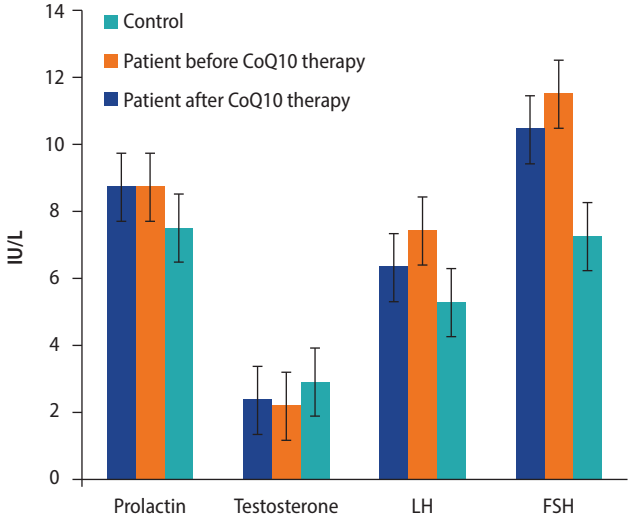

Figure 1. Hormonal profiles of controls and infertile patients with oligoasthenoteratospermia before and after coenzyme Q10 (CoQ10) therapy. LH, luteinizing hormone; FSH, follicle-stimulating hormone.

Table 3. Correlation of SDF and CoQ10 levels with sperm parameters in infertile patients with idiopathic oligoasthenoteratozoospermia

Variable	*r* (*p*-value)		
	Concentration	Total motility	Normal morphology
SDF	–0.09 (0.31)	–0.62 (0.001)	–0.18 (0.16)
CoQ10	0.23 (0.12)	0.56 (0.005)	0.14 (0.22)

SDF, sperm DNA fragmentation; CoQ10, coenzyme Q10.

OS-induced sperm damage [27]. OS-mediated disruptions in fertility parameters entail sperm DNA and cell membrane damage [2].

The role of CoQ10 in cellular respiration and energy generation underscores its usefulness as an antioxidant [28]. In our previous studies, we found that CoQ10 at the dose of 200 mg/day for 3 months improved semen quality and antioxidant status in infertile patients with idiopathic OAT, but the underlying mechanism by which CoQ10 improves fertility parameters are unknown [1,15]. In the present study, we found that CoQ10 therapy in patients with idiopathic OAT significantly improved sperm parameters and antioxidant status, while decreasing OS markers and SDF.

It has been widely reported that SDF is a critical factor that causes male infertility [29]. Thus, the results of the present study may indicate that at the molecular level, CoQ10 acts to ameliorate sperm DNA damage and mitigates OS, thereby leading to improvements in sperm parameters. Similar observations have been published by Suliga and Gluszek [2], who showed that CoQ10 concentrations in seminal plasma were linearly associated with sperm count and motility. Moreover, in a case-control study of 65 idiopathic OA patients, CoQ10 therapy significantly increased progressive motility, GPx levels, sperm concentration, seminal fluid CoQ10 concentration, total motility, and TAC compared with the control group consisting of 45 healthy men [1]. The study concluded that CoQ10 supplementation for 12 weeks led to improvements in OS markers, enhancement of semen parameters, and reduction of SDF in infertile patients with idiopathic OA [1]. Another study conducted in 212 infertile patients with idiopathic OAT, who were on 300 mg of oral CoQ10 for 182 days, showed substantial increases in sperm motility and concentration after CoQ10 treatment [14]. A similar study showed that CoQ10 therapy (200– 300 mg per day) could significantly increase sperm motility and concentration [30]. CoQ10 therapy has also been reported to improve SOD and CAT levels, as well as sperm parameters [14]. A meta-analysis conducted by Lafuente et al. [26], also showed that supplementation with CoQ10 enhanced sperm parameters. Furthermore, our recent meta-analysis of three randomized clinical trials demonstrated that CoQ10 improved sperm motility [18]. In a non-controlled trial [31] in 287 infertile patients with idiopathic OAT, it was found that CoQ10 therapy given orally at the dose of 300 mg twice daily for 1 year significantly improved progressive motility, the proportion of normal morphology, and sperm concentration [2].

A controlled trial conducted among 228 idiopathic infertile OAT patients who received CoQ10 supplementation (200 mg daily for 26 weeks) concluded that CoQ10 increased sperm motility, sperm concentration, and morphology [31]. Another controlled trial including

60 infertile patients with idiopathic OAT treated with CoQ10 (200 mg per day or placebo for 3 months) concluded that there were improvements in semen parameters [32]. The various results from meta-analyses and clinical trials on CoQ10 therapy in men with infertility align with our findings that supplementation with CoQ10 increased seminal CoQ10 levels, sperm motility, and concentration [33]. Generally, controlled clinical trials among men with idiopathic infertility treated with CoQ10 have shown that this treatment leads to a reduction in OS in seminal plasma and lipid peroxidation, as well as an increase in ubiquinol levels and seminal enzymatic antioxidant levels [32].

In a study assessing the effect of 2 doses of CoQ10 on semen quality and OS markers in men with idiopathic OAT, it was found that CoQ10 treatment increased SOD activity, TAC, and CAT activity [34]. CoQ10 inherently inhibits the action of OS by antagonizing any system that increases OS and enhancing systems that could inhibit OS. A prior study showed a strong negative relationship between CoQ10 and hydrogen peroxide levels [6]. Our results, in agreement with the study by Safarinejad et al. [32], showed that CoQ10 therapy decreased FSH and LH, whereas it increased serum prolactin levels. Hyperprolactinemia is among the causes of hypogonadotropic hypogonadism [35].

A study of 20 infertile patients with high SDF levels treated for 3 months with a preparation containing various antioxidants, including CoQ10, showed that SDF levels significantly decreased and sperm concentration significantly increased [36]. In a study of 20 infertile patients with high SDF levels and low-grade varicocele who were treated for 3 months with a preparation containing CoQ10 and other antioxidants, SDF levels significantly decreased and sperm concentration significantly increased [37]. These results support our finding of a significant decrease in SDF levels when infertile patients with idiopathic OAT were placed on CoQ10 therapy (200 mg/day) [1]. It was also shown that treatment with CoQ10 substantially enhanced CAT, TAC, and seminal CoQ10 levels in idiopathic OAT, and that total ROS levels decreased when compared with pretreatment values. These findings suggest that CoQ10 therapy could improve sperm parameters in infertile patients with idiopathic OAT.

The present study showed that CoQ10 increased sperm concentration and sperm progressive motility and decreased SDF levels in infertile patients with idiopathic OAT. These findings suggest that CoQ10 therapy improves sperm parameters by reducing OS and OS-induced sperm damage. Therefore, CoQ10 is a potentially useful antioxidant for the treatment of infertile patients with idiopathic OAT.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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CERM

Comparison of the effects of coenzyme Q10 and Centrum multivitamins on semen parameters, oxidative stress markers, and sperm DNA fragmentation in infertile men with idiopathic oligoasthenospermia

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Objective: Oxidative stress and sperm DNA fragmentation (SDF) have been linked to idiopathic male infertility (IMI). Various antioxidants have been tried to improve semen parameters and fertility potential in IMI patients, but with inconsistent results. The study aimed to compare the effects of coenzyme Q10 (CoQ10) and Centrum multivitamins on semen parameters, seminal antioxidant capacity, and SDF in infertile men with idiopathic oligoasthenospermia (OA).

Methods: This prospective controlled clinical study involved 130 patients with idiopathic OA and 58 fertile controls. The patients were divided randomly into two groups: the first group received CoQ10 (200 mg/day orally) and the second group received Centrum multivitamins (1 tablet/day) for 3 months. Semen parameters, CoQ10 levels, reactive oxygen species (ROS), total antioxidant capacity (TAC), catalase, SDF, and serum hormone levels (follicle-stimulating hormone, luteinizing hormone, testosterone, and prolactin) were compared at baseline and after 3 months.

Results: Both CoQ10 and Centrum improved sperm concentration and motility, but the improvement was greater with Centrum therapy ($p<0.05$). Similarly, both therapies improved antioxidant capacity, but TAC and catalase improvement was greater ($p<0.01$ and $p<0.001$ respectively) with CoQ10, whereas ROS ($p<0.01$) and SDF ($p<0.001$) improvements were greater with Centrum administration. Centrum therapy was associated with reduced serum testosterone ($p<0.05$).

Conclusion: In conclusion, both CoQ10 and Centrum were effective in improving semen parameters, antioxidant capacity, and SDF, but the improvement was greater with Centrum than with CoQ10. Therefore, Centrum—as a source of combined antioxidants—may provide more effective results than individual antioxidants such as CoQ10 in the treatment of infertile men with idiopathic OA.

Keywords: Centrum; Coenzyme Q10; Idiopathic oligoasthenozoospermia; Oxidative stress

Introduction

Infertility is defined as the failure to achieve pregnancy after 12 months or more of regular unprotected intercourse [1]. Infertility is a global health issue affecting approximately 48 million couples worldwide [2], and male factors are considered to be the contributor in approximately 50% of infertile couples [3]. The etiology of male infertility is complex and includes underlying factors such as cryptorchidism, varicocele, genital tract infections, genetic mutations, immunological, endocrine disorders, systemic diseases, and environmental

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factors [4]. However, in 30%–50% of all male infertility cases, the underlying cause of semen abnormalities remains unknown, leading to their classification as idiopathic male infertility (IMI) [5]. Oxidative stress (OS) is also a contributing factor in a significant number of infertile men. OS is defined as the distortion of the prooxidant-antioxidant balance, resulting in elevated levels of reactive oxygen species (ROS) [6]. Increased levels of seminal ROS have been found in 30%–80% of men with infertility [7]. Excessive ROS could be due to immature sperm, white blood cells, testicular torsion, cryptorchidism, varicocele, aging, infection, tumors, radiation, chemotherapy, environmental toxins, smoking, alcohol use, or systemic disease [8]. An increase in OS levels can lead to sperm plasma membrane damage due to lipid peroxidation, reduced sperm motility, reduced fertilization, and sperm DNA fragmentation (SDF) [9].

Spermatozoa possess limited-capacity antioxidant defense mechanisms that protect against ROS-induced damage [10]. Intrinsic protection is mediated by enzymatic antioxidants such as superoxide dismutase, catalase (CAT), glutathione peroxidase (GPx), and glutathione S-transferase, and non-enzymatic antioxidants including urate, carnitine, glutathione, coenzyme Q10 (CoQ10), and vitamins C and E [11]. A low total antioxidant capacity (TAC) in infertile men has been attributed to a significant reduction in both enzymic and non-enzymic antioxidants [12]. A reduction in the antioxidant defense and OS may also result in SDF, which has been associated with abnormal semen parameters, reduced fertilization, and conception rates, as well as increased rates of malignancies and neurological disorders in offspring [13].

CoQ10, which is a component of the mitochondrial respiratory chain, exerts antioxidant effects [14]. CoQ10 deficiency has been associated with lower sperm motility, count, and male infertility, while CoQ10 supplementation has been shown to improve sperm count and sperm morphology in infertile men [15]. We and others have reported that CoQ10 therapy increased antioxidant capacity in men with idiopathic oligoasthenospermia (OA) [16-18]. However, studies exploring the impacts of CoQ10 on seminal antioxidant capacity and SDF in men with idiopathic OA are limited. Other studies have tried combinations of multiple antioxidants to treat male infertility and have reported variable effects on semen parameters, antioxidant capacity, and SDF [19,20]. Furthermore, there is a lack of agreement on the type, dose, or the use of the individual or combined antioxidants in IMI [17]. Therefore, the present study aimed to compare the effects of CoQ10 and Centrum multivitamins (containing 26 vitamins and minerals) on semen parameters, OS markers, and SDF in infertile men with idiopathic OA.

Methods

This prospective clinical study was conducted with a 3-month follow-up period. One hundred and thirty patients with idiopathic OA and 58 fertile men as the control group were enrolled at the Fertility Clinic, Babyl, Iraq, from August 2018 to February 2019. A comprehensive fertility assessment was performed for all participants. Nine patients did not complete the study and were excluded. Patients were allocated randomly to one of two groups (each containing 65 patients). The first group received a daily dose of 200 mg of CoQ10 (in the form of ubiquinol) (AMS, Woodinville, WA, USA) as a single oral dose for 3 months [21]. The second group received Centrum multivitamins (Pfizer, New York, NY, USA) as 1 tablet per day orally containing 26 vitamins and minerals for 3 months. Semen analysis findings, seminal CoQ10 levels, ROS, TAC, CAT, SDF, and serum hormone levels (follicle-stimulating hormone [FSH], luteinizing hormone [LH], testosterone, and prolactin) levels were compared at baseline and after 3 months. The primary endpoint was improvement in semen parameters, and the secondary endpoint was improvement in seminal antioxidant markers. The sample size was calculated using 80% power and a 5% level of error, yielding 54 for each group.

The study was approved by the Research Ethical Committee of the University of Sumer (EC/2018/8879). All the participants provided an informed consent before enrollment. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

1. Eligibility criteria

Fertile controls had a history of fathering a child in the last 2 years, with normal seminal fluid analysis findings and normal female fertility assessment. The patients included in the study had a history of infertility of 1 year or more without the use of contraception. OA was defined according to the World Health Organization (WHO) 2010 criteria [22]. Patients were excluded if they had genital infections, azoospermia, cryptorchidism, varicocele, testicular trauma or surgery, an endocrine disorder, systemic disease, relevant medications, smoking, alcohol, recent administration of antioxidants, and female factor infertility.

2. Semen analysis

Semen samples were collected by masturbation following a period of abstinence of 2–3 days. The semen sample was collected in a special container, followed by incubation at 37°C until semen liquefaction, and then semen analysis was performed within 1 hour following the WHO manual criteria (fifth edition, 2010) [22]. Two semen analyses were performed at baseline and after 3 months, and mean

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values were analyzed as the results of both analyses. All semen analysis tests were performed by the same investigator (ATA) to ensure data consistency.

3. Measurements of seminal CoQ10 concentrations

Seminal CoQ10 levels were measured using high-performance liquid chromatography (HPLC) using an ultraviolet (UV) detector at 275 nm and calculated using a published method [23]. Reverse-phase HPLC with UV detection using coenzyme Q9 as the internal standard was utilized to obtain seminal CoQ10 levels.

4. Seminal ROS measurements

Semen samples were centrifuged at 3,000 rpm for 5 minutes to obtain seminal plasma and then stored at –20°C. A manual method was used for ROS measurement as previously described by Venkatesh et al. [24]. To 400 µL of liquefied neat semen, 10 µL of luminol (5-amino-2,3,-dihydro-1,4-phthalazinedione; Sigma, St. Louis, MO, USA), prepared as 5 mM stock in dimethyl sulfoxide (DMSO), was added. Furthermore, 10 µL of 5 mM luminol in DMSO served as the blank, and 25 µL of H_2O_2 with 10 µL of luminol was used as a positive control. The luminol-dependent chemiluminescence served as an indicator of ROS levels.

5. Measurement of seminal TAC and CAT

TAC was estimated with a colorimetric method using a total antioxidant capacity assay kit (#E-BC-K136; Elabscience, Houston, TX, USA). Seminal plasma CAT activity was assessed using a CAT Assay Kit (#E-BC-K031, Elabscience) using the protocol recommended by the manufacturer.

6. Chromatin dispersion test

Sperm chromatin dispersion was tested using the Halosperm kit (Halotech DNA, Madrid, Spain). The test principle is that sperm with SDF do not exhibit the halo of dispersed DNA loops that is observed in sperm without SDF, after acid denaturation and removal of nuclear proteins. The nucleoids from spermatozoa with SDF show no or minimal dispersion halo. Bright-field microscopy with Diff-Quik staining was utilized to examine the halos. SDF, defined as the ratio (expressed as a percentage) of sperm with SDF to total spermatozoa, was calculated using a previously published method [25].

7. Hormonal assays

Venipuncture was performed to collect blood samples (5 mL) using clean, plain labeled tubes. The samples were allowed to clot, and centrifugation was performed at 3,000 rpm for 10 minutes for analysis of hormones. Serum FSH, LH, testosterone, and prolactin levels were estimated using enzyme-linked fluorescent assays with the Mini Vidas system (bioMérieux, Marcy l'Etoile, France).

8. Statistical analysis

IBM SPSS ver. 24 (IBM Corp., Armonk, NY, USA) was used for the data analysis. The results were expressed as mean ± standard deviation. Data normality was assessed using Kolmogorov-Smirnov test. The paired *t*-test was used to compare pre- and post-treatment values. The unpaired *t*-test was used to compare means between independent groups. Pearson correlation coefficients were calculated to evaluate the correlations of seminal fluid parameters with CoQ10 levels and SDF. A false discovery rate (FDR) correction for multiple comparisons was performed using the Benjamini–Hochberg procedure. A *p*-value lower than 0.05 was considered to indicate statistical significance.

Results

Semen parameters in patients were significantly lower than in the control group. Both CoQ10 and Centrum improved sperm concentration ($p < 0.05$), progressive motility ($p < 0.01$), and total motility ($p < 0.05$); however, sperm motility showed better improvement with Centrum therapy ($p < 0.05$) (Table 1).

Infertile patients showed poorer seminal antioxidant status than the fertile control group (Table 2). Seminal CoQ10 levels were significantly lower in infertile men than in controls and significantly increased following CoQ10 therapy (FDR $p < 0.01$). CoQ10 and Centrum therapy both reduced ROS and DNA fragmentation and improved TAC and CAT activity, but the reduction in ROS (FDR $p < 0.01$) and CAT (FDR $p < 0.001$) was significantly lower with Centrum therapy and the improvement of TAC (FDR $p < 0.01$) was significantly higher with CoQ10 treatment (Table 2).

Patients had higher FSH, LH, and prolactin and lower testosterone levels than controls. Centrum therapy resulted in a decrease in serum testosterone in patients ($p < 0.05$) (Figure 1). Correlations were also found between total sperm motility and SDF ($r = –0.51$, $p = 0.002$) and between total sperm motility and seminal CoQ10 levels ($r = 0.42$, $p = 0.007$) (Table 3).

Discussion

With an increasing body of evidence linking OS and SDF to male infertility, antioxidant supplementation has been recommended for the treatment of IMI [6]. Both individual and combined antioxidant treatments have been attempted in men with IMI [26]. Recent systematic reviews have examined several studies and reported that antioxidants exert beneficial effects on semen parameters, antioxidant status, and fertility potential in men with IMI [26-28]. However,

Table 1. Semen parameters in fertile and infertile men before and after administration of CoQ10 and Centrum

Varaible	Fertile control (n=58)	Patient before CoQ10 (n=65)	Patient after CoQ10	Patient before Centrum	Patient after Centrum
Age (yr)	36.4±15.2	33.6±14.1		32.4±13.6	
Infertility duration (yr)		6.4±5.2		7.1±6.4	
Volume (mL)	3.1±1.6	3.3±1.8	3.5±1.6	3.6±2.0	3.6±1.7
Concentration (million/mL)	50.4±27.3	8.9±5.1[c]	10.6±.6.4[a,c]	9.8±5.7[c]	11.8±7.0[a,c]
Progressive motility (%)	46.1±9.7	20.8±8.4[c]	25.7±12.5[b,c]	22.9±9.3[c]	30.2±13.6[b,c,d]
Total motility (%)	63.5±12.6	28.8±8.2[c]	35.1±14.6[a,c]	31.6±9.2[c]	41.4±16.2[a,c,d]
Normal morphology (%)	6.3±2.9	2.7±2.1[c]	3.3±1.6[c]	2.5±1.3[c]	3.7±1.6[c]

Values are presented as mean±standard deviation.
CoQ10, coenzyme Q10.
[a]vs. patients before CoQ10, $p<0.05$; [b]vs. patients before CoQ10, $p<0.01$; [c]vs. fertile control group, $p<0.001$; [d]vs. patients after CoQ10, $p<0.05$.

Table 2. Seminal plasma CoQ10, oxidative stress markers, and sperm DNA fragmentation levels in fertile and infertile men before and after administration of CoQ10 and Centrum

Variable	Fertile control	Patient before CoQ10	Patient after CoQ10	Patient before Centrum	Patient after Centrum
CoQ10 level (ng/mL)	56.2±38.5	41.6±29.8[d]	76.9±26.3[b,e]	38.9±27.6[d]	40.2±28.1[d,g]
ROS ($\times 10^4$ RLU/min/20 million spermatozoa)	0.07±0.03	3.52±1.29[e]	2.68±1.31[c,e]	2.8±0.96[e]	2.08±1.04[c,e,f]
TAC (mmol/L)	1.12±0.21	0.73±0.36[d]	0.92±0.4[b,d]	0.56±0.24[d]	0.73±0.3[c,d,f]
Catalase (U/mL)	12.45±2.49	8.42±2.21[e]	9.8±2.06[b,e]	6.72±1.75[e]	7.8±1.4[b,e,g]
Sperm DNA fragmentation (%)	13.2±3.8	35.2±6.4[e]	32.1±7.9[a,e]	28.3±5.1[e]	25.7±4.1[b,e,g]

CoQ10, coenzyme Q10; ROS, reactive oxygen species; TAC, total antioxidant capacity; FDR, false discovery rate.
[a]vs. patients before CoQ10, FDR $p<0.05$; [b]vs. patients before CoQ10, FDR $p<0.01$; [c]vs. patients before CoQ10, FDR $p<0.001$; [d]vs. fertile control group, FDR $p<0.01$; [e]vs. fertile control group, FDR $p<0.001$; [f]vs. patients after CoQ10, FDR $p<0.01$; [g]vs. patients after CoQ10, FDR $p<0.001$.

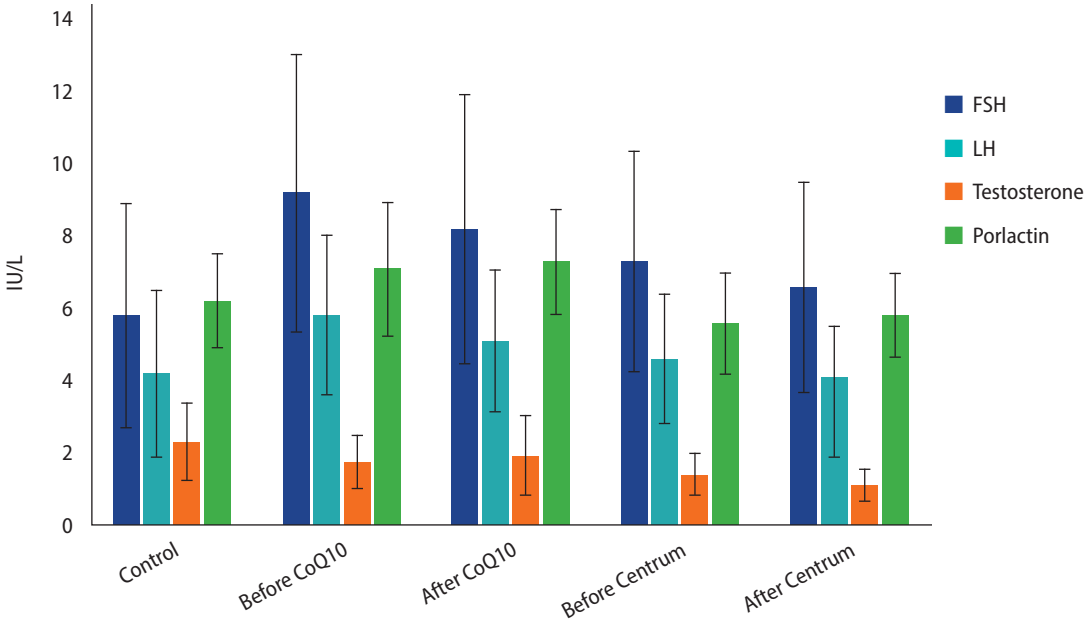

Figure 1. Hormones in fertile and infertile men before and after administration of coenzyme Q10 (CoQ10) and Centrum. FSH, follicle-stimulating hormone; LH, luteinizing hormone.

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Table 3. Correlations between SDF, CoQ10 levels, and semen parameters in infertile subjects after CoQ10 treatment

Variable	*r* (*p*-value)		
	Concentration	Total motility	Normal morphology
SDF	–0.07 (0.34)	–0.51 (0.002)	–0.14 (0.24)
CoQ10	0.23 (0.16)	0.42 (0.007)	0.11 (0.26)

r: Pearson correlation coefficient.
CoQ10, coenzyme Q10; SDF, sperm DNA fragmentation.

there is a lack of consensus on the type, dosing, target group, and the use of individual or combined antioxidants in IMI [27]. To our knowledge, this study is the first to compare the effects of CoQ10 and Centrum on semen parameters, seminal antioxidant capacity, and SDF in men with idiopathic OA.

In our study, both CoQ10 and Centrum improved sperm concentration, progressive motility, and total motility; however, sperm motility showed better improvement with Centrum therapy. A randomized, double-blind, placebo-controlled trial of 212 men with idiopathic oligoasthenoteratospermia (OAT) who received CoQ10 (300 mg/day) for 26 weeks reported improvements in sperm concentration and motility post-therapy [29]. Balercia et al. [21,30], in two studies of 82 men with idiopathic asthenospermia treated with CoQ10 (200 mg/day) for 6 months, also confirmed higher sperm progressive and total motility and an increase in seminal CoQ10 level after treatment. Furthermore, our recent systematic review and another systematic review including three randomized clinical trials in infertile men who received CoQ10 therapy confirmed improvements in sperm concentration and motility in these men, although there was no increment in pregnancy rates [31,32]. Another study, however, reported no improvement in semen parameters in men with idiopathic OAT following CoQ10 therapy [33].

We could not find studies on the effects of Centrum on semen parameters or antioxidant status or in comparison with the effects of CoQ10 therapy. However, many studies have tried combinations of multiple antioxidants in men with IMI and reported improvement in 1 or more semen parameters [34,35] and in the pregnancy rate [36]. One study reported no improvement in semen parameters or pregnancy rates after combined antioxidant treatment [37]. As expected, infertile men had lower semen parameters than fertile controls. The improvement in semen parameters observed in our study could be attributed to the antioxidant properties of CoQ10 and Centrum multivitamins, which counteract OS and its detrimental effects on sperm in men with idiopathic OA [6]. Nevertheless, a comparison with the results of the aforementioned studies is challenging due to the heterogeneity of design, antioxidants, doses, and treatment duration.

Seminal CoQ10 levels were significantly lower in infertile men than in controls and significantly increased following CoQ10 therapy. CoQ10 and Centrum therapy both reduced ROS and DNA fragmentation and improved TAC and CAT activity, but the reduction in ROS and SDF was significantly lower with Centrum therapy and the improvement of TAC and CAT was significantly higher with CoQ10 treatment. Our results are consistent with previous studies that demonstrated lower seminal antioxidant capacity [12] and higher SDF in infertile men than in controls [38]. Our previous studies on men with idiopathic OA and OAT treated with CoQ10 (200 mg/day) for 3 months demonstrated improvements in semen parameters, ROS, TAC, CAT, and GPx, as well as a reduction in SDF following CoQ10 therapy [13,18,39,40]. Our findings are consistent with other studies showing that CoQ10 therapy resulted in improvement in antioxidant capacity and reduced SDF levels in infertile men [13,41]. Our study also demonstrated a correlation between seminal CoQ10 and SDF levels and sperm total motility. This finding aligns with our previous studies, which have also shown correlations between CoQ10 levels, SDF, and sperm motility [13,18]. Other studies have also reported similar correlations in men with IMI [42,43]. We could not find studies on the effect of Centrum on SDF, but several studies have explored the impact of different combinations of antioxidants in IMI and reported reductions in SDF levels [44,19]. Other studies, however, reported no alterations in SDF levels after antioxidant treatment [45,46]. CoQ10 treatment in our study resulted in decreased FSH and LH levels, but this change was not statistically significant, and there was a reduction in serum testosterone with Centrum treatment. The reduction in serum testosterone after Centrum therapy was an unexpected finding, which might have been due to the direct effects of 1 or more of the 26 antioxidants in Centrum on seminiferous tubules. This possibility requires further investigation. A previous study of men with idiopathic OAT treated with CoQ10 (300 mg/day) reported reductions in FSH, LH, and inhibin levels [29].

The improvement in antioxidant capacity and reduction in SDF level detected in our study after CoQ10 and Centrum therapy could be due to lower antioxidant capacity in infertile men and the antioxidant properties of CoQ10 and Centrum multivitamins [12]. These properties may counteract OS, increase seminal antioxidant defense, and reduce OS-induced SDF and therefore may enhance fertility potential in men with idiopathic OA [14]. The greater improvement after Centrum therapy could have been due to the synergistic antioxidant action of the 26 combined antioxidants. Comparisons of the studies discussed above are also limited by the heterogeneity of studies' designs and the different combinations of antioxidants used. The correlations between semen parameters and antioxidant capacity and SDF may establish the foundation for the use of oral antioxidants, including CoQ10, in the treatment of infertile men with IMI and idiopathic OA to enhance their pregnancy outcomes [7]. Fur-

thermore, these measures could also be used as diagnostic biomarkers for male fertility and pregnancy outcomes. The limitations of our study include the small sample size and the lack of long-term follow-up, which meant that we could not report the pregnancy rate for the participants. Therefore, further large-scale studies are warranted to consolidate the findings of this study.

Both CoQ10 (200 mg/day) and Centrum (1 tablet/day) treatment for 3 months were effective in improving semen parameters, antioxidant capacity, and reducing SDF, but the improvement was greater with Centrum than with CoQ10. Therefore, Centrum combined antioxidants may provide more effective results than individual antioxidants such as CoQ10 in the treatment of infertile men with idiopathic OA due to the potential synergistic antioxidant action of the combined antioxidants. Furthermore, semen parameters, seminal antioxidant capacity, and SDF could be used as diagnostic biomarkers in men with IMI.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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Conceptualization: ATA. Data curation: ATA. Formal analysis: ATA. Methodology: ATA. Project administration: ATA. Visualization: ATA. Writing–original draft: ATA. Writing–review & editing: RS.

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Predictors of pregnancy and time to pregnancy in infertile men with idiopathic oligoasthenospermia pre- and post-coenzyme Q10 therapy

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Abstract

Different antioxidants including coenzyme Q10 (CoQ10) have been tried to treat idiopathic male infertility (IMI) with variable results. Therefore, this study aimed to determine the clinical and biochemical predictors of pregnancy outcome and time to pregnancy (TTP) in infertile men with idiopathic oligoasthenospermia (OA) pre- and post-CoQ10 therapy. This prospective controlled clinical study included 178 male patients with idiopathic OA and 84 fertile men (controls). Patients received 200 mg of oral CoQ10 once daily for 6 months. Demographics, semen parameters, seminal CoQ10 levels, reactive oxygen species (ROS) levels, total antioxidant capacity (TAC), catalase (CAT), glutathione peroxidase (GPx), sperm DNA fragmentation (SDF) and body mass index were measured and compared at baseline and after 6 months. All participants were followed up for another 18 months for pregnancy outcome and TTP. CoQ10 therapy for 6 months significantly improved semen parameters, antioxidant measures and reduced SDF. The pregnancy rate was 24.2% and TTP was 20.52 ± 6.72 months in patients as compared to 95.2% and 5.73 ± 6.65 months in fertile controls. After CoQ10 therapy, CoQ10 level, sperm concentration, motility and ROS were independent predictors of pregnancy outcome and CoQ10 level, male age, sperm concentration, motility, ROS and GPx were independent predictors of TTP in patients. In conclusion, CoQ10 therapy of 6 months is a potential treatment for men with idiopathic OA. CoQ10 level, male age, semen parameters, ROS and GPx could potentially be used as diagnostic biomarkers for male fertility and predictors for pregnancy outcome and TTP in these patients.

KEYWORDS
coenzyme Q10, idiopathic oligoasthenospermia, pregnancy, time to pregnancy

Correction added on 10 February 2022 after first online publication: Corrections have been made in this article.

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1 | INTRODUCTION

Infertility is defined as the failure to achieve pregnancy after 12 months of regular unprotected sexual intercourse (Ko et al., 2014). It affects around 8%–15% of couples within the reproductive age globally, with half of these cases are associated with male factor. Male infertility could be attributed to varicocele, genital tract infections, congenital abnormalities, endocrine disorders and genetic, immunological and systemic diseases as well as environmental factors (Elsheikh et al., 2015). Oligoasthenospermia (OA) is defined as a reduction in sperm concentration below 15 million/ml and sperm progressive motility below 32% or total motility below 40% according to World Health Organization (WHO) 2010 5th criteria W. H. O, (2010).

Approximately 25% of infertility cases is of idiopathic origin (Punab et al., 2017). Potential mechanisms for idiopathic male infertility (IMI) and idiopathic OA include genetic, epigenetic, posttranslational modifications, sperm DNA fragmentation (SDF) and oxidative stress (OS) (Santi et al., 2018). A low level of reactive oxygen species (ROS) is necessary for several physiological processes, including sperm capacitation, hyperactivation, acrosomal reaction and fertilization (Gulcin, 2020; Gülçin et al., 2012). However, the overproduction of ROS causes an imbalance between oxidants and antioxidants leading to OS. Sperm cells are sensitive to OS due to the presence of unsaturated fatty acids which makes them prone to lipid peroxidation (Agarwal et al., 2006; Kose & Gulcin, 2021; Köse et al., 2015). Oxidative stress has been linked to reduced sperm membrane fluidity, motility, vitality, fertilization potential as well as high SDF (Kao et al., 2008; Nowicka-Bauer & Nixon, 2020). Further, approximately 30%–80% of infertile men exhibits OS semen characteristics and, therefore, may serve as a potential biomarker of male fertility (Huang et al., 2018).

Another mechanism suggested for IMI is sperm DNA fragmentation (Selvam et al., 2020). Causes of SDF encompass extrinsic factors such as smoking, environmental toxins, radiation and chemotherapy as well as intrinsic factors such as defective germ cell maturation, leukocytes, abortive apoptosis and OS (Esteves et al., 2021). Elevated SDF has been associated with reduced sperm motility, recurrent abortions and reduced fertilization (Aktan et al., 2013; Alahmar et al., 2021). Additionally, SDF has been recently linked to increased incidence of genetic diseases, childhood malignancies and neurological disorders in offspring (Agarwal & Bui, 2017; Alahmar, 2019).

Seminal fluid is a major source of antioxidants that play key roles in protecting sperm from oxidative injury (Zini et al., 2009). The endogenous antioxidants include enzymatic antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione S-transferase (GST) and catalase (CAT), and non-enzymatic antioxidants including urate, carnitine, glutathione, coenzyme Q10 (CoQ10) and vitamins C and E (Nakamura et al., 2010). Oral antioxidants have been tried to improve semen parameters, antioxidant capacity, SDF and fertility potential of men with IMI (Ahmadi et al., 2016). The treatment of men with unexplained idiopathic infertility, however, remains a challenge as different medications have been tried individually or in combination with inconsistent results (Alahmar, 2018; Majzoub & Agarwal, 2018). Some studies have reported that antioxidant therapy may be beneficial and improve several sperm parameters (Alahmar, Calogero, Singh, et al., 2021; Alahmar & Sengupta, 2021). Other studies, on the contrary, reported no improvements in semen parameters (Ahmadi et al., 2016; Alahmar, 2018). Further, there is a lack of consensus on the type, dosing, duration of treatment, target patient groups and the use of individual or combination antioxidants (Majzoub et al., 2017).

Coenzyme Q10 is a component of the mitochondrial respiratory chain with antioxidant properties that counteract lipid peroxidation and OS (Showell et al., 2014). In healthy males, seminal fluid CoQ10 concentrations positively correlate with sperm concentration and motility (Alahmar, Calogero, Singh, et al., 2021). We and others have reported improvement in sperm concentration and motility following CoQ10 therapy (Alahmar, 2019; Alahmar, Calogero, Sengupta, et al., 2021; Safarinejad, 2009). Further, our recent meta-analysis (Vishvkarma et al., 2020) and another meta-analysis (Lafuente et al., 2013) of three randomized controlled trials confirmed improvement of semen parameters but not improvement of pregnancy rates. Other studies, however, demonstrated no improvement in one or more of the seminal fluid parameters following CoQ10 therapy (Imamovic Kumalic & Pinter, 2014).

Many previous clinical studies on the effect of CoQ10 therapy in men with IMI had semen parameters improvement but not pregnancy as a primary endpoint. Further, the results of these studies were limited by a small number of participants, heterogeneity of the patients' groups, a short period of follow-up and the lack of exploration of the predictors of pregnancy outcomes (Lafuente et al., 2013; Safarinejad, 2009). Additionally, data on the impact of CoQ10 therapy on seminal antioxidant capacity, SDF and pregnancy outcomes are limited. Therefore, this study aimed to determine the clinical, antioxidant and other biochemical predictors of pregnancy outcome and time to pregnancy (TTP) in infertile men with idiopathic OA following 6 months of coenzyme Q10 therapy and another 18 months of follow-up.

2 | MATERIALS AND METHODS

2.1 | Participants

In this prospective controlled clinical study, one hundred and seventy-eight patients with idiopathic OA and 84 fertile men (controls) were recruited at the Fertility Clinic, Babyl, Iraq, from September 2018 to February 2019. Eight patients and five controls dropped out of the study and, therefore, were excluded. The participants underwent comprehensive fertility assessment by fertility specialists at the Fertility Clinic at baseline as well as during follow-up visits. All patients received a daily dose of 200 mg of CoQ10 (as ubiquinol) (America Medic and Science AMS, WA, USA) as a single oral dose for 6 months (Balercia et al., 2009). The controls did not receive treatment and served as no treatment group. Clinical demographics, weight, height, body mass index (BMI), semen parameters, seminal CoQ10 level, ROS, TAC, GPx, CAT and SDF were measured

compared at baseline and after 6 months. All participants were followed up for another 18 months for pregnancy outcome and TTP and follow-up visits which were scheduled at 3-month intervals. Sample size calculation was performed using 80% power and 5% level of significance and was 72 for each group. Study approval was obtained from the University of Sumer local research ethical committee (EC/2018/8866/8876/8878/8879).

2.2 | Eligibility criteria

Patients had a history of infertility of at least one year in spite of regular unprotected intercourse and semen analysis shows OA. OA was defined according to the WHO 2010 (5th criteria) (W. H. O, 2010). Men with varicocele, genital infection, azoospermia, anatomical abnormalities, testicular injury or surgery, endocrine disease, renal, hepatic or other systemic illness, relevant medications, smoking, alcohol intake, recent antioxidant intake and the existence of female cause were excluded. Fertile controls enrolled in the study had a history of having had a child in the last 24 months, normal semen analysis, normal female fertility assessment and they were trying to get pregnant. All the participants provided informed consent before enrolment in the study.

2.3 | Semen analysis

Semen samples were collected by masturbation following abstinence of 2–3 days. A special wide-mouth container was used to collect semen, incubated at 37°C until semen was liquefied and then semen analysis was performed within an hour following the WHO manual criteria (5th edition, 2010) (W. H. O, 2010). Duplicate semen analyses were performed at baseline and after 6 months, and the average of the two values was used to analyse the results. The same investigator performed all semen analyses to optimize repeatability.

2.4 | Measurement of seminal CoQ10 concentrations

Semin CoQ10 level was measured using high-performance liquid chromatography (HPLC) using a UV detector at 275 nm and calculated using a published method (Li et al., 2006). Reversed-phase HPLC with UV detection using coenzyme Q9 as the internal standard are utilized to obtain seminal CoQ10 level.

2.5 | Seminal ROS measurement

Semen samples were centrifuged at 3000 rpm (1008 g) for 5 minutes to obtain seminal plasma and then were stored at −20°C. A manual method was used for ROS measurement as previously described by Venkatesh *et.al*. (Venkatesh et al., 2011). To 400 μl of liquefied neat semen, 10 μl of luminol (5-amino-2,3,-dihydro-1,4-phthalazinedione; Sigma), prepared as 5 mM stock in dimethyl sulfoxide (DMSO), was added. Ten microlitres of 5 mM luminol in DMSO served as blank. Twenty-five microlitres H_2O_2 with 10 μl luminol was used as a positive control. The luminol-dependent chemiluminescence served as an indicator of ROS levels.

2.6 | Measurement of seminal total antioxidant capacity (TAC), Glutathione peroxidase (GPx) and catalase (CAT) activity

TAC was estimated with a colorimetric method using the Total Antioxidant Capacity Assay Kit (#E-BC-K136, Elabscience, Texas, USA). Seminal plasma GPx activity was assessed using GPx Assay Kit (#E-BC-K096, Elabscience, Texas, USA), and seminal plasma CAT activity was assessed using CAT Assay Kit (#E-BC-K031, Elabscience, Texas, USA) using a colorimetric method and the protocol recommended by the manufacturer.

2.7 | Sperm chromatin dispersion test

Sperm chromatin dispersion test was applied using the Halosperm kit (Halotech DNA, S.L. Madrid, Spain). The test principle is that sperm with SDF do not exhibit the halo of dispersed DNA loops that is observed in sperm without SDF, after denaturation of acid and removal of nuclear proteins. The nucleoids from spermatozoa with SDF show no or minimal dispersion halo. Bright-field microscopy with Diff-Quik staining was utilized to examine the halos. SDF, defined as the percentage ratio of sperm with SDF to total spermatozoa, was calculated using a previously published method (Alahmar et al., 2021; Zaazaa et al., 2018).

2.8 | Statistical analysis

SPSS software (SPSS, v. 24, IBM, USA) was used for data analysis. Results were expressed as mean ± SD. Data normality was assessed using Shapiro–Wilk test and indicated a non-normal distribution ($p < 0.05$). Wilcoxon signed-rank test was used to compare pre- and post-treatment values in patients and controls. Mann–Whitney U test was used to compare means for independent groups (patients and controls at baseline). Chi-square test was used to compare proportions of family history, education and pregnancy outcome in patients and controls. Spearman's correlation coefficient was applied to find the relationships between seminal fluid parameters, antioxidant measures, CoQ10 level, SDF, age, BMI, pregnancy outcome and TTP in patients and controls. Univariate and multivariate logistic regressions were used to explore the predictors of pregnancy outcome in patients and controls (by estimating pre- and post-values for each group). Univariate and multivariate Cox regression tests

were used to perform survival analysis to estimate the predictors of TTP in patients and controls (by estimating pre- and post-values for each group). Kaplan–Meier curve was used to examine the survival analysis between family history and education with TTP in patients and controls. P-value lower than 0.05 was considered statistically significant.

3 | RESULTS

3.1 | CoQ10 therapy improved sperm parameters and antioxidant levels in infertile men as compared to baseline values with a pregnancy rate of 24.2%

Following 6 months of CoQ10 therapy, patients exhibited significant improvement in semen parameters and an increment in semen volume, concentration, total and progressive motility and normal morphology as compared to baseline (Table 1). The patients also demonstrated a significant increase in seminal antioxidant capacity and higher CoQ10 level, TAC, GPX, CAT, BMI and lower ROS and SDF after CoQ10 therapy. Family history was positive in 13.5%. The pregnancy rate was 24.2%, and TTP was 20.52 ± 6.72 months in the infertile patients' group. The controls, on the contrary, demonstrated higher total motility, CoQ10 level, TAC, ROS, SDF and BMI and lower progressive motility, normal morphology, GPx and CAT after 6 months as compared to baseline. The improvement, however, was mild and levels remained within the normal range. Family history was positive in 4.8% in the control group. The pregnancy rate in controls was 95.2%, and TTP was 5.73 ± 6.65 months. As expected, infertile men had lower semen parameters, antioxidant capacity, pregnancy rate and higher SDF and TTP as compared to fertile controls.

3.2 | Correlations between semen parameters, antioxidant measures, SDF and pregnancy outcome in patients and controls after 6 months

In patients, semen parameters (sperm concentration, progressive motility, total motility and normal morphology) correlated significantly with CoQ10 levels, antioxidant measures (ROS, TAC, GPx and CAT), SDF, BMI, female age, pregnancy rate and TTP after CoQ10 therapy (Table 2). Antioxidant measures correlated significantly with CoQ10 level, semen parameters, SDF, BMI and pregnancy measures. SDF correlated significantly with semen parameters, CoQ10 level, antioxidant measures, female age and pregnancy measures. Pregnancy rate and TTP correlated significantly with semen parameters, antioxidant measures, SDF and BMI. Controls, on the contrary, showed similar but weaker correlations between semen parameters and antioxidant measures, SDF, female age and BMI after 6 months of follow-up. Many of the correlations between antioxidant measures and SDF, female age, BMI and pregnancy measures were not statistically significant (Table 3).

3.3 | Predictors of pregnancy outcome in patients and controls (pre and post)

Using univariate regression analysis, factors associated with pregnancy outcome in patients before CoQ10 therapy were sperm concentration, progressive and total motility, CoQ10 level, ROS, GPx, CAT, SDF, BMI and patient education (Table 4). After CoQ10 therapy, factors in patients were sperm concentration, progressive and total motility, normal morphology, TAC, CAT, SDF, BMI and patient education. In the multivariate logistic regression model, factors that independently predicted pregnancy outcome in patients before CoQ10 therapy were sperm progressive motility, CoQ10 level and patients' education (Table 5). Post-CoQ10 therapy, the independent factors in patients were CoQ10 level, sperm concentration, total motility and ROS. Univariate regression analysis for pregnancy outcome in controls at baseline and after 6 months showed that none of the variables of the study was associated with pregnancy outcome (Table 6). Using a multivariate regression model, factors that independently predicted pregnancy outcome in controls at baseline were male age and total motility. Following 6 months, none of the variables predicted pregnancy outcomes (Table 5).

3.4 | Predictors of time to pregnancy in patients and controls (pre and post)

Univariate Cox regression for TTP in patients before CoQ10 therapy demonstrated that factors associated with TTP were sperm concentration, progressive motility, total motility, CoQ10 level, ROS, GPx, CAT, SDF, BMI and patients' education all in the condition before CoQ10 treatment (Table 7). Following CoQ10 therapy, factors that predicted TTP in patients were sperm concentration, progressive and total motility, CAT, SDF, BMI and patient education. Additionally, normal morphology and TAC were also associated with TTP. Using multivariate Cox regression, factors that independently predicted TTP in patients were age, sperm concentration, progressive motility, CoQ10 level and patient education (Table 8). After CoQ10 therapy, independent predictors of TTP were male age, sperm concentration, total motility, CoQ10 level, ROS and GPx. Kaplan–Meier curve for patients showed that patient education was associated with TTP ($p < 0.001$). Kaplan–Meier curve for family history of male infertility versus TTP in patients demonstrated no association between family history and TTP (P value = 0.67) (Figure 1-A and 1-C).

Univariate Cox regression in controls at baseline showed that male age and education were the only predictors of TTP in this group (Table 9). After 6 months, the predictors of TTP were male age, sperm concentration, normal morphology, SDF and education. Multivariate Cox regression in controls at baseline demonstrated that the independent predictors of TTP were sperm normal morphology, ROS, GPx, CAT and education (Table 8). After 6 months, the independent predictors in controls were male age, sperm concentration, progressive motility, ROS and GPx. Kaplan–Meier curve for controls demonstrated that education was associated with TTP

TABLE 1 Clinical, seminal and biochemical characteristics of patients and controls at baseline and after CoQ10 therapy

Parameter	Controls (No = 84)				Patients (N = 178)				
	Pre	Post	Per cent change	P value Pre vs post	Pre	Post	Per cent change	P value Pre vs post	P value patient vs controls baseline
Male age (year)	31.69 ± 7.79	32.19 ± 7.7	1.58	0.001	29.46 ± 6.49	29.69 ± 6.4	0.78	0.06	0.06
Infertility duration (year)					6.38 ± 3.55				
Volume (ml)	3.00 ± .51	3.28 ± .060	9.33	0.058	2.88 ± 1.14	3.06 ± 0.28	6.25	0.001	0.37
Concentration ($*10^6$/ml)	45.16 ± 21.13	47.61 ± 26.43	5.43	0.167	9.41 ± 3.53	9.70 ± 3.73	3.08	0.001	0.001
Progressive motility (%)	46.88 ± 7.80	42.32 ± 7.07	−9.73	0.001	21.53 ± 6.89	28.84 ± 8.88	33.95	0.001	0.001
Total motility (%)	65.66 ± 10.91	72.26 ± 12.01	10.05	0.001	29.03 ± 8.09	37.33 ± 11.41	28.59	0.001	0.001
Normal morphology (%)	44.08 ± 7.47	39.64 ± 6.83	−10.07	0.001	36.98 ± 11.58	39.75 ± 6.72	7.49	0.047	0.001
CoQ10 level (ng/ml)	60.62 ± 28.45	66.30 ± 31.48	9.37	0.000	44.28 ± 22.11	79.57 ± 17.46	79.70	0.001	0.001
ROS (×104 RLU/min/20million spermatozoa)	0.10 ± 0.05	0.162 ± 0.018	62.00	0.001	4.62 ± 1.34	4.03 ± 1.16	−12.77	0.001	0.001
TAC (mmol/L)	1.80 ± 0.20	1.98 ± 0.22	10.00	0.001	0.89 ± 0.40	1.16 ± 0.45	30.34	0.001	0.001
GPx (U/ml)	0.65 ± 0.07	0.58 ± 0.06	−10.77	0.001	0.23 ± 0.06	0.39 ± .044	69.57	0.001	0.001
CAT (U/ml)	14.72 ± 2.40	13.22 ± 2.16	−10.19	0.001	10.25 ± 1.59	12.34 ± 1.69	20.39	0.001	0.001
SDF (%)	15.38 ± 3.13	16.83 ± 3.63	9.43	0.001	34.78 ± 5.57	32.71 ± 7.19	−5.95	0.001	0.001
Female age (year)	25.54 ± 6.09	26.04 ± 6.09	1.96	0.001	23.36 ± 5.03	23.86 ± 5.03	2.14	0.01	0.002
BMI (Kg/m^2)	26.32 ± 5.43	27.63 ± 5.70	4.98	0.001	27.70 ± 4.82	29.92 ± 5.2	8.01	0.04	0.03
Family history Of male infertility	Yes 4 (4.8%) No 80 (95.2%)				24 (13.5%), 154 (86.5%)			0.03	0.03

(Continues)

TABLE 1 (Continued)

Parameter	Controls (No = 84)				Patients (N = 178)				
	Pre	Post	Per cent change	P value Pre vs post	Pre	Post	Per cent change	P value Pre vs post	P value patient vs controls baseline
Education	Primary 18 (21.4%) Secondary 45 (53.6%) Tertiary 21 (25.0%)				Primary 127 (71.3%) Secondary 31 (17.4%) Tertiary 19 (10.7%)			0.001	0.001
Pregnancy (%)	Yes 80 (95.2%) No 4 (4.8%)				Yes 43 (24.2%) No 135 (75.8%)			0.001	0.001
TTP (month)	5.73 ± 6.65				20.52 ± 6.72			0.001	0.001

Abbreviations: Statistical tests: Wilcoxon signed-rank test (for dependent samples), Mann–Whitney U (for independent samples), Chi-square test to compare proportions.
ROS, reactive oxygen species; TAC, total antioxidant capacity; GPx, glutathione peroxidase; CAT, catalase; SDF, sperm DNA fragmentation; CoQ10, Coenzyme Q10; BMI, Body Mass Index; TTP: Time To Pregnancy.

($p < 0.01$). Kaplan–Meier curve for family history of male infertility versus TTP in controls demonstrated no association between family history and TTP (P value = 0.88) (Figure 1-B and 1-D).

4 | DISCUSSION

Men with IMI represent a real challenge in medical practice as the exact mechanisms underlying semen abnormalities are unknown. Further, several therapies have been tried to improve semen measures and men's fertility potential with variable results (Imamovic Kumalic & Pinter, 2014). The rationale for some of these therapeutics such as oral antioxidants including CoQ10 is based on the proposed association between IMI and OS and SDF and lower seminal antioxidant capacity in infertile men (Agarwal et al., 2019). Data on the predictors of pregnancy and time to pregnancy in men with idiopathic OA before and after receiving oral antioxidants are limited. To our knowledge, this study is the first study to explore these predictors in men with idiopathic OA before and after CoQ10 therapy.

Our study demonstrated a beneficial effect for CoQ10 therapy of 6 months on improving semen parameters and antioxidant capacity in men with idiopathic OA as compared to fertile controls.

The main improvement was in sperm concentration, motility, normal morphology, markers of antioxidant capacity and reduction in SDF following CoQ10 treatment. Our findings are consistent with previous studies which have reported similar improvement in men with IMI (Balercia et al., 2009; Safarinejad, 2009). In a randomized clinical trial on 228 men with idiopathic OA, treatment with CoQ10 (200 mg/day) was associated with improvement in semen parameters, and these parameters also correlated with antioxidant capacity (Safarinejad et al., 2012). Another clinical trial that involved treatment with CoQ10 (200 mg/day) for 3 months in men with idiopathic oligoasthenoteratospermia (OAT) reported an increment in sperm motility, CoQ10, CAT and SOD (Nadjarzadeh et al., 2014). Further, a randomized double-blind placebo-controlled study observed an increase in forward and total motility after 6 months of CoQ10 treatment (Balercia, 2004), which may suggest that a longer treatment regimen may be more effective in improving sperm parameters. Our previous studies have also demonstrated a beneficial effect for CoQ10 on sperm concentration, motility as well as antioxidant capacity(Alahmar, 2019; Alahmar, Calogero, et al., ; Alahmar & Sengupta,). However, in one RCT in men with idiopathic OAT who received CoQ10 for 3 months, there was no improvement in semen parameters following CoQ10 therapy (Nadjarzadeh et al., 2011). The control group also showed mild improvement in semen parameters and antioxidant capacity and correlations between semen parameters and other study variables, but these correlations were weak correlations. The enhancement of semen parameters and antioxidant capacity observed in our study could be attributed to higher CoQ10 level, the antioxidant properties of CoQ10 and its role in mitochondrial chain reaction kinetic, higher levels of seminal antioxidant which counteract OS as well as the longer duration of treatment as compared to shorter periods in other studies.

TABLE 2 Correlations between semen parameters, antioxidants and time to pregnancy in patients post-CoQ10 therapy

	Male age	Volume	Concentration	Progressive motility	Total motility	Normal morphology	CoQ10 level	ROS	TAC	GPx	CAT	SDF	Female age	BMI	TTP	Pregnancy
	r P value	r P value	r P value	r P value	r P value	r P value	r P value	r P value	r P value	r P value	r P value	r P value	r P value	r P value	r P value	r P value
Male age		NS	−0.20 0.007	−0.42 0.000	−0.19 0.01	0.21 0.003	NS	NS	NS	NS	−0.23 0.002	0.42 0.000	0.97 0.000	0.35 0.000	NS	NS
Volume	NS		NS	NS	NS	0.32 0.000	0.22 0.002	0.36 0.000	NS	0.69 0.000	NS	NS	NS	NS	NS	NS
Sperm concentration	−0.20 0.007	NS		0.38 0.000	0.26 0.000	−0.41 0.000	0.20 0.006	0.16 0.02	0.35 0.000	NS	0.60 0.000	−0.38 0.000	−0.20 0.007	−0.18 0.01	−0.65 0.000	−0.63 0.000
Progressive motility	−0.42 0.000	NS	0.38 0.000		0.44 0.000	NS	0.44 0.000	0.42 0.000	0.41 0.000	NS	0.61 0.000	−0.99 0.000	−0.39 0.000	−0.71 0.000	−0.35 0.000	−0.34 0.000
Total motility	−0.19 0.01	NS	0.26 0.000	0.44 0.000		NS	0.46 0.000	0.28 0.000	0.47 0.000	NS	0.46 0.000	−0.43 0.000	−0.16 0.03	−0.39 0.000	−0.42 0.000	−0.39 0.000
Normal morphology	0.21 0.003	0.32 0.000	−0.41 0.000	NS	NS		0.23 0.000	0.32 0.000	NS	0.23 0.000	NS	0.14 0.04	0.21 0.003	NS	0.34 0.000	0.33 0.000
CoQ10 level	NS	0.22 0.002	0.20 0.006	0.44 0.000	0.46 0.000	0.23 0.000		0.73 0.000	0.61 0.000	0.38 0.000	0.52 0.000	−0.43 0.000	NS	−0.32 0.000	NS	NS
ROS	NS	0.36 0.000	0.16 0.02	0.42 0.000	0.28 0.000	0.32 0.000	0.73 0.000		0.38 0.000	0.22 0.003	0.59 0.000	−0.41 0.000	NS	−0.25 0.001	−0.17 0.02	0.17 0.02
TAC	NS	NS	0.35 0.000	0.41 0.000	0.47 0.000	NS	0.61 0.000	0.38 0.000		0.17 0.01	0.66 0.000	−0.41 0.000	NS	−0.46 0.000	−0.31 0.000	−0.28 0.000
GPx	NS	0.69 0.000	NS	NS	NS	0.23 0.000	0.38 0.000	0.22 0.003	0.17 0.01		NS	NS	NS	NS	NS	NS
CAT	−0.23 0.002	NS	0.60 0.000	0.61 0.000	0.46 0.000	NS	0.52 0.000	0.59 0.000	0.66 0.000	NS		−0.61 0.000	−0.21 0.004	−0.43 0.000	−0.49 0.000	−0.46 0.000
SDF	0.42 0.000	NS	−0.38 0.000	−0.99 0.000	−0.43 0.000	0.14 0.04	−0.43 0.000	−0.41 0.000	−0.41 0.000	NS	−0.61 0.000		0.39 0.000	0.72 0.000	0.35 0.000	0.35 0.000
Female age	0.97 0.000	0.97 0.000	−0.20 0.007	−0.39 0.000	−0.16 0.03	0.21 0.003	NS	NS	NS	NS	−0.21 0.004	0.39 0.000		0.32 0.000	NS	NS
BMI	0.35 0.000	NS	−0.18 0.01	−0.71 0.000	−0.39 0.000	NS	−0.32 0.000	−0.25 0.001	−0.46 0.000	NS	−0.43 0.000	0.72 0.000	0.32 0.000		0.25 0.001	0.21 0.004
TTP	NS	NS	−0.65 0.000	−0.35 0.000	−0.42 0.000	0.34 0.000	NS	−0.17 0.02	−0.31 0.000	−0.49 0.000	NS	0.35 0.000	NS	0.25 0.001		0.95 0.000
Pregnancy	NS	NS	−0.63 0.000	−0.34 0.000	−0.39 0.000	0.33 0.000	NS	0.17 0.02	−0.28 0.000	NS	−0.46 0.000	0.35 0.000	NS	0.21 0.004	0.95 0.000	

Abbreviations: r, Spearman correlation coefficient; ROS, reactive oxygen species; TAC, total antioxidant capacity; GPx, glutathione peroxidase; CAT, catalase; SDF, sperm DNA fragmentation; BMI, body mass index; TTP, time to pregnancy; CoQ10, Coenzyme Q10; NS, non-significant.

TABLE 3 Correlations between semen parameters, antioxidants and time to pregnancy in controls post-CoQ10 therapy

	Male age	Volume	Concentration	Progressive motility	Total motility	Normal morphology	CoQ10 level
	r P value	r P value	r P value	r P value	r P value	r P value	r P value
Male age		0.27 0.01	0.33 0.002	0.22 0.037	0.23 0.031	−0.38 0.000	0.25 0.01
Volume	0.27 0.01		NS	NS	NS	NS	NS
Sperm concentration	0.33 0.002	NS		0.21 0.04	NS	NS	NS
Progressive motility	0.22 0.03	NS	0.21 0.04		0.98 0.000	NS	0.90 0.000
Total motility	0.23 0.031	NS	NS	0.98 0.000		NS	0.90 0.000
Normal morphology	−0.38 0.000	NS	NS	NS	NS		NS
CoQ10 level	0.25 0.019	NS	NS	0.90 0.000	0.90 0.000	NS	
ROS	NS	0.25 0.02	0.27 0.01	0.79 0.000	0.79 0.000	0.21 0.04	0.58 0.000
TAC	NS	0.25 0.01	0.27 0.01	0.79 0.000	0.79 0.000	0.22 0.04	0.58 0.000
GPx	NS	0.23 0.03	0.31 0.004	0.72 0.000	0.74 0.000	NS	0.49 0.000
CAT	0.24 0.02	NS	NS	0.88 0.000	0.88 0.000	NS	0.86 0.000
SDF	NS	NS	−0.25 0.02	−0.41 0.000	−0.31 0.000	NS	−0.59 0.000
Female age	0.99 0.000	0.26 0.01	0.32 0.003	0.21 0.04	0.22 0.04	−0.39 0.000	0.25 0.02
BMI	−0.29 0.006	NS	−0.29 0.006	−0.86 0.000	−0.84 0.000	NS	−0.85 0.000
TTP	−0.31 0.000	NS	−0.70 0.000	NS	NS	0.31 0.004	NS
Pregnancy	NS	NS	−0.34 0.001	NS	NS	0.21 0.04	NS

Abbreviations: r, Spearman correlation coefficient; ROS, reactive oxygen species; TAC, total antioxidant capacity; GPx, glutathione peroxidase; CAT, c SDF, sperm DNA fragmentation; BMI, body mass index, TTP, time to pregnancy; CoQ10, Coenzyme Q10; NS, non-significant.

We have also identified higher SDF in infertile patients and significant correlations between semen parameters and antioxidant measures, SDF, BMI and pregnancy outcomes. These correlations were stronger in patients with IMI as compared to fertile controls.

Our findings are consistent with previous studies which reported correlations between semen parameters and antioxidant measures such as CAT, TAC, GPx, ROS and seminal CoQ10 level (Alahmar & Sengupta, ; Nadjarzadeh et al., 2014). High SDF level has been observed in infertile men and correlated with CoQ10 level and semen parameters (Alahmar, Calogero, Sengupta, et al., 2021). These findings are supported by the observations of reduced antioxidant capacity and higher SDF levels among infertile men (Huang et al., 2018; Safarinejad, 2012). Further, CoQ10 therapy resulted in improvement in antioxidant capacity and reduced SDF levels (Alahmar, Calogero, Sengupta, et al., 2021; Kumar & Sharma, 2010). Obesity and high BMI also correlate with semen parameters, OS and high SDF levels among infertile men (Dubeux et al., 2016). Correlation of pregnancy rate with semen parameters and antioxidant capacity have been reported previously as well as an increase in pregnancy rate following antioxidant treatment in infertile men (Huang et al., 2018). A study reported that men with elevated seminal ROS levels have a sevenfold decrease in conception rates when compared to men having low ROS (Aitken et al., 1991). Male and female age, as well as BMI, may reduce semen parameters and clinical pregnancy rate (Dubeux et al., 2016). The correlations between semen parameters and antioxidant capacity and SDF may establish the foundation for the use of oral antioxidants including CoQ10 in the treatment of infertile men with IMI and idiopathic OA to enhance their pregnancy outcomes.

ROS	TAC	GPx	CAT	SDF	Female age	BMI	TTP	Pregnancy
r P value	r P value	r P value	r P value	r P value	r P value	r P value	r P value	r P value
NS	NS	NS	0.24 0.02	NS	0.99 0.000	−0.29 0.006	−.031 0.004	NS
0.25 0.02	0.25 0.01	0.23 0.03	NS	NS	0.26 0.01	NS	NS	NS
0.27 0.01	0.27 0.01	0.31 0.004	NS	−0.25 0.02	0.32 0.003	−0.29 0.006	−.070 0.000	−0.34 0.001
0.79 0.000	0.79 0.000	0.72 0.000	0.88 0.000	−0.41 0.000	0.21 0.04	−0.86 0.000	NS	NS
0.79 0.000	0.79 0.000	0.74 0.000	0.88 0.000	−0.31.004	0.22 0.04	−0.84 0.000	NS	NS
0.21 0.04	0.22 0.04	NS	NS	NS	−0.39 0.000	NS	0.31 0.004	0.21 0.04
0.58 0.000	0.58 0.000	0.49 0.000	0.86 0.000	−0.59 0.000	0.25	−0.85	NS	NS
	0.99 0.000	0.98 0.000	0.74 0.000	NS	NS	−0.62 0.000	NS	NS
0.99 0.000		0.98 0.000	0.75 0.000	NS	NS	−0.62 0.000	NS	NS
0.98 0.000	0.98 0.000		0.67 0.000	NS	NS	−0.54 0.000	NS	NS
0.74 0.000	0.75 0.000	0.67 0.000		−.36 0.001	0.23 0.03	−0.78 0.000	NS	NS
NS	NS	NS	−0.36 0.001		NS	0.45 0.000	0.29 0.006	NS
NS	NS	NS	0.23 0.03*	NS		−0.29 0.007	−0.29 0.006	NS
−0.62 0.000	−0.62 0.000	−0.54 0.000	−0.78 0.000	0.45 0.000	−0.29 0.007		0.23 0.03	NS
NS	NS	NS	NS	0.29 0.006	−0.29 0.006	0.23 0.03		0.37 0.000
NS	NS	NS	NS	NS	NS	NS	0.37 0.000	

Further, these measures could be also used as diagnostic biomarkers for male fertility and pregnancy outcome.

The pregnancy rate in patients in the current study was 24.2%, and TTP was 20.52 ± 6.72 months following 6 months of CoQ10 therapy and another 18 months of follow-up. We have also identified many independent predictors for pregnancy and TTP.

Our results are consistent with the results of an uncontrolled study in men with idiopathic OAT treated with CoQ10 300 mg twice daily for 12 months that reported a pregnancy rate of 34.1% and time to pregnancy of 8.4 ± 4.7 months (Safarinejad, 2012). Another RCT in men with IMI reported a pregnancy rate of 10% in patients following CoQ10 therapy (200 mg/day) for 6 months and a period of follow-up of 9 months (Balercia et al., 2009). In contrast, a systematic review and meta-analysis that looked at several studies that supplemented infertile men with CoQ10 did not observe an increase in pregnancy rates (Lafuente et al., 2013). Although the findings of this meta-analysis are in contrast to our study and others, the number of events included in the meta-analysis is relatively small, and both live births and pregnancy rates were not the primary outcomes of the included trials. The high pregnancy rate in men with idiopathic OA after CoQ10 therapy could be attributed to improvement in semen parameters, antioxidant capacity and reduction in OS and SDF and, therefore, enhanced fertility potential in these patients.

In multivariate logistic regression, factors that independently predicted pregnancy in patients before and after CoQ10 therapy in our study were CoQ10 level and sperm motility. Additional factors that independently predicted pregnancy post-CoQ10 therapy were sperm concentration and ROS. Our results are in agreement

TABLE 4 Logistic regression analysis for predictors of pregnancy in patients

	Before CoQ10 therapy			After CoQ10 therapy		
	OR	95% CI	P value	OR	95% CI	P value
Male age	0.95	0.9–1.01	0.1	0.95	0.9–1.01	0.1
Infertility duration	0.96	0.87–1.06	0.53	0.85	0.8–1.03	0.71
Volume	0.85	0.62–1.16	0.31	0.46	0.13–1.57	0.21
Concentration	1.15	1.04–1.27	0.006	1.75	1.47–2.08	0.001
Progressive motility	1.12	1.06–1.19	0.001	1.08	1.03–1.13	0.001
Total motility	1.09	1.05–1.15	0.001	1.1	1.05–1.14	0.001
Normal morphology	1.02	0.99–1.05	0.17	0.83	0.77–0.9	0.001
CoQ10 level	1.03	1.01–1.04	0.001	1.01	0.99–1.03	0.22
ROS	0.61	0.46–0.81	0.001	1.32	0.97–1.79	0.07
TAC	1.64	0.72–3.7	0.23	3.6	1.6–8.1	0.001
GPx	0.001	0.001–0.44	0.03	0.33	0.01–7.32	0.77
CAT	1.25	1.01–1.56	0.04	1.97	1.51–2.5	0.001
SDF	0.93	0.87–0.99	0.02	0.9	0.86–0.95	0.001
Female age	0.93	0.86–1.01	0.06	0.93	0.87–1.01	0.06
BMI	0.92	0.86–0.99	0.03	0.9	0.87–0.99	0.03
Family history	1.24	0.43–3.5	0.68	1.24	0.4–3.5	0.68
Education	3.3	2.01–5.5	0.001	3.3	2.01–5.5	0.001

Abbreviations: OR, odds ratio; ROS, reactive oxygen species; TAC, total antioxidant capacity; GPx, glutathione peroxidase; CAT, catalase; SDF, sperm DNA fragmentation; BMI, body mass index; CoQ10, Coenzyme Q10.

TABLE 5 Multivariate Logistic regression analysis for predictors of pregnancy in patients and controls

Controls				Patients			
Baseline	OR	After 6 months	OR	Baseline	OR	After CoQ10 therapy	OR
Male age	6.9*			Progressive motility	1.77**	Sperm Concentration***	1.55
Total motility	0.88*			CoQ10	0.87**	Total motility**	1.09
				Education	3.9***	CoQ10*	0.93
						ROS**	2.7

Abbreviations: OR, odds ratio; ROS, reactive oxygen species; CoQ10, Coenzyme Q10.
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

with previous studies which showed an association between sperm concentration, motility, normal morphology and pregnancy outcome (Aboutorabi et al., 2018; Jedrzejczak et al., 2008). Semen analysis and semen parameters, however, have limitations as WHO reference values of semen analysis were obtained from fertile couples, unequal distribution of population and inability to assess sperm function and fertilization (Agarwal et al., 2018). Therefore, additional biomarkers of sperm function and male fertility are essential. In our study, antioxidant measures also correlated and predicted pregnancy. Studies have reported lower levels of antioxidants in infertile men (Huang et al., 2018) as well as higher pregnancy rates following oral antioxidant therapy including CoQ10 (Ahmadi et al., 2016; Majzoub & Agarwal, 2018). Our previous studies have also demonstrated lower antioxidant measures and higher SDF in infertile men with idiopathic OA or OAT, and these abnormalities were ameliorated with CoQ10 therapy (Alahmar, Calogero, Sengupta, et al., 2021; Alahmar, Calogero, Singh, et al., 2021). High levels of SDF have been linked to IMI, abnormal semen parameters, pregnancy loss and poor fertilization (Arafa et al., 2020). Further, different cut-off values from 4% to 56% have been proposed for SDF prediction of pregnancy in infertile men (Agarwal et al., 2020). Obesity and high BMI have been associated with IMI, poor semen parameters, OS and reduced fertilization and pregnancy rates (Palmer et al., 2012). Our findings suggest that CoQ10 level, sperm motility and ROS could be diagnostic biomarkers for male fertility as well as predictors of pregnancy outcome in men with idiopathic OA with CoQ10 therapy.

In multivariate Cox regression, factors that independently predicted TTP in patients before and after CoQ10 treatment were male age, sperm concentration, sperm motility and CoQ10 level. Additional factors that predicted TTP post-therapy were sperm concentration, ROS and GPx. Our results are consistent with a follow-up study on 501 couples that showed longer TTP and lower fecundability odds

TABLE 6 Logistic regression analysis for predictors of pregnancy in controls

	Baseline			After 6 months		
	OR	95% CI	P value	OR	95% CI	P value
Male age	1.02	0.89–1.17	0.7	1.02	0.89–1.17	0.7
Volume	0.49	0.06–3.6	0.49	2.1	0.37–12.1	0.38
Concentration	1.02	0.96–1.08	0.42	1.45	0.45–4.65	0.53
Progressive motility	0.92	0.8–1.05	0.23	0.91	0.78–1.05	0.21
Total motility	0.94	0.85–1.03	0.19	0.94	0.87–1.03	0.2
Normal morphology	1.02	0.87–1.14	0.98	0.85	0.72–1.02	0.08
CoQ10 level	0.97	0.93–1.01	0.16	0.97	0.94–1.01	0.15
ROS	1882.4	0.001–13700	0.17	0.68	0.01–1.15	0.69
TAC	2.6	0.01–397.9	0.7	2.4	0.02–2.8	0.6
GPx	82.5	0.001–26882	0.56	0.05	0.01–1.3	0.47
CAT	0.74	0.48–1.14	0.18	0.72	0.44–1.16	0.17
SDF	1.01	0.85–1.2	0.85	0.87	0.65–1.16	0.35
Female Age	1.01	0.85–1.2	0.85	1.01	0.85–1.2	0.85
BMI	1.1	0.91–1.34	0.29	1.1	0.9–1.3	0.29
Family history	0.001	0.001–1.1	0.99	0.1	0.05–1.1	0.9
Education	3.7	0.68–20.9	0.12	3.7	0.68–20.9	0.12

Abbreviations: OR, odds ratio; ROS, reactive oxygen species; TAC, total antioxidant capacity; GPx, glutathione peroxidase; CAT, catalase; SDF, sperm DNA fragmentation; BMI, body mass index; CoQ10, Coenzyme Q10.

TABLE 7 Univariate Cox regression analysis for predictors of time to pregnancy in patients

	Before CoQ10 therapy			After CoQ10 therapy		
	HR	95% CI	P value	HR	95% CI	P value
Male age	0.96	0.91–1.01	0.12	0.96	0.91–1.01	0.12
Infertility duration	0.97	0.89–1.06	0.59	0.88	0.81–1.01	0.64
Volume	0.9	0.68–1.18	0.45	0.47	0.16–1.38	0.17
Concentration	1.13	1.04–1.24	0.003	1.5	1.4–1.7	0.001
Progressive motility	1.11	1.06–1.17	0.001	1.07	1.03–1.12	0.001
Total motility	1.09	1.04–1.13	0.001	1.09	1.05–1.13	0.001
Normal morphology	1.02	0.99–1.04	0.16	0.84	0.79–0.9	0.001
CoQ10 level	1.03	1.01–1.04	0.001	1.01	0.99–1.02	0.15
ROS	0.63	0.49–0.8	0.001	1.3	0.99–1.7	0.05
TAC	1.69	0.83–3.41	0.14	3.4	1.7–6.8	0.001
GPx	0.0005	0.001–0.005	0.03	0.3	0.01–266.6	0.75
CAT	1.25	1.02–1.51	0.02	1.83	1.5–2.23	0.001
SDF	0.93	0.88–0.98	0.01	0.91	0.87–0.95	0.001
Female age	0.94	0.88–1.01	0.08	0.94	0.88–1.00	0.08
BMI	0.92	0.87–0.98	0.01	0.93	0.99–0.98	0.01
Family history	1.2	0.47–3.08	0.68	1.2	0.47–3.08	0.68
Education	2.5	1.7–3.5	0.001	2.51	1.7–3.5	0.001

Abbreviations: OR, odds ratio; ROS, reactive oxygen species; TAC, total antioxidant capacity; GPx, glutathione peroxidase; CAT, catalase; SDF, sperm DNA fragmentation; BMI, body mass index; CoQ10, Coenzyme Q10.

ratios (FORs) were associated with normal sperm morphology, male age and female BMI (Buck Louis et al., 2014). Further, a multicentre study demonstrated that sperm concentration, normal morphology and multiple anomalies index (MAI) can predict pregnancy and TTP among infertile couples (Slama et al., 2002). Elevated ROS can be associated with a sevenfold decrease in pregnancy rate (Aitken et al.,

TABLE 8 Multivariate Cox regression analysis for predictors of time to pregnancy in patients and controls

Controls				Patients			
Baseline	**HR**	**After 6 months**	**HR**	**Baseline**	**HR**	**After CoQ10 therapy**	**HR**
Normal morphology	0.96*	Male age	1.04*	Male age	1.006*	Male age	1.05*
ROS	0.001*	Sperm Concentration	0.66***	Sperm Concentration	1.13*	Sperm Concentration	1.46***
GPx	44392**	Progressive motility	1.03*	Progressive motility	1.62***	Total motility	1.06*
CAT	0.59**	ROS	1.833**	CoQ10	0.9**	CoQ10	0.94**
Education	1.54*	GPx	0.001*	Education	3.14***	ROS	1.96**
						GPx	761837*

Abbreviations: HR, hazard ratio; ROS, reactive oxygen species; GPx, glutathione peroxidase; CAT, catalase; CoQ10, Coenzyme Q10.
*$p < 0.05$,; **$p < 0.01$,; ***$p < 0.001$.

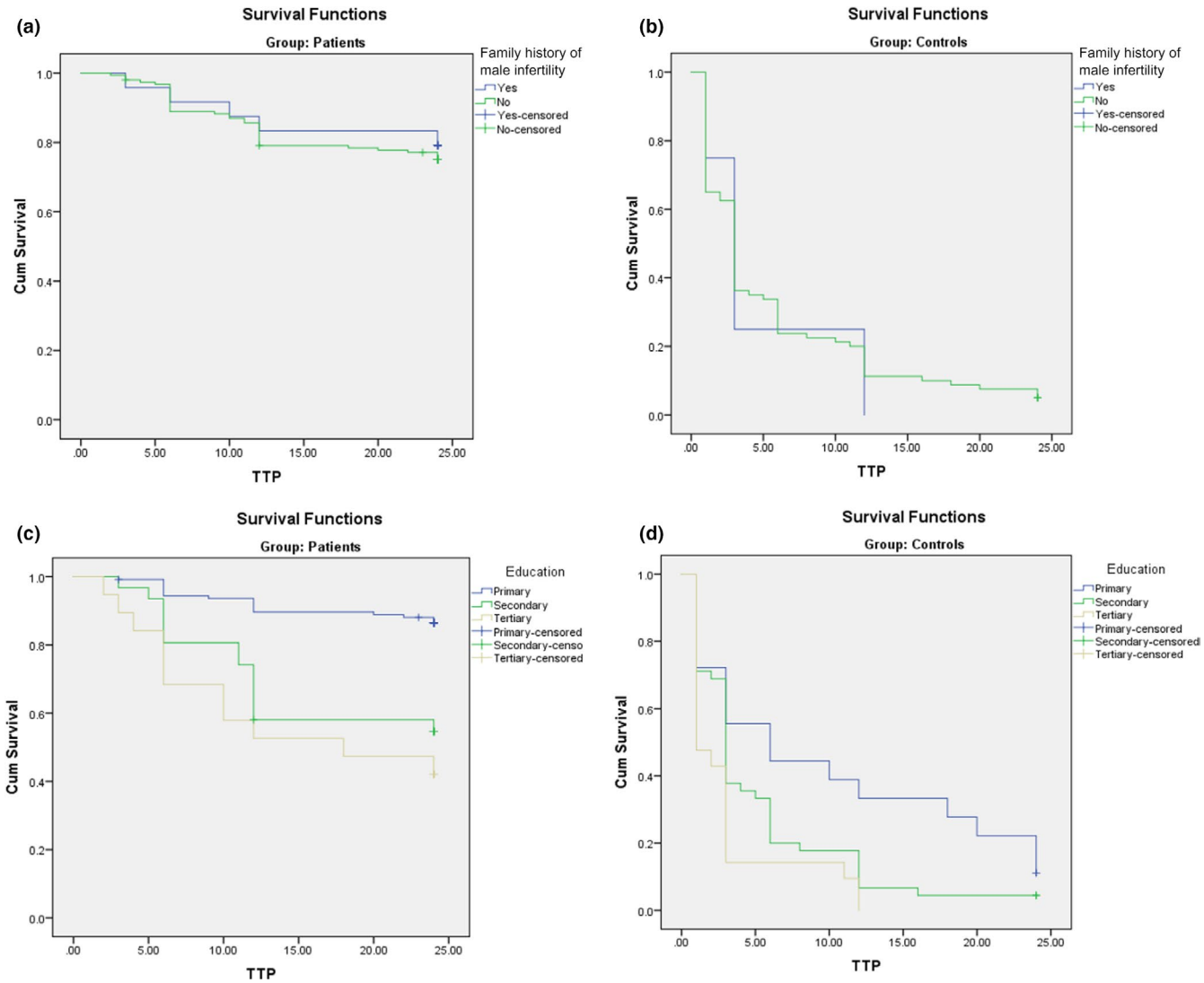

FIGURE 1 A. Kaplan–Meier curve for family history of male infertility versus time to pregnancy (TTP) in patients; B. Kaplan–Meier curve for family history of male infertility versus time to pregnancy (TTP) in controls; C. Kaplan–Meier curve for education versus time to pregnancy (TTP) in patients; D. Kaplan–Meier curve for education versus time to pregnancy (TTP) in controls

TABLE 9 Univariate Cox regression analysis for predictors of time to pregnancy in controls

	Baseline			After 6 months		
	HR	95% CI	P value	HR	95% CI	P value
Male age	1.03	1.01–1.06	0.03	1.03	1.00–1.06	0.03
Volume	0.65	0.40–1.05	0.07	1.12	0.8–1.7	0.29
Concentration	1.01	0.99–1.01	0.12	1.03	1.02–1.04	0.001
Progressive motility	1.01	0.97–1.03	0.92	1.00	0.97–1.03	0.96
Total motility	1.002	0.98–1.02	0.97	1.00	0.98	0.9
Normal morphology	0.99	0.95–1.02	0.54	0.96	0.93–0.99	0.01
CoQ10 level	0.99	0.99–1.01	0.73	1.01	0.99–1.01	0.96
ROS	3.1	0.05–187.7	0.58	552.2	0.003–11319	0.31
TAC	1.96	0.66–5.7	0.22	1.7	0.62–4.6	0.29
GPx	10.9	0.42–282.7	0.15	10.4	0.25–425.9	0.21
CAT	0.98	0.89–1.07	0.71	0.99	0.89–1.09	0.85
SDF	0.92	0.85–1.01	0.06	0.93	0.87–0.99	0.04
Female age	1.04	1.01–1.08	0.053	1.04	1.00–1.08	0.05
BMI	0.98	0.94–1.02	0.47	0.98	0.95–1.02	0.47
Family history	0.94	0.34–2.5	0.9	0.94	0.34–2.5	0.9
Education	1.5	1.1–2.1	0.01	1.5	1.1–2.1	0.01

Abbreviations: HR, hazard ratio; ROS, reactive oxygen species; TAC, total antioxidant capacity; GPx, glutathione peroxidase; CAT, catalase; SDF, sperm DNA fragmentation; BMI, body mass index; CoQ10, Coenzyme Q10.

1991). High SDF levels among infertile men were associated with idiopathic infertility, recurrent IUI failure, recurrent pregnancy loss and IVF/ICSI outcomes (Cho & Agarwal, 2018). The association between obesity and high BMI with longer TTP could be attributed to abnormal semen parameters, OS, low testosterone/estradiol ratio and increased SDF among infertile men with obesity (Le et al., 2020). A study has also reported a link between CoQ10 intake and altered serum testosterone level which was attributed to the antioxidant properties of CoQ10 that protect against gonadal toxicity (Banihani, 2018). Our previous study, however, reported the lack of altered hormonal profile post-CoQ10 therapy in men with IMI (Alahmar, Calogero, et al.,). A lower level of education was a significant factor in the occurrence of infertility in our patient group, which also correlated with pregnancy outcomes. A lower level of education has previously been linked to infertility in males (Moridi et al., 2019). The link between a low level of education and infertility could be attributed to lack of awareness about reproductive organs and fertilization physiology, factors that may cause infertility, early diagnosis and treatment and the available treatment options and health care facilities (Mahanta, 2016). Our results point out that male age, sperm concentration, motility, ROS and GPx could be used as diagnostic biomarkers as well as independent predictors of TTP in men with idiopathic OA with CoQ10 therapy. Therefore, our study has highlighted the possible role of CoQ10 in improving semen parameters, seminal antioxidant status and SDF in men with idiopathic OA. Potential predictors of pregnancy and time to pregnancy have also been suggested. Our observations are consistent with previous studies, which have reported similar results of antioxidants in men with IMI (Arafa et al., 2020; Balercia et al., 2009; Imamovic Kumalic & Pinter, 2014). Previous studies have also explored the link between dietary intake of CoQ10 and semen parameters and male fertility (Torres-Arce et al., 2021; Vishvkarma et al., 2020). Another study, however, reported the lack of association between dietary sources of CoQ10 and semen measures among infertile men (Tiseo et al., 2017). Although dietary assessment of CoQ10 could be useful, we and others have not assessed it due to the subjective nature of dietary questionnaires with a potential recall bias and also it can be limited by the complex nature of multiple dietary micronutrients (Mirmiran et al., 2021).

Limitations of our study include a smaller number of controls in comparison with patients and the lack of placebo arm due to ethical considerations although we have used fertile controls as no treatment group. Another limitation is that the participants were recruited from one location, so our findings may not be generalized as there are genetic, racial and geographical variations in semen parameters; so further multicentre studies are warranted to consolidate the evidence provided in this study.

5 | CONCLUSIONS

Our findings demonstrate that 6 months of CoQ10 therapy significantly increase CoQ10 levels in seminal plasma and improve semen parameters, antioxidant capacity and SDF with a pregnancy rate of 24.2% in men with idiopathic OA. CoQ10 level, male age, semen parameters, ROS and GPx could be used as diagnostic biomarkers for male fertility and predictors for pregnancy outcome and time to pregnancy in these men. Further, CoQ10 therapy for 6 months could be a potential therapy for men with idiopathic OA and may enhance their fertility and pregnancy outcomes.

FUNDING STATEMENT

None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest associated with this study.

ETHICAL APPROVAL

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. The study protocol was approved by the local ethical committee at the University of Sumer, Iraq (EC/2018/8866/8876/8878/8879), and all participants consented for participation in the study.

DATA AVAILABILITY STATEMENT

Data will be available on request from the authors.

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To / Graduate School

School of Health, Science and Wellbeing

Staffordshire University

UK

15/11/2021

Dear sir,

I am writing in regard to my co-author Ahmed T Alahmar who is doing a PhD by Publication at Staffordshire University. I endorse his co-authorship of the paper entitle:

'Impact of Coenzyme Q10 and Selenium on Seminal Fluid Parameters and Antioxidant Status in Men with Idiopathic Infertility'

I confirm that Ahmed T Alahmar had a leading role in this publication and had lead authorship, undertook conceptualization, formulation, execution, analysis, and designed the study, searched the literature, collected and analyzed data, wrote the manuscript draft, and handled the submission and publication. If you need anything further, please let me know.

Kind regards.

List of authors (in the order shown in publication)

No.	Co-author	Affiliation	Email	Signature
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2	Dr. Pallav Sengupta	MAHSA University, Malaysia	pallav_cu@yahoo.com	

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'Coenzyme Q10 effect on semen parameters: Profound or meagre?'

I confirm that Ahmed T Alahmar contributed as second author for conceptualization, formulation, execution, data collection and analysis. If you need anything further, please let me know.

Kind regards.

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3	Dr. Gopal Gupta	Central Drug Research Institute, Lucknow, India	ggupta.cdri@gmail.com	
4	Dr. Rajender Singh	Central Drug Research Institute, Lucknow, India	nainrs@gmail.com	

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'Coenzyme Q10, oxidative stress, and male infertility: A review'

I confirm that Ahmed T Alahmar had a leading role in this publication and had lead authorship, undertook conceptualization, formulation, execution, analysis, and designed the study, searched the literature, collected and analyzed data, wrote the manuscript draft, and handled the submission and publication. If you need anything further, please let me know.

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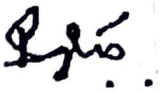
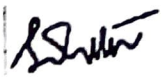
5	Dr. Pallav Sengupta	MAHSA University, Selangor, Malaysia	pallav_cu@yahoo.com	
6	Dr. Sulagna Dutta	MAHSA University, Selangor, Malaysia	duttasulagna@mahsa.edu.my	

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15/11/2021

Dear sir,

I am writing in regard to my co-author Ahmed T Alahmar who is doing a PhD by Publication at Staffordshire University. I endorse his co-authorship of the paper entitle:

'Comparing the effects of Coenzyme Q10 and Centrum Multivitamin on Semen Parameters, Oxidative Stress Markers and Sperm DNA Fragmentation in Infertile Men with Idiopathic Oligoasthenospermia'

I confirm that Ahmed T Alahmar had a leading role in this publication and had lead authorship, undertook conceptualization, formulation, execution, analysis, and designed the study, searched the literature, collected and analyzed data, wrote the manuscript draft, and handled the submission. If you need anything further, please let me know.

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15/11/2021

Dear sir,

I am writing in regard to my PhD student and co-author Ahmed T Alahmar who is doing a PhD by Publication at Staffordshire University. I endorse his co-authorship of the paper entitle:

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