Nutrient supplementation in Assisted Reproductive Technologies: A review

Isabel Sofia Andrade da Silva Castelo
Master in Cell and Molecular Biology
Department of Biology
Faculty of Sciences of the University of Porto
2025/2026

Nutrient supplementation in Assisted Reproductive Technologies: A review

Isabel Sofia Andrade da Silva Castelo

Internship Report carried out as part of the Master in Cell and Molecular Biology

Department of Biology

2025/2026

Supervisor
Luís Filipe Costa de Castro, Associate Professor, FCUP

External Host Supervisors
Maria Sofia Pinto Gama Lobo Xavier, Laboratory Director, CHUSJ
Ione Gonçalves Tavares Reis, Embryologist, CHUSJ

U.PORTO
FACULDADE DE CIÊNCIAS
UNIVERSIDADE DO PORTO

UNIDADE LOCAL DE SAÚDE
SÃO JOÃO

Acknowledgements

This dissertation is not solely a reflection of my work, but also a reflection of all the support, guidance and encouragement I have experienced throughout the process.

Firstly, I would like to express my gratitude to my Host Supervisors Sofia Xavier and Yone Reis for their daily guidance, patience and invaluable mentorship during both the experimental work and the writing of this dissertation. I would also like to thank all the members of CRI-MR, and especially Ana Patrícia Martins, Renata Leite, Maria García and Beatriz Araújo, for their kindness, support, and willingness to help whenever needed. Furthermore, I would like to extend my sincere appreciation to my Academic Supervisor, Assistant Professor Luís Filipe Castro, for all the continuous support in the writing of the dissertation and the constructive feedback.

I am also grateful to FCUP, Hospital Universitário de São João, and specifically to the Master's in Cell and Molecular Biology programme for providing the academic and institutional environment that made this work possible.

My friends, with special mention to Filipa Carneiro, Teresa Simões, Pedro Maciel, and José Silva, were an important part of this journey. Thank you for listening to my concerns, motivating me when I needed it most, and making even the most challenging moments easier to face.

Without my family and their endless encouragement and belief in me this achievement would never have been possible. They have celebrated every success with me and stayed by my side in every challenge, which gave me the strength to pursue my life and academic goals.

I would especially like to express my deepest gratitude to my boyfriend, who has helped me every step in the way. He shared with me his extensive knowledge and supported me in countless ways. Thank you for believing in me even when I doubted myself and for everything you have done for me.

Resumo

As Tecnologias de Reprodução Assistida (TRA) têm emergido nos últimos anos, sendo que um número crescente de casais enfrenta dificuldades em conceber de forma natural. No entanto, estas técnicas ainda enfrentam desafios consideráveis e beneficiariam de um aumento de eficiência. Está bem estabelecido que a infertilidade é influenciada por diversos fatores, alguns deles relacionados com estilo de vida e exposição ambiental. Os efeitos de suplementação dietária têm sido objeto de estudo em casais que recorrem a TRA, com o objetivo de melhorar os desfechos reprodutivos dessas técnicas, com resultados promissores. Apesar disso, ainda há muitos resultados contrastantes na literatura, o que restringe a sua implementação prática em ambientes clínicos. Tendo isto em conta, esta tese teve o objetivo de sumarizar e analisar criticamente a literatura existente relacionada com a suplementação dietária em homens e mulheres sujeitos a TRA e resultados reprodutivos, assim como identificar objetivamente estratégias de suplementação com potencial aplicação prática. Para este propósito, uma pesquisa extensiva por literatura científica foi efetuada, tendo resultado em diversos artigos relevantes, sob os quais a análise foi executada. Os resultados sugeriram que a suplementação dietária com Coenzima Q10 (CoQ10) está associada a resultados reprodutivos positivos tanto em homens como mulheres, enquanto o mio-inositol (MI) e L-Carnitina (LC) aparentam ser benéficos em mulheres e homens, respetivamente. A Vitamina D (VitD) demonstrou efeitos positivos quando utilizada especificamente na correção de deficiência em ambos os géneros. Para além disso, os resultados também revelaram que provavelmente a suplementação com melatonina (MEL) em mulheres e com Vitamina E (VitE) em homens não está diretamente correlacionada com melhores desfechos reprodutivos. Com bases nestes resultados, protocolos de suplementação específicos foram apresentados para várias condições clínicas.

Palavras-chave: Tecnologias de Reprodução Assistida, infertilidade, infertilidade masculina, infertilidade feminina, suplementação nutricional dietária, resultados reprodutivos, coenzima Q10, mio-inositol, L-carnitina, vitamina D, melatonina, vitamina E

Abstract

Assisted Reproductive Technologies (ART) emerged in the last years, as more couples struggle to conceive naturally. However, ART still face considerable challenges, with low success rates and would thus benefit from enhanced efficiency. It is well known that infertility is influenced by multiple factors, some of them related to lifestyle and environmental exposure. The effects of dietary nutrient supplementation have been an object of study in couples going through ART treatments as a means of improving the reproductive outcomes of such techniques, with promising results. Nevertheless, there are still multiple conflicting results in the literature, which restricts its practical implementation in clinical environments. Given this background, this thesis aimed to summarize and critically analyze the existing literature regarding nutrient supplementation in both men and women undergoing ART and its association with reproductive outcomes, as well as objectively identify supplementation strategies with potential practical application. For this purpose, an extensive search for scientific literature was conducted, which resulted in several relevant articles, upon which the analysis was performed. The results highlight that dietary supplementation with Coenzyme Q10 (CoQ10) is associated with positive reproductive outcomes in both women and men, while Myo-inositol (MI) and L-carnitine (LC) appear to be beneficial in women and men, respectively. Vitamin D (VitD) shows positive effects when used specifically to correct deficiency in both genders. Moreover, the results also reveal that melatonin (MEL) in women and Vitamin E (VitE) in men cannot be directly correlated with improved reproductive outcomes. Based upon these findings, specific supplementation protocols were presented for several clinical conditions.

Keywords: Assisted reproductive technologies, infertility, male infertility, female infertility, dietary nutrient supplementation, Coenzyme Q10, myo-inositol, L-carnitine, vitamin D, melatonin, vitamin E

Table of Contents

List of Tables vii

List of Figures viii

List of Abbreviations x

Introduction 1

1. Infertility 1

2. The process of the initial approach 1

3. Gender specific analyses 2

3.1. Assessing female factor 2

3.1.1. Ovulatory factor 3

3.1.2. Tubal factor 3

3.1.3. Uterine factor 3

3.1.4. Cervical factor 4

3.2. Assessing male factor 4

3.3. Genetic factors 5

4. Clinical interventions 6

4.1. Intrauterine insemination (IUI) 6

4.2. In vitro fertilization (IVF) 6

4.2.1. cIVF 6

4.2.2. ICSI 7

4.3. Post-IVF assessment 7

4.3.1. Fertilization 7

4.3.2. Embryo development 7

4.3.3. Embryo quality 7

4.4. Embryo final destination 8

4.4.1. Embryo transfer 8

4.4.2. Embryo cryopreservation 8

4.4.3. Preimplantation Genetic Testing 8

5. Objectives 9
Methods 10
Results and discussion 13
1. Coenzyme Q10 13
1.1. Effects of Coenzyme Q10 supplementation in women 14
1.1.1. Ovarian response and oocyte quality 14
1.1.2. Fertilization rates and embryo quality 15
1.1.3. Implantation, clinical pregnancy rates and live birth rates 16
1.1.4. Miscarriage rates 17
1.1.5. Overall interpretation 17
1.2. Effects of Coenzyme Q10 supplementation in men 18
1.2.1. Sperm quality and reproductive outcomes 18
1.2.2. Overall interpretation 20
2. Myo-inositol 22
2.1. Effects of Myo-inositol supplementation in women 23
2.1.1. Ovarian response and oocyte quality 23
2.1.2. Fertilization rates and embryo quality 24
2.1.3. Implantation, clinical pregnancy rates and live birth rates 24
2.1.4. Miscarriage rates 25
2.1.5. Overall interpretation 25
3. Melatonin 27
3.1. Effects of Melatonin supplementation in women 28
3.1.1. Ovarian response and oocyte quality 28
3.1.2. Fertilization rates and embryo quality 28
3.1.3. Implantation, clinical pregnancy rates and live birth rates 29
3.1.4. Overall interpretation 29
4. Vitamin D 30
4.1. Effects of Vitamin D supplementation in women 31
4.1.1. Fertilization rates and embryo quality 31

4.1.2. Implantation, clinical pregnancy rates and live birth rates 31
4.1.3. Miscarriage rates 32
4.1.4. Overall interpretation 33
4.2. Effects of Vitamin D supplementation in men 36
4.2.1. Sperm quality and reproductive outcomes 36
4.2.2. Overall interpretation 37
5. L-Carnitine 40
5.1. Effects of L-Carnitine supplementation in men 41
5.1.1. Sperm quality and reproductive outcomes 41
5.2.2. Overall interpretation 41
6. Vitamin E 42
6.1. Effects of Vitamin E supplementation in men 43
6.1.1. Sperm quality and reproductive outcomes 43
6.2.2. Overall interpretation 44
Conclusion 45
References 46
Attachments 57
Attachment 1 57

List of Tables

Table 1 - Table summarizing main semen parameters and respective reference values used in the assessment of male fertility [23]. 4
Table 2 - Table summarizing clinical classifications of semen abnormalities based on deviations from reference semen parameters [24]. 5
Table 3 - Types of preimplantation genetic testing and clinical indications [37]. 9
Table 4 - Table summarizing keyword groups used in the general and targeted searches. Terms within the same keyword group were combined with OR, and keyword groups were combined with AND. 12
Supplementary Table 1 - Table summarizing the characteristics of the analysed articles for female patients. 57
Supplementary Table 2 - Table summarizing the characteristics of the analysed articles for male patients.. 60

List of Figures

Figure 1 - Schematic representation of the proposed role of CoQ10 in both female and male reproductive health.... 14
Figure 2 - Graphical representation summarizing the studied articles and the main outcomes of CoQ10 supplementation in female patients on assisted reproductive outcomes across different patient populations. 18
Figure 3 - Graphical representation summarizing the studied articles and the main outcomes of CoQ10 supplementation in male patients on assisted reproductive outcomes on assisted reproductive outcomes across different patient populations.... 21
Figure 4 - Schematic representation of the proposed role of MI in female reproductive health.... 22
Figure 5 - Graphical representation summarizing the studied articles and the main outcomes of MI supplementation in female patients on assisted reproductive outcomes across different patient populations.... 26
Figure 6 - Schematic representation of the proposed role of MEL in female reproductive health.... 27
Figure 7 - Graphical representation summarizing the studied articles and the main outcomes of MEL supplementation in female patients on assisted reproductive outcomes on assisted reproductive outcomes across different patient populations 29
Figure 8 - Schematic representation of the proposed role of VitD in both female and male reproductive health.. 30
Figure 9 - Graphical representation summarizing the studied articles and the main outcomes of VitD supplementation in female patients on assisted reproductive outcomes across different patient populations.... 34
Figure 10 - Graphical representation summarizing the studied articles and the main outcomes of enhanced VitD levels in female patients on assisted reproductive outcomes across different patient populations.... 36
Figure 11 - Graphical representation summarizing the studied articles and the main outcomes of VitD supplementation in male patients on assisted reproductive outcomes across different patient populations.... 38
Figure 12 - Graphical representation summarizing the studied articles and the main outcomes of enhanced VitD levels in male patients on assisted reproductive outcomes across different patient populations.... 39
Figure 13 - Schematic representation of the proposed role of LC in male reproductive health.... 40

Figure 14 - Graphical representation summarizing the studied articles and the main outcomes of LC supplementation in male patients on assisted reproductive outcomes across different patient populations..... 42
Figure 15 - Schematic representation of the proposed role of VitE in male reproductive health..... 43
Figure 16 - Graphical representation summarizing the studied articles and the main outcomes of VitE supplementation in male patients on assisted reproductive outcomes in unspecified subfertile/infertile men population. 44

List of Abbreviations

AFC	ANTRAL FOLLICLE COUNTS
ART	ASSISTED REPRODUCTIVE TECHNOLOGIES
CIVF	CONVENTIONAL IN VITRO FERTILIZATION
ATP	ADENOSINE TRIPHOSPHATE
COQ10	COENZYME Q10
CPR	CLINICAL PREGNANCY RATES
DOR	DIMINISHED OVARIAN RESERVE
FSH	FOLLICLE-STIMULATING HORMONE
ICSI	INTRACYTOPLASMIC SPERM INJECTION
IUI	INTRAUTERINE INSEMINATION
IVF	IN VITRO FERTILIZATION
LBR	LIVE BIRTH RATES
LC	L-CARNITINE
MEL	MELATONIN
MI	MYO-INOSITOL
MII	METAPHASE II
MR	MISCARRIAGE RATES
NOR	NORMAL OVARIAN RESERVE
OA	OLIGOASTHENOZOOSPERMIA
OAT	OLIGOASTHENOTERATOZOOSPERMIA
PMOS	POLYENDOCRINE METABOLIC OVARIAN SYNDROME
PGT	PREIMPLANTATION GENETIC TESTING
POR	POOR OVARIAN RESPONSE
TEAS	TRANSCUTANEOUS ELETRICAL ACUPOINT STIMULATION
TRA	TECNOLOGIAS DE REPRODUÇÃO ASSISTIDA
UI	UNEXPLAINED INFERTILITY
VITD	VITAMIN D
VITE	VITAMIN E
WHO	WORLD HEALTH ORGANIZATION

Introduction

1. Infertility

The definition of infertility, according to the World Health Organization (WHO), consists of the inability to achieve pregnancy following 12 months or more of regular and unprotected sexual intercourse [1]. There are two types of infertility considered [1]. The first is known as primary infertility and is defined by the individual's lack of prior pregnancies [1]. The second type, referred to as secondary infertility, happens when a person or couple achieved a pregnancy, but is struggling to conceive again [1]. The WHO has estimated that roughly 1 in 6 people worldwide have experienced infertility during their lifetime, corresponding to 17.5% of the global population [2]. Infertility causes vary between female factor, male factor, combined male and female, and unexplained (idiopathic) [1], [2]. The latter is characterized by the absence of a physiological explanation [1]. Identifiable causes underlying infertility correspond to approximately 85% of all cases, while 15% correspond to unexplained infertility [3].

ART has emerged as a clinical answer to infertility cases in the past few decades, along with an increased interest in strategies to improve reproductive outcomes [4]. One of those latter strategies consists in the use of dietary nutritional supplementation as a potential ART adjuvant, based on the fact that infertility is often multifactorial and influenced by hormonal and metabolic factors [5]. Antioxidants, vitamins and micronutrients may attenuate oxidative stress and inflammation, which contribute to impaired reproductive function for both men and women [6]. Even though these supplements are being increasingly used in clinical practice, specific recommendations are lacking, and the protocols are heterogeneous [5]. Nevertheless, the first medical approach in infertility cases remains the evaluation of the physiological characteristics and lifestyle of patients [3], [7].

2. The process of the initial approach

One of the first factors to be considered when approaching a couple with infertility is the age of the members, especially of the woman [7]. It is well known that women fertility declines steeply with age, being less probable to achieve a pregnancy as they age, especially after 35 years of age [8]. This is primarily because oocytes are finite, being

produced in fetal development and monthly released through the menstrual cycle [9], [10]. In addition to the reduction in quantity, the remaining oocytes undergo a reduction in quality caused by their natural aging [11], [12]. Oocytes from older women were associated with increased oxidative damage caused by lipid and protein peroxidation [13]. Furthermore, an imbalanced cellular redox state, change in energy sources and compromised mitochondrial function were also correlated with higher age [13]. Advanced maternal age has also been reported to higher risks of having an aneuploidy embryo [14], lower fecundability [15] and higher miscarriage rates (MR) [16].

Male fertility may also be impacted by advanced age, particularly regarding sperm quality [17]. In older men, lower sperm motility, total progressive motility, total progressive motile count, and normal sperm morphology were reported [17]. Higher MR have also been observed [16].

Lifestyle choices are also an important factor in both female and male fertility [18]. Smoking, alcohol or drugs consumption, poor nutrition, obesity, physical inactivity, psychological factors, and environmental exposures, have been associated with impaired fertility in both genders [18].

Nutritional status should also be considered during the initial evaluation of infertile couples. Deficiencies in vitamins, minerals and other micronutrients may impair reproductive function by disrupting mitochondrial function and antioxidant defenses, leading to increased oxidative stress and reduced gamete quality [5], [6]. Therefore, identifying and correcting clinically relevant nutritional deficiencies before ART has been proposed as a strategy to optimize reproductive outcomes [5].

After evaluating these factors, in the case of undisclosed health issues, additional gender specific steps are performed to underly the causes of infertility.

3. Gender specific analyses

3.1. Assessing female factor

Among all the causes of female infertility, the main ones are ovulatory, cervical, uterine and tubal-peritoneal factors.

3.1.1. Ovulatory factor

Ovulation is characterized by the release of a mature oocyte by the ovary into fallopian tubes so it can be fertilized. [19], [20]. Thus, the functionality of this process is critical in the establishment of pregnancy. Ovulatory disorders constitute approximately 25% of infertility causes [3]. When menstrual cycles are irregular, considering less than 21 days and more than 35 days, or are abnormal bleeding or amenorrhea, anovulation can be suspected [3]. To confirm, serum progesterone level may be obtained in the expected midluteal phase, approximately one week before the expected menses [1], [3]. Among the causes of anovulation, Polyendocrine Metabolic Ovarian Syndrome (PMOS) is the most frequent, being estimated to affect 70% of women with anovulation [3]. Additional causes may be idiopathic, thyroid or pituitary disease-related, adrenal hyperplasia or tumor, and functional hypothalamic amenorrhea [3]. Anovulation is more common in people who are obese or suffer from eating disorders [3]. As aforementioned, the oocyte reserve diminishes with age, being the main reason for Diminished Ovarian Reserve (DOR) [3]. Other contributing factors include a family history of early menopause, ovarian surgery, gonadotoxic cancer treatments, and the FMR1 premutation (fragile X) [3]. Ovarian reserve can be assessed using serum anti-Müllerian hormone levels, antral follicle count (AFC) by transvaginal ultrasound, and early follicle-stimulating hormone (FSH) levels on day two or three of the menstrual cycle [3].

3.1.2. Tubal factor

Functional fallopian tubes are essential in the establishment of pregnancy, since they have functions ranging from ovulation to fertilization and embryo development [19]. Bilateral tubal blockage has been associated with 17% of causes of female infertility [1], [2]. Another cause for tubal infertility is impaired oocyte capture by the fallopian tube [3]. Hysterosalpingography is often used to diagnose tubal disease. The most important risk factor for such problems is infection from sexually transmitted diseases [3].

3.1.3. Uterine factor

The uterus is where implantation and pregnancy occur. Thus, uterine abnormalities are related to difficulty in obtaining a pregnancy and to a higher risk of miscarriage [3]. The most common causes are endometrial polyps, leiomyomas, intrauterine synechiae, and congenital uterine malformations such as septate uterus [3]. Surgery is common to correct the uterine cavity disorders [3].

3.1.4. Cervical factor

Cervical factors are not very common and are often correlated to congenital irregularities or postsurgical problems [3]. Most cervical factors are related to mucous dysfunction or to problems in the interaction between mucous and sperm, which have a negative impact in sperm progression to the uterus [3], [21], [22].

3.2. Assessing male factor

The assessment of male fertility is done by a seminogram [23]. This exam consists in the obtainment of a semen sample and posterior analysis of both microscopic and macroscopic parameters [23]. The macroscopic parameters analyzed are sperm volume, pH, color, scent, viscosity and liquefaction [23]. As for microscopic parameters, the ones analyzed are motility, concentration, morphology and sometimes vitality (in the case of less than 40% of total progressive motility) [23]. In some cases, the assessment of deoxyribonucleic acid (DNA) fragmentation can also be done [23]. Male factor is considered when the values obtained for one or more the parameters is below the WHO reference values, represented in Table 1 [23]. The most important diagnosis for each impairment is represented in Table 2 [24].

Table 1 - Table summarizing main semen parameters and respective reference values used in the assessment of male fertility [23].

Semen volume	>1,4 ml
Sperm concentration * 10^6/ml	≥16 million
Total sperm number * 10^6/ejaculate	≥39 million
Total motility (progressive+non-progressive)	≥42 %
Progressive motility	≥30 %
Immotile	≥20 %
Normal forms	≥4,0 %
Vitality	≥54 %
Leucocytes * 10^6/ml	1 - 2

Table 2 - Table summarizing clinical classifications of semen abnormalities based on deviations from reference semen parameters [24].

Normozoospermia	Normal sperm values
Asthenozoospermia	Progressive motility ≤ 30%
Teratozoospermia	Normal morphology ≤ 4%
Oligozoospermia	Total number or concentration below reference values
Cryptozoospermia	Absence of spermatozoa in fresh preparations, but present after centrifugation
Asthenoteratozoospermia	Progressive motility ≤ 30% and normal morphology ≤ 4%
Oligoasthenozoospermia	Total number or concentration below reference values and progressive motility ≤ 30%
Oligoteratozoospermia	Total number or concentration below reference values and normal morphology ≤ 4%
Oligoasthenoteratozoospermia	Total number or concentration below reference values, progressive motility ≤ 30% and normal morphology ≤ 4%
Azoospermia	Absence of spermatozoa in the ejaculate
Aspermia	No semen collected

3.3. Genetic factors

Genetic abnormalities are an important cause of both men and women infertility, affecting reproductive function through gene mutations, chromosomal alterations or epigenetic mechanisms [25], [26]. Genetic factors could contribute up to 30% of male infertility cases, particularly in men diagnosed with severe oligozoospermia or azoospermia [27].

4. Clinical interventions

4.1. Intrauterine insemination (IUI)

Intrauterine insemination (IUI), due to its cost-effective and noninvasive nature, is the first approach of therapy in couples with unexplained infertility, infertility caused by ovulatory disorders, cervical factors, mild male infertility, ejaculatory disorders or immunological factors [28]. However, is not commonly used in tubal factor, severe cases of men infertility, endometriosis and women of advanced age [28]. The procedure consists in the processing of a sperm sample and subsequent deposition into the uterus, with or without prior ovarian stimulation [3], [28]. The main objective is to bypass the barriers within the female reproductive trait [28]. It is estimated to contribute to up to 20 % of clinical pregnancy rates (CPR) per cycle [28].

4.2. In vitro fertilization (IVF)

IVF is a second line intervention treatment that involves a process of controlled ovarian stimulation in order to enhance the number of follicles and consequently of mature oocytes [1]. The patients undergo an intervention named oocyte retrieval, in which a doctor tries to extract all follicles large enough to have responded to ovarian stimulation [29]. After the procedure, an embryologist identifies all the oocytes [29]. Another common thing among IVF cycles is the process of a semen sample through gradients, wash, and swim up or through a microfluidic chamber [23]. These treatments are divided into conventional *in vitro* fertilization (cIVF) and intracytoplasmic sperm injection (ICSI). In an ART cycle, one of these techniques or their combination can be used [30].

4.2.1. cIVF

cIVF is characterized by the incubation of an oocyte with a defined concentration of spermatozoa, previously processed [30]. In this procedure, the cumulus remains intact after oocyte retrieval, allowing the natural penetration of the spermatozoa into the oocyte, and therefore its fertilization [30]. Compared with ICSI, cIVF is a more natural procedure, as sperm selection and fertilization occur with minimal embryologist intervention, without the direct injection of a spermatozoa into the oocyte [30].

4.2.2. ICSI

ICSI is a treatment more commonly used in couples with severe male factor [30]. The cumulus is removed through mechanical and chemical processes with hylaruronidase [29]. A spermatozoon for each oocyte is chosen based on its morphology and motility and immobilized by stroking the tale [30]. The injection pipette will collect the spermatozoon and subsequently inject it into the oocyte through zona pelucida, positioned by a holder pipette in a way that the globular polar is at ninety degrees from the pipette, due to the perturbation otherwise [30].

4.3. Post-IVF assessment

4.3.1. Fertilization

After cIVF or ICSI, the culture plates are stored in incubators at 37ºC and controlled atmospheric conditions, optimal for embryo development [31]. Normal fertilization is confirmed after 16 to 18 hours, through the visualization of polar bodies and pronuclei [1]. If the oocyte has two of each, the fertilization was successfully accomplished [29], [30].

4.3.2. Embryo development

After an oocyte has been fertilized, it undergoes consecutive cellular divisions [30]. Ideally, there must be a total of four cells on day two and a total of eight cells on day three [30]. If the embryo on day three has good quality, and there is medical recommendation, fresh embryo transfer can be considered [30]. On day four, the embryo is supposed to start to compact and form a morula, which forms a unity [30]. Subsequently, a blastocyst is formed, by day five or six of culture [30]. However, embryos can stay in culture until day seven [30].

4.3.3. Embryo quality

The main parameters analyzed to determine embryo quality until day three or four are the number of cells, symmetry and the percentage of fragmentation [30]. At the blastocyst stage, embryo quality is primarily based on its expansion, morphology of

trophectoderm and inner cell mass [30]. Embryo quality assessment is essential for selecting embryos with the highest implantation potential [30].

4.4. Embryo final destination

4.4.1. Embryo transfer

Embryo transfer is the final step of an ART cycle and can be divided into fresh or frozen embryo transfer. The first consists of transferring one or more embryos directly from culture into the uterine cavity, and the second involves thawing of a previously frozen embryo [32]. The final step of both types of embryo transfer is done recurring to a soft catheter which will collect the embryo and place it in the uterus [30]. The timing of embryo transfer depends on several factors, such as embryo development, patient characteristics and medical recommendations. It is commonly performed at the cleavage stage (day three) or blastocyst stage (day five) [33]. Single embryo transfer is recommended to reduce the multiple pregnancy risk [30], [34].

4.4.2. Embryo cryopreservation

Embryo cryopreservation allows the preservation of embryos for future use, mantaining their viability [30]. It is mostly used in cases of excedent embryos after fresh embryo transfer, in the risk of ovarian hyperstimulation syndrome, and in preimplantation genetic testing [35].

4.4.3. Preimplantation Genetic Testing

Preimplantation genetic testing (PGT) is performed when couples have a medical recommendation, normally due to genetic abnormalities or recurrent pregnancy loss [36], [37]. The main objective is to ensure that the embryo has a normal genetic profile before its transfer [36]. The technique consists of an extraction of embryo cells, normally from the trophectoderm at the blastocyst stage, through laser biopsy, followed by genetic screening [38]. This technique is divided into different categories, and the choice depends on the case, as represented in Table 3 [37].

Table 3 - Types of preimplantation genetic testing and clinical indications [37].

Types	Abbreviations	Description	Indications
Preimplantation genetic testing for aneuploidies	PGT-A	Identification of numerical chromosomal abnormalities in embryos	Couples with recurrent pregnancy loss or implantation failure, advanced maternal age, and unexplained severe teratozoospermia
Preimplantation genetic testing for monogenic/single-gene disorders	PGT-M	Detection of specific inherited genetic diseases in embryos	Couples with genetic diseases that can affect the progeny
Preimplantation genetic testing for structural rearrangements	PGT-Sr	Detection of chromosomal structural abnormalities	Couples carrying balanced chromosomal abnormalities

5. Objectives

Even though the use of supplements in the context of fertility has increased substantially, the recommendations for clinical practice remain unspecified and heterogeneous. This dissertation aims to summarize and critically analyze the current knowledge regarding supplementation in women and men undergoing ART treatments, and where possible, propose practical recommendations. Firstly, evidence from scientific literature for both genders will be gathered and presented. Secondly, the results will be summarized and discussed.

Methods

This dissertation was written in parallel with laboratory and technical work in the host institution Centro de Responsabilidade Integrada de Medicina da Reprodução do Hospital de São João in Oporto. This professional experience provided the technical background required to critically assess the scientific evidence regarding nutrient supplementation in assisted reproductive technologies (ART).

Information Sources and Search Strategy

A comprehensive literature search was conducted to identify studies evaluating the effects of nutrient supplementation on ART outcomes. The search was performed using the literature databases Google Scholar, PubMed and Cochrane Library. The search strategy combined terms related to assisted reproductive technologies, infertility and nutrient supplementation. The complete search strategy, including all search terms, is provided in Table 4.

Study Selection and Eligibility Criteria

Studies were selected according to predefined eligibility criteria. Original clinical studies, systematic reviews and meta-analyses published from 2015 were considered eligible if they evaluated the effects of a single nutrient supplement in women or men undergoing ART. Studies investigating combinations of multiple supplements were excluded to minimise potential confounding effects and enable the specific contribution of individual nutrients to be evaluated. However, studies evaluating inositol combined with folic acid were also included, as folic acid represents standard periconceptual supplementation [39]. Thus, the potential for a combined effect of inositol and folic acid was acknowledged. Animal studies, conference abstracts, editorials and publications not available in English were also excluded. The selection of nutrients was performed in two parts. Firstly, a broad literature search was conducted to identify the supplements most frequently investigated in the context of ART, while focusing on studies evaluating individual supplementation. Subsequently, supplements were selected based on the availability of the evidence, prioritising those investigated independently across multiple studies and study populations.

Data Extraction and Outcomes

Data was extracted manually from all eligible studies, including study design, participant characteristics, type of supplementation, ART procedure and reported outcomes. For female supplementation, outcomes of interest included ovarian response, oocyte quality, fertilization rate, embryo quality, implantation rate, clinical pregnancy rate, live birth rate and miscarriage rate. For male supplementation, the extracted outcomes included semen parameters and reproductive outcomes, whenever these were reported.

Evidence Synthesis

As this dissertation constitutes a narrative review, studies were synthesized according to the supplement evaluated rather than through quantitative meta-analysis. The evidence was organized separately for female and male supplementation. The female chapter was further divided according to the reproductive outcomes assessed, whereas the male chapter was organized by individual nutrient, due to the more limited and heterogeneous available evidence.

Risk of Bias

Given the narrative nature of this review, no formal risk-of-bias assessment was performed. However, greater emphasis was placed on evidence derived from systematic reviews, meta-analyses and higher-quality clinical studies to ensure that the discussion reflected the best available evidence. The quality of the clinical studies was evaluated according to the availability of the metrics for the populations used (e.g. number of patients) and clear identification of study bias by the authors.

Table 4 - Table summarizing keyword groups used in the general and targeted searches. Terms within the same keyword group were combined with OR, and keyword groups were combined with AND.

Search category	First keyword group	Second keyword group
General supplementation search	Supplementation Nutrient supplementation Antioxidant supplementation Dietary supplementation Micronutrient supplementation	ART Assisted reproductive technology Assisted reproductive technologies IVF ICSI Fertility Infertility Reproductive outcomes IVF outcomes ICSI outcomes Fertilization rate Embryo quality Oocyte quality Sperm quality
Targeted search for female fertility Outcomes	Coenzyme Q10 CoQ10 Vitamin D Melatonin Myo-inositol Folic acid Folate	ART Assisted reproductive technology Assisted reproductive technologies IVF ICSI Female fertility Female infertility Ovarian response Oocyte quality Embryo quality Implantation rate Fertilization rate Clinical pregnancy Live birth rate Miscarriage
Targeted search for male fertility outcomes	Coenzyme Q10 CoQ10 Vitamin D Vitamin E Vitamin C Melatonin Myo-inositol Folic acid Folate L-carnitine Zinc	ART Assisted reproductive technology Assisted reproductive technologies IVF ICSI Male fertility Male infertility Sperm quality Semen parameters Sperm concentration Sperm count Sperm motility Progressive motility Sperm morphology Sperm DNA fragmentation Clinical pregnancy Live birth rate

Results and discussion

The literature search performed in the databases Google Scholar, Cochrane Library and PubMed using the keywords represented in Table 4. Some searches originated an unfeasible number of articles to evaluate in the one-year time span of the internship (e.g. the keywords 'Coenzyme Q10 AND Assisted Reproductive Technologies' originated 16600 results using Google Scholar). In these cases, the chosen articles were represented in the first five pages when ordered by relevance criteria, defined by the database algorithm (50 articles). From these, several articles were excluded by title (e.g. when the title referred to combination of supplements and language). Using this approach, a total of 285 articles from the different databases were selected to evaluate the clinical effects of the six selected supplements, with approximately 48 articles per supplement. After excluding articles based on the abstract content and relevant findings, a total of 114 articles were fully analyzed. Of these, 60 were excluded according to exclusion criteria mentioned in the Methods section. From the remaining 54 articles, 4 studies investigated Coenzyme Q10 (CoQ10) in women and 7 in men; 7 investigated Myo-inositol (MI) in women; 6 investigated melatonin (MEL) in women; 14 investigated vitamin D (VitD) in women and 9 in men; 4 investigated L-carnitine (LC) in men; and 3 investigated vitamin E (VitE) in men. The characteristics of the studies are represented in Supplementary Table 1 and 2.

1. Coenzyme Q10

CoQ10 is a lipid-soluble molecule endogenously synthesized by human cells, essential in the mitochondrial electron transport chain, since it facilitates electron transfer between complexes I/II and III [40]. Consequently, CoQ10 supports ATP production required for oocyte maturation, fertilization and embryonic development [40]. In addition, CoQ10 acts as a potent lipid-soluble antioxidant, reducing reactive oxygen species (ROS) [40], [41], which diminishes lipid peroxidation and DNA oxidation [41]. In the male reproductive system, CoQ10 is highly concentrated in spermatozoa and contributes to mitochondrial energy production and antioxidant protection, processes that are particularly relevant to sperm motility and overall sperm quality [42]. Consistent with these biological functions,

studies in men with impaired semen parameters suggest that CoQ10 supplementation may improve sperm motility and reduce oxidative stress [43], [44]. Studies in animal models reported that CoQ10 supplementation improves mitochondrial function and oocyte quality during reproductive ageing, suggesting a protective role against age-related mitochondrial dysfunction [40], [45], [46]. Interestingly, CoQ10 endogenous levels decline with age [40], [45], [46], which may partly explain the beneficial effects of supplementation during the ageing process in both men and women.

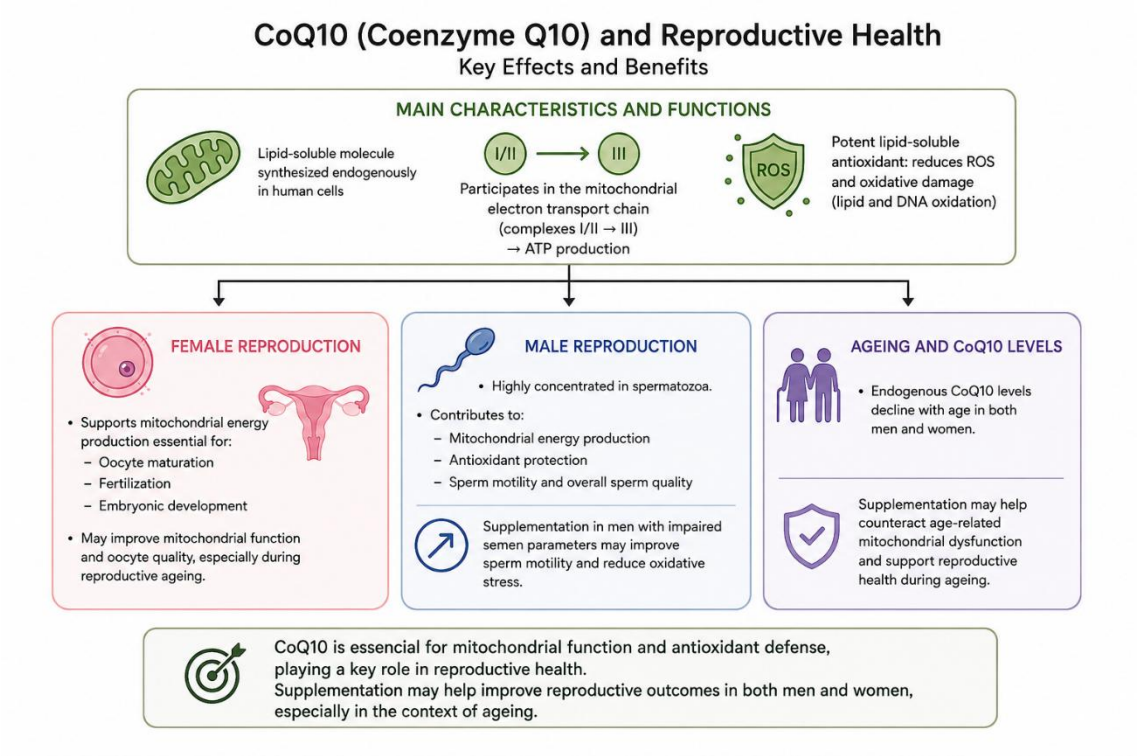


Figure 1 - Schematic representation of the proposed role of CoQ10 in both female and male reproductive health. Created using artificial intelligence (AI).

1.1. Effects of Coenzyme Q10 supplementation in women

1.1.1. Ovarian response and oocyte quality

CoQ10 supplementation has emerged as a promising intervention in improving the ovarian response to stimulation [41], [47], [48]. Studies have demonstrated that CoQ10 pretreatment increases the number of retrieved oocytes, particularly in women with poor ovarian response (POR) [41], [48] or diminished ovarian reserve (DOR) [47]. In a

prospective randomized controlled trial, Xu *et al.* evaluated women younger than 35 years with POR who received oral CoQ10 at a dose of 200 mg three times daily for 60 days before ovarian stimulation [41]. The study found that CoQ10 pretreatment significantly increased the number of retrieved oocytes while reducing the total dose of gonadotrophins required for stimulation. [41]. Nonetheless, the antral follicle count and cycle cancellation rate were not significantly different between groups [41]. These findings suggest that CoQ10 may improve ovarian responsiveness, which is consistent with the enhancement of the mitochondrial function and cellular energy production.

Another study reported that CoQ10 administration significantly increased antral follicle count, the total number of retrieved oocytes and the number of metaphase II (MII) oocytes in women with (POR) compared to the control group. [48]. Nevertheless, the same was not observed in regard to MII oocytes rate [48]. Regarding women with DOR, CoQ10 pretreatment appears to increase the number of oocytes retrieved across IVF/ICSI cycles and reduce cycle cancellation rate and total dose of gonadotrophins [47].

Overall, supplementation with CoQ10 was associated with an enhanced number of oocytes retrieved in both POR [41], [48] and DOR [47] populations, as demonstrated in Figure 1. Evidence regarding the number of oocytes retrieved is lacking in mixed populations. Concerning the gonadotrophins required for stimulation, one article approaching POR [41] and another DOR [47] reported that CoQ10 supplementation lowered the necessary dose, which suggests a positive influence in ovarian response. Contrarily, the retrospective controlled trial performed by Qui *et al.* did not find any difference with regard to the dose of gonadotrophins needed in women diagnosed with POR [48]. However, the daily dose of CoQ10 administered by the authors was 20 times lower than the one used by Xu *et al.*, and possibly insufficient to induce an effect [41], [48]. Contrarily, AFC were higher in Qi *et al.*, either because of a beneficial effect of low doses of CoQ10 or due to inner study differences, such as baseline patients' characteristics [48].

1.1.2. Fertilization rates and embryo quality

Supplementation with CoQ10 was systematically correlated with enhanced number of embryos with good quality either in POR, [41], [48] or DOR [47] populations (Figure 1). Nonetheless, the rate of good quality embryos was not unanimously associated with positive outcomes in POR population upon CoQ10 supplementation [48]. This may be due to the fact that the number of embryos was higher alongside the number of oocytes

retrieved, resulting in a proportional enhancement in both total and good-quality embryos without affecting embryo quality rate. The effects regarding embryo quality were not reported in mixed population. The fertilization rate was only evaluated in POR and was enhanced when the dose of CoQ10 supplementation was higher [41].

1.1.3. Implantation, clinical pregnancy rates and live birth rates

CoQ10 has been correlated with the enhancement of the aforementioned parameters, although not consistently [41], [47], [48], [49]. The systematic review and meta-analysis by Florou *et al.*, which analyzed five RCT's, demonstrated that CoQ10 supplementation significantly enhanced CPR, both generally and particularly among women with POR and PMOS when assessed separately [49]. Nevertheless, LBR and miscarriage rate did not exhibit a significant difference between the groups analyzed. In a different study by Qi *et al.*, the findings indicated that implantation, CPR and LBR improved with CoQ10 treatment in women with POR [48]. Nonetheless, the research conducted by Xu *et al.* indicated a positive trend of CPR towards enhancement, however it was not statistically significant [41]. LBR also did not show a statistically significant difference [41]. The study conducted by Lin *et al.* has associated the oral supplementation of women with DOR with increased CPR and lower miscarriage rates [47].

Broadly speaking, CoQ10 appeared to enhance CPR in both mixed population and DOR, without any noted effect in POR, likely due to specific biological characteristics of women in this group [41], [47], [48], [49]. Even though the number of good quality embryos is improved intragroup after supplementation, it may not still be comparable to the other two groups and may not be sufficient to enhance CPR, as this condition is often associated with less transferable embryos [41], [48]. Cycle cancellation rates may corroborate this hypothesis, since they are lower in DOR [47], but not significantly different in POR [41], [48]. Nevertheless, data regarding mixed population is missing. Implantation rate was solely studied in one research centered on POR, with CoQ10 providing a statistically significant enhancement [48]. LBR were not improved in any of the analyzed studies, likely because this outcome is influenced by multiple factors beyond improvements in the aforementioned biological parameters, such as fetal development.

1.1.4. Miscarriage rates

Supplementation with CoQ10 lowered MR in a systematic review and meta-analysis conducted by Lin *et al.* in women diagnosed with DOR undergoing IVF/ICSI [47]. However, the same was not observed in other studies either in women with POR [41], [48], or women with diverse characteristics [49]. These results underscore the need for more studies in order to formulate any hypothesis.

1.1.5. Overall interpretation

Remarkably, several outcomes in women with DOR [47] were ameliorated with CoQ10 supplementation, with greater benefits observed compared with POR and mixed population [41], [48], [49]. Women with DOR were reported to have, in comparison with women with NOR, increased oxidative stress and inflammation on follicular fluid, along with reduced antioxidant capacity [50]. Moreover, DOR has also been associated with disrupted energy metabolism in granulosa cells, as reduced glucose uptake, downregulated main substances in energy pathways, and impaired mitochondrial function were observed [51]. Taking this into account, the overall better efficacy of CoQ10 supplementation in women diagnosed with DOR may be due to the fact that it has the ability to counteract the increased oxidative stress and mitochondrial alterations in these individuals.

Taking all the evidence into account, CoQ10 may be considered as an adjuvant intervention, and particularly in DOR, as an attempt to improve reproductive outcomes in women undergoing ART. Regarding dose and duration, only two articles provided specific information on these parameters, with considerable different in the reported regimens. Consequently, the available evidence is insufficient to recommend a specific dose or treatment duration.

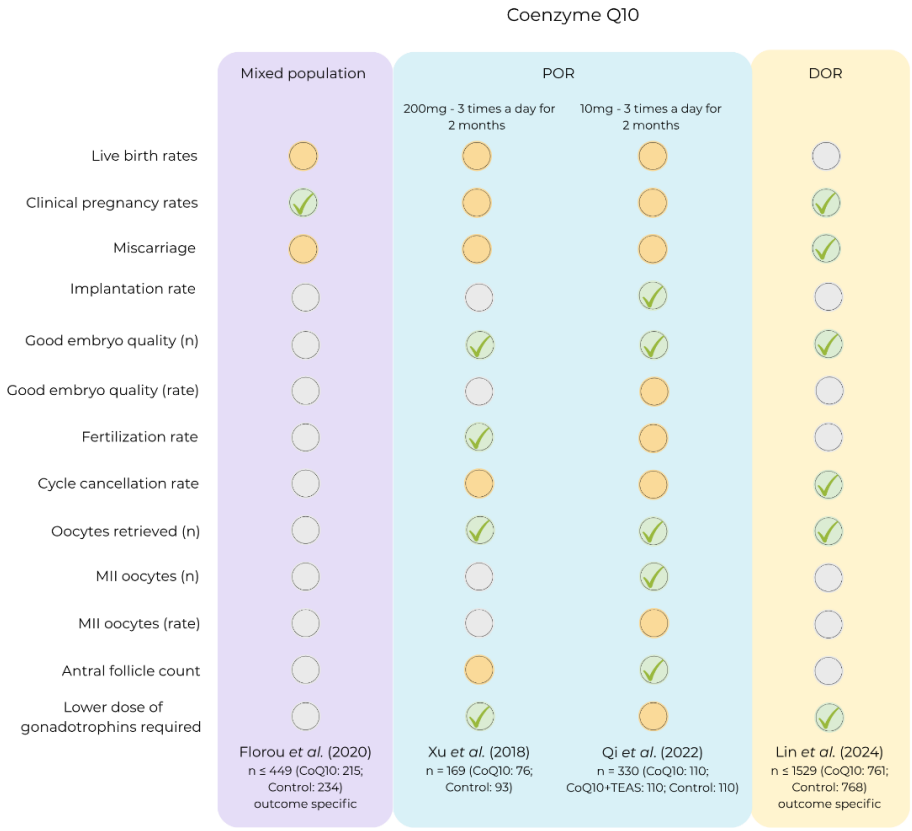


Figure 2 - Graphical representation summarizing the studied articles and the main outcomes of CoQ10 supplementation in female patients on assisted reproductive outcomes across different patient populations (Mixed population in lilac, POR in light blue, and DOR in light yellow. Columns represent group population and the respective doses and treatment duration in the case in which they were explicitly discriminated in the articles. The respective references and number of patients are below the columns. The articles which analysed for each outcome a different sample number, are indicated. Rows display main reproductive outcomes and the results for each one in the specific article. Green circles correspond to a statistically significant enhancement after treatment, orange circles to a non-statistically significant difference upon treatment and grey circles to not evaluated outcomes.

1.2. Effects of Coenzyme Q10 supplementation in men

1.2.1. Sperm quality and reproductive outcomes

Akhigbe *et al.* conducted a systematic review and meta-analysis of eight RCTs in which the semen parameters of a total of 877 male subjects with ages ranging from 18 and 50 were evaluated (462 underwent CoQ10 supplementation and 415 were untreated) [52]. Interestingly, Co10Q10 supplementation led to an increase in total sperm count, total and progressive motility and in the ratio of sperm with normal morphology [52]. The latter parameter was observed in men supplemented from over three months with single therapy, but not in men with shorter treatments and as a combined therapy [52]. However, no differences were observed in sperm concentration and ejaculate volume [52]. It should be taken into account that publication bias was observed in all the

mentioned parameters. A similar pattern was observed relatively to inner-study heterogeneity, with the exception of ejaculate volume [52]. The systematic review and network meta-analysis of randomized controlled trials by Su *et al.* demonstrated that sperm concentration was increased by CoQ10 supplementation, with statistically significant differences when compared to the placebo [53]. In one of the statistical tests, it was the only supplement between the analyzed ones (folic acid, zinc, VitE, LC, selenium, CoQ10, N-acetylcysteine, and vitamin C) to achieve a statistical difference [53]. Regarding motility and normal morphology, both were significatively enhanced by CoQ10 supplementation in one test, but only motility had a significant difference in another [53]. The randomized controlled trial study by Bahar *et al.* analyzed 30 participants with idiopathic infertility in each CoQ10 and placebo groups, with the mean ages of 34.07 (5.26) and 34.83 (6.22), respectively [54]. The treatment consisted in daily capsules containing 100 mg CoQ10 for three months [54]. It was reported that only the normal morphology achieved a significant increase in the CoQ10 supplemented group [54]. The remaining analyzed parameters, such as the volume and viscosity of semen, sperm count and sperm motility, had a non-significant increase [54]. In another study, a systematic review and meta-analysis of randomized controlled trials, 781 participants with idiopathic infertility were divided in two groups, in which 430 received CoQ10 treatment and 351 corresponded to the control [44]. The concentration, ejaculate volume, total sperm motility and morphology (in treatment with duration superior to three months) were increased, along with seminal CoQ10 levels [44].

Alahmar *et al.* published several articles over the last years related to CoQ10 supplementation of men with idiopathic oligoasthenoteratozoospermia (OAT) [43], [55], [56]. In a prospective study, the supplementation of men with idiopathic OAT for three months with 200 mg/day (n=35) or 400 mg/day (n=30) of CoQ10 was correlated with a significant increase in sperm concentration as well as in total and progressive motility [55]. These improvements were more accentuated in the 400 mg group, which suggests that a greater dosage may have more beneficial effects [55]. In a subsequent prospective controlled study, 50 patients with idiopathic OAT and 50 fertile men who served as controls received 200 mg of CoQ10 orally and daily for a total of three months [43]. The sperm concentration and total and progressive motility were also enhanced when compared to baseline values [43]. In another prospective controlled study, 50 patients with idiopathic oligoasthenozoospermia (OA) and 35 fertile controls were analyzed [56]. All patients received oral CoQ10 (300 mg/day) daily for three months [56]. After CoQ10 therapy, total sperm and progressive motility were significantly higher [56]. Nonetheless,

there was a non-significant reduction in sperm normal morphology after CoQ10 treatment [56].

1.2.2 Overall interpretation

In general, CoQ10 supplementation in men appears to improve several parameters of sperm quality. As shown in Figure 2, total sperm motility was systematically enhanced across all the populations, when studied [43], [44], [52], [53], [55], [56]. The two articles analyzed regarding OAT reported higher progressive motility [43], [55]. Moreover, the only article that evaluated OA also showed higher progressive motility [56]. However, conflicting results were observed in non-specific subfertility or infertility [52], [54]. These outcomes are probably the result of enhanced mitochondrial ATP production and reduced oxidative stress mediated by CoQ10 [40]. The more pronounced effects in OAT and OA could be due to higher baseline levels of oxidative stress and mitochondrial dysfunction in these conditions, although evidence to support it is missing. Sperm concentration was consistently improved on idiopathic male infertility [44], idiopathic OAT [43], [55], and OA [56]. As for unspecific infertile or subfertile men, the results were inconsistent between studies [52], [53]. Normal morphology was enhanced in non-specific infertility [52], [53], [54] and idiopathic male infertility [44], but not in OAT [43], [55] or OA [56], likely because in the latter two, the abnormalities are more severe and cannot be reversed by CoQ10 supplementation. Interestingly, two studies reported that this effect was only noticed after three months of supplementation [44], [52]. Sperm volume was only improved in idiopathic infertility [44], whereas two studies in non-specific infertility [52], [54], two in OAT [43], [55] and one in OA reported no difference [56]. Total sperm counts were only higher in one article [52], without any differences in the others that assessed this parameter [44], [54]. Higher clinical pregnancy rates were only reported in one article in idiopathic male infertility, with a positive correlation with supplementation [44]. More studies including this outcome are necessary in order to understand if CoQ10 supplementation also has effects beyond upstream outcomes. Sperm viscosity does not seem to be improved by CoQ10 supplementation [44], [54].

Taking this evidence into account, CoQ10 supplementation may be prescribed to the general men with any type of infertility or subfertility, and specifically in men with severe forms of infertility, such as idiopathic OAT and OA. The best dose and duration probably range between 100 and 300 mg daily for a minimum of three months.



Figure 3 - Graphical representation summarizing the studied articles and the main outcomes of CoQ10 supplementation in male patients on assisted reproductive outcomes on assisted reproductive outcomes across different patient populations:
A: Unspecific subfertile/infertile men in light blue and idiopathic male infertility in darker blue;
B: OAT in light green and OA in darker green.
Columns represent group population and the respective doses and treatment duration in the case in which they were explicitly discriminated in the articles. The respective references and number of patients are below the columns. The articles which analysed for each outcome a different sample number, are indicated. Rows display main reproductive outcomes and the results for each one in the specific article. Green circles correspond to a statistically significant enhancement after treatment, orange circles to a non-statistically significant difference upon treatment and grey circles to not evaluated outcomes.

2. Myo-inositol

MI is a naturally occurring cyclic sugar alcohol which functions as an insulin-sensitizing agent [57], [58]. Since it reduces insulin resistance, a condition associated with PMOS, it can be beneficial in ovulation induction in those patients [59], [60]. Moreover, MI acts as a second messenger of FSH in the ovary, supporting the maturation and proliferation of granulosa cells [57], [59]. Furthermore, MI has also been reported to enhance aromatase (CYP19A1) expression and activity, thereby promoting the conversion of androgens into estrogens [57]. These mechanisms may improve oocyte maturation and embryo development, supporting the use of MI as an adjuvant in assisted reproductive techniques

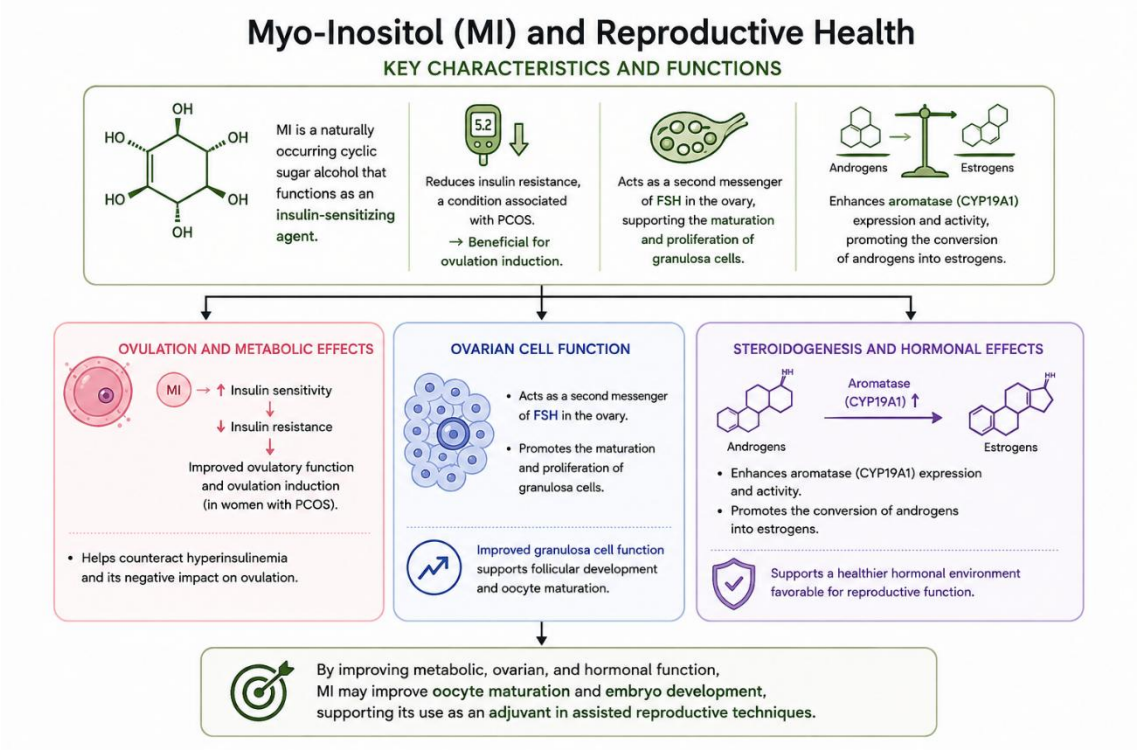


Figure 4 - Schematic representation of the proposed role of MI in female reproductive health. Created using artificial intelligence (AI).

2.1. Effects of Myo-inositol supplementation in women

2.1.1. Ovarian response and oocyte quality

Beneficial effects on ART outcomes have been linked to MI [61], [62], [63], [64], [65], [66]. A study correlated significantly higher mean number of oocytes and MII oocytes to MI supplementation in women who went through IVF or ICSI cycles [61]. Contrarily, the meta-analysis and systematic review by Zheng *et al.* did not confirm any impact MI supplementation on the quantity of oocytes recovered and MII stage oocytes obtained during ICSI or IVF with embryo transfer [62]. Nevertheless, the MI group had substantially fewer degenerated oocytes and the dose of gonadotrophins required was lower [62].

Regarding women with PMOS, the study by Tabatabaie *et al.* linked supplementation to a higher percentage of MII oocytes but found no difference relatively to the number of oocytes retrieved [63]. Zhang *et al.* demonstrated that the MII stage oocyte rate was higher when women were supplemented with MI, including in women with PMOS, but not in those with POR [64]. In accordance, MI in women with POR did not appear to have any effect on the quantity of oocytes or the MII stage ones, according to *Nazari et al.* [65]. The double-blinded randomized controlled study by Mohammadi *et al.* indicated that in the group supplemented with Inofolic (4 g MI + 400 µg folic acid) the number of oocytes retrieved and MII oocytes was higher, although not statistically significantly, when compared with folic acid supplementation (400 µg) for the same period [66]. Nevertheless, the ovarian sensitivity index and the required dose of gonadotropin were significantly lower in the MI group [66].

Generally, the rate of MII oocytes was improved by MI supplementation in women with PMOS [63], [64]. Contrarily, in POR, neither MII oocytes rate nor number was enhanced [64], [65], [66]. Regarding mixed populations, the results were inconsistent, as well as regarding the number of oocytes retrieved [61], [62]. The difference in sample number could be the reason for discrepancy between these analyzed outcomes. The improvement in MII oocyte rates in women with PMOS, but not in POR and inconsistently in mixed population are results probably associated with MI-specific mechanisms of action in women diagnosed with PMOS. Increased oocyte retrieval in POR and PMOS was not observed in any of the analyzed articles [63], [64], [65], [66]. The dose of gonadotrophins required was lower in mixed population, POR and PMOS after supplementation [62], [64], [66]. However, only one article for each population evaluated the dose of gonadotrophins needed [62], [64], [66].

2.1.2. Fertilization rates and embryo quality

Supplementation with MI appears to improve fertilization rates in both POR and PMOS populations [63], [64], [65], [66], as illustrated in Figure 3. Further research supports that the number of fertilized embryos was higher in mixed population, without evaluating fertilization rates itself [61]. The number of embryos with good quality was substantially higher in the study by Nazari *et al.*, in women with POR [65]. Nevertheless, no other article on a similar population supported that outcome, and one article did not corroborate any difference regarding good embryo quality rates [64]. As for mixed population, an article which assessed the number of good quality embryos reported that there was no enhancement elicited by MI supplementation [61], but another observed a higher good embryo quality rate in the supplemented group [62]. In PMOS, only one study evaluated the rate of good quality embryos, with a positive effect [63].

2.1.3. Implantation, clinical pregnancy rates and live birth rates

MI is reported to have an effect on clinical pregnancy rates [61], [62], [64], [65], [66]. The double blind randomized controlled trial by Seyedoshohadaei *et al.* verified an enhancement in CPR and LBR in women undergoing IVF/ICSI cycles [61]. Nonetheless, in a meta-analysis and systematic review of published articles concerning IVF or ICSI with embryo transfer, CPR improved, while LBR did not [62]. As for women with POR, a double-blinded randomized controlled study reported a non-significant increase in chemical and CPR in the MI intervention group [66]. Moreover, in another randomized controlled trial conducted in women with POR, implantation and CPR were not enhanced among the supplemented group [65]. Regarding women with PMOS, MI's supplementation was not correlated with a statistically significant higher CPR [64].

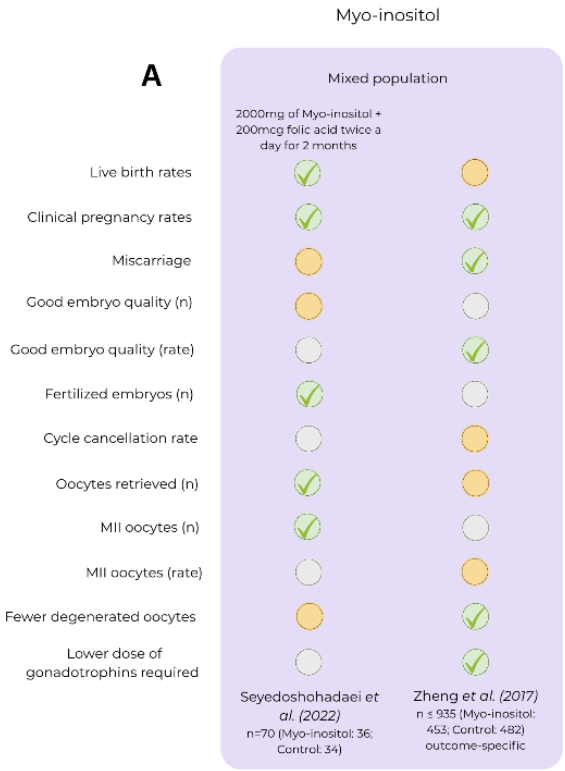
However, in POR and PMOS, none of the articles were able to correlate MI to enhanced clinical pregnancy rates [63], [64], [65], [66]. The results regarding LBR in patients with diverse characteristics were not homogeneous, since one article associated supplementation with MI to enhanced live birth rates [61], but another did not [62]. Although in one article LBR were higher in the supplemented group in women diagnosed with POR [64], more studies are necessary in order to determine the effect of supplementation on those patients.

2.1.4. Miscarriage rates

The systematic review and meta-analysis by Zheng *et al.* reported that MR were diminished after MI supplementation, in a heterogeneous group of patients [62]. Contrarily, this evidence was not supported by any other evaluated article, either in diversified population [61] or POR [66].

2.1.5. Overall interpretation

MI appears to enhance general and population-specific outcomes, and so its administration can be thought-out. The most consistent observation was the enhancement of fertilization rates, which suggests a beneficial effect in oocyte competence. In women with PMOS, supplementation was also associated with a higher rate of MII oocytes, good embryo quality, higher MII oocytes rate and lower dose of gonadotrophins needed. In POR, improvements in LBR, embryo quality and lower doses of gonadotrophins were reported. In mixed population, CPR were higher, as well as the rates of good embryo quality, number of fertilized embryos, and number of MII oocytes. Moreover, the dose of gonadotrophins was lower. Regarding dose and duration of treatment, more studies are needed in order to determine the best option.



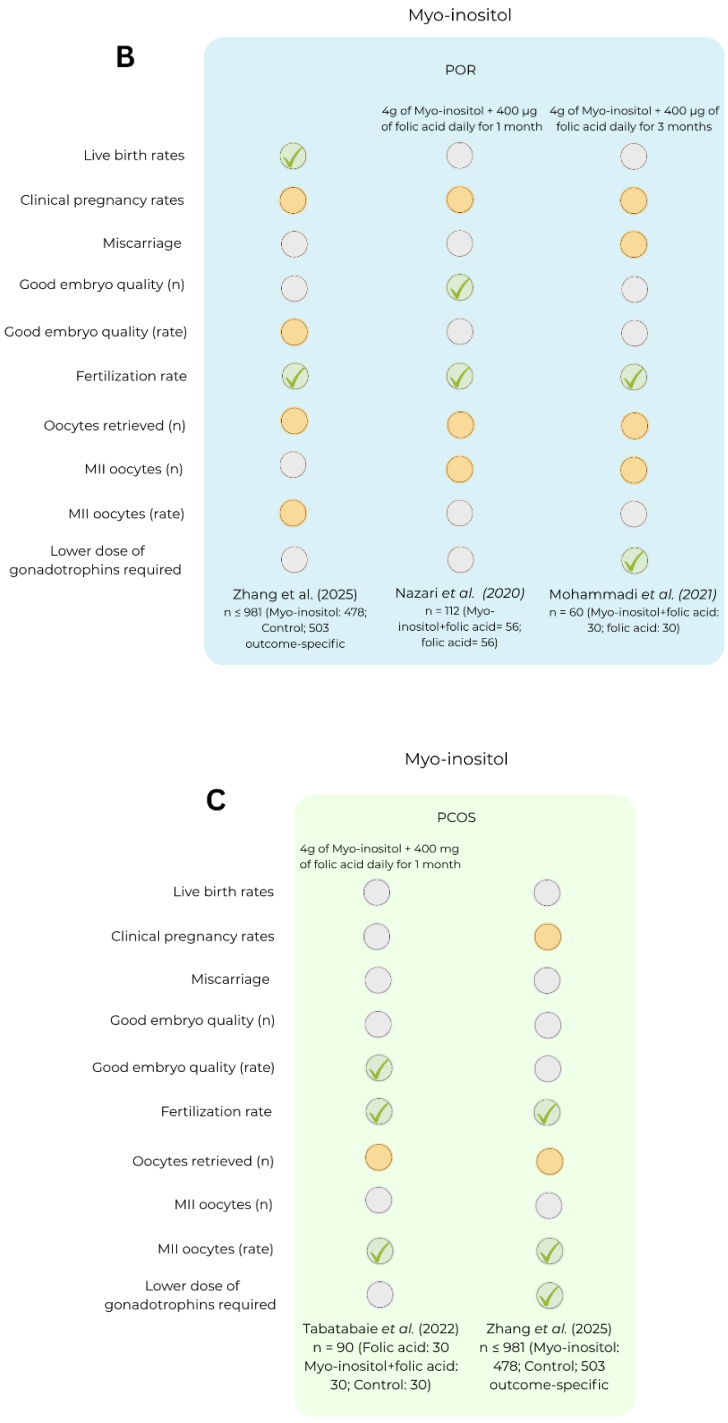


Figure 5 - Graphical representation summarizing the studied articles and the main outcomes of MI supplementation in female patients on assisted reproductive outcomes across different patient populations.
A: Mixed population in lilac,
B: POR in light blue;
C: PMOS in light green.
Columns represent group population and the respective doses and treatment duration in the case in which they were explicitly discriminated in the articles. The respective references and number of patients are below the columns. The articles which analysed for each outcome a different sample number, are indicated. Rows display main reproductive outcomes and the results for each one in the specific article. Green circles correspond to a statistically significant enhancement after treatment, orange circles to a non-statistically significant difference upon treatment and grey circles to not evaluated outcomes.

3. Melatonin

MEL is a neurohormone secreted by the pineal gland, although it is also produced in oocytes, granulosa cells and luteal cells, contributing to a higher MEL concentration in the follicular fluid of Graafian follicles than in the blood of healthy women [67]. Beyond its role in regulating circadian rhythms, MEL is a potent antioxidant that directly scavenges reactive oxygen and nitrogen species (ROS and RNS), thereby reducing oxidative stress and protecting mitochondrial and nuclear DNA from oxidative damage [68]. Consequently, MEL supplementation has been investigated as an adjuvant therapy in IVF, where it has shown promising effects on oocyte quality and fertilization rates in women undergoing IVF [68].

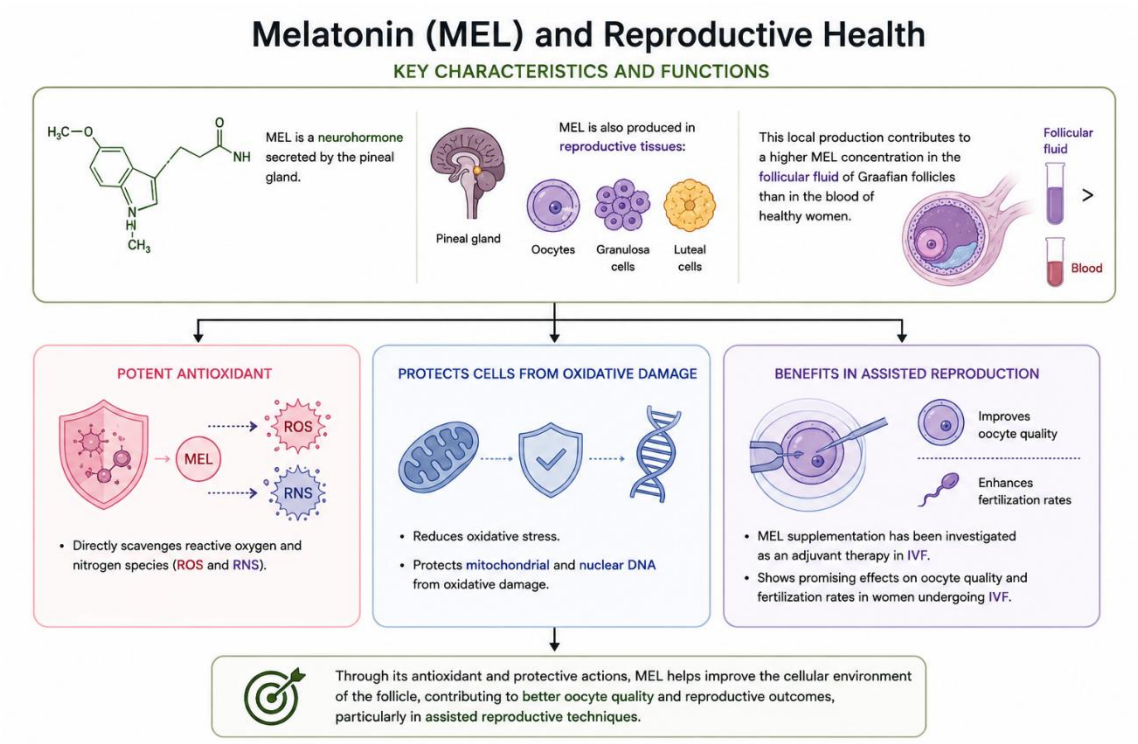


Figure 6 - Schematic representation of the proposed role of MEL in female reproductive health. Created using artificial intelligence (AI).

3.1. Effects of Melatonin supplementation in women

3.1.1. Ovarian response and oocyte quality

MEL has been studied as a possible contributor to enhanced number of oocytes retrieved and maturated oocytes [69], [70], [71], [72], [73]. Espino *et al.* verified that MEL significantly improved the number of oocytes retrieved, independently of the dose administered in women with unexplained infertility [69]. Moreover, the proportion of MII oocytes was also higher in the MEL supplemented group [69]. In line with this, Hu *et al.* reported an increased number of oocytes collected and maturated oocytes [74]. The results, however, are not homogeneous. Tang *et al.* observed a significantly enhanced number of MII oocytes in MEL supplemented group, but not on the number of oocytes retrieved [70]. Moreover, MEL in addition to folic acid, increased the number of MII oocytes [70]. These authors also studied the MEL supplementation in women with PMOS and normal ovarian reserve (NOR) and conclude that it increased the number of MII oocytes in the first group, but not in the latter [70]. In another article, a higher mature oocyte count was observed, but the total number of oocytes and mature oocytes were not verified [71]. An article showed no difference in total oocyte number and number of mature oocytes in women who underwent IVF [72], and another reported a statistically significant increase in number of patients with DOR who had mature MII oocytes, but not in mean number of MII oocytes, although a positive trend has been observed [73].

3.1.2. Fertilization rates and embryo quality

MEL has been studied regarding its effect on embryo quality and fertilization rates [69], [70], [71], [72], [73], [74]. Fertilization rates upon MEL supplementation were consistently increased in three analyzed articles concerning mixed populations and in the only article in women with unexplained infertility (UI), as represented in Figure 4 [69], [70], [72], [74]. Evidence for this outcome is missing from DOR patients. The number of good quality embryos was enhanced in both women with UI and DOR [69], [73]. Nevertheless, only one article analyzed this outcome in UI and DOR, and so any inference should be cautiously done [69], [73]. As for mixed population, two articles correlated MEL supplementation with enhanced number of good quality embryos [70], [74], but two articles stated that there was no difference [71], [72]. Regarding retrieved oocytes, a higher number was observed in two articles on mixed populations [71], [74], but Tang *et al.* and Fernando *et al.* iterated that there was no difference [70], [72]. Antral follicle counts were not different in DOR [73].

3.1.3. Implantation, clinical pregnancy rates and live birth rates

Some studies associated supplementation with MEL in mixed population with enhanced CPR [70], [74]. However, there are studies which did not demonstrate a statistically significant difference [71], [72]. Moreover, LBR do not seem to be affected by MEL intake [70], [71], [72], [74]. In women with unexplained infertility or DOR, supplementation with MEL did not enhance CPR [69], [73].

3.1.4. Overall interpretation

Based on the evidence, MEL may have a more relevant impact in downstream outcomes, probably due to effects on ovarian response and oocyte quality and competence, which is consistent with its antioxidant properties [68]. Nonetheless, it should be noted that the results were heterogeneous, and more studies are required for further inferences and to determine an effective regimen.

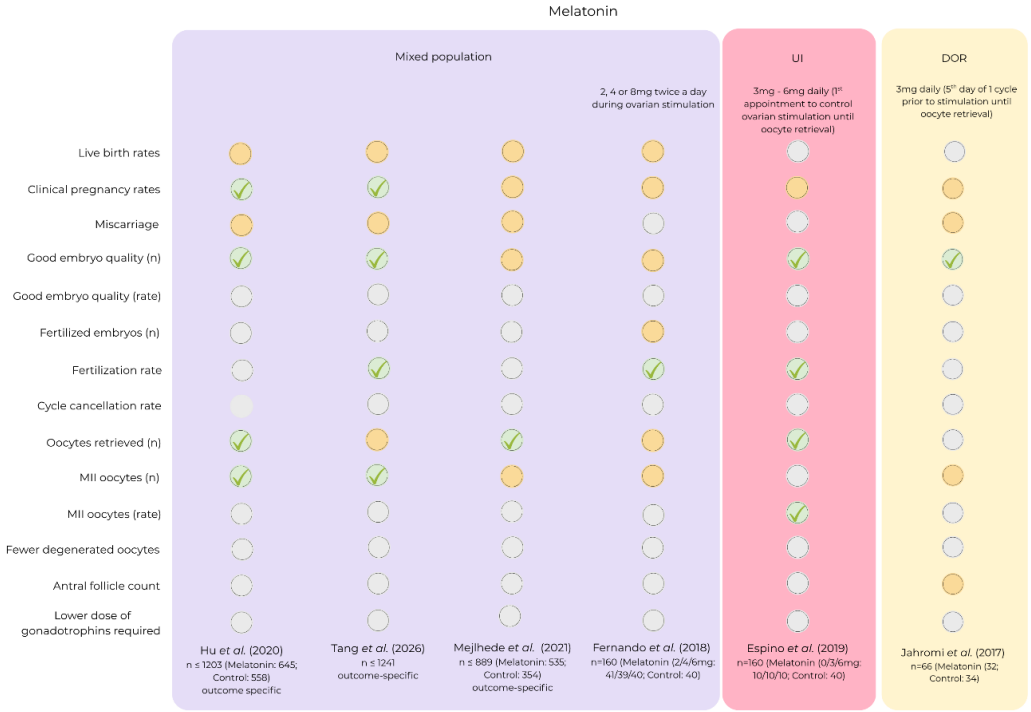


Figure 7 - Graphical representation summarizing the studied articles and the main outcomes of MEL supplementation in female patients on assisted reproductive outcomes on assisted reproductive outcomes across different patient populations: Mixed population in lilac; UI in pink and DOR in light yellow. Columns represent group population and the respective doses and treatment duration in the case in which they were explicitly discriminated in the articles. The respective references and number of patients are below the columns. The articles which analysed for each outcome a different sample number, are indicated. Rows display main reproductive outcomes and the results for each one in the specific article. Green circles correspond to a statistically significant enhancement after treatment, orange circles to a non-statistically significant difference upon treatment and grey circles to not evaluated outcomes.

4. Vitamin D

VitD is a fat-soluble secosteroid hormone synthesised mainly in the skin following ultraviolet B (UVB) exposure, although it can also be obtained through dietary sources and supplementation [75]. Following hepatic and renal hydroxylation, VitD is converted into its biologically active form, calcitriol [76]. In addition to its classical role in calcium homeostasis, VitD may contribute to reproductive function through the VitD receptor (VDR), which is expressed in ovarian tissue and involved in follicular development, steroidogenesis and oocyte maturation [76]. VitD deficiency has been associated with insulin resistance and ovulatory dysfunction, particularly in women with PMOS [76]. In men, VitD status has also been associated with semen quality, particularly sperm concentration and motility [77], [78].

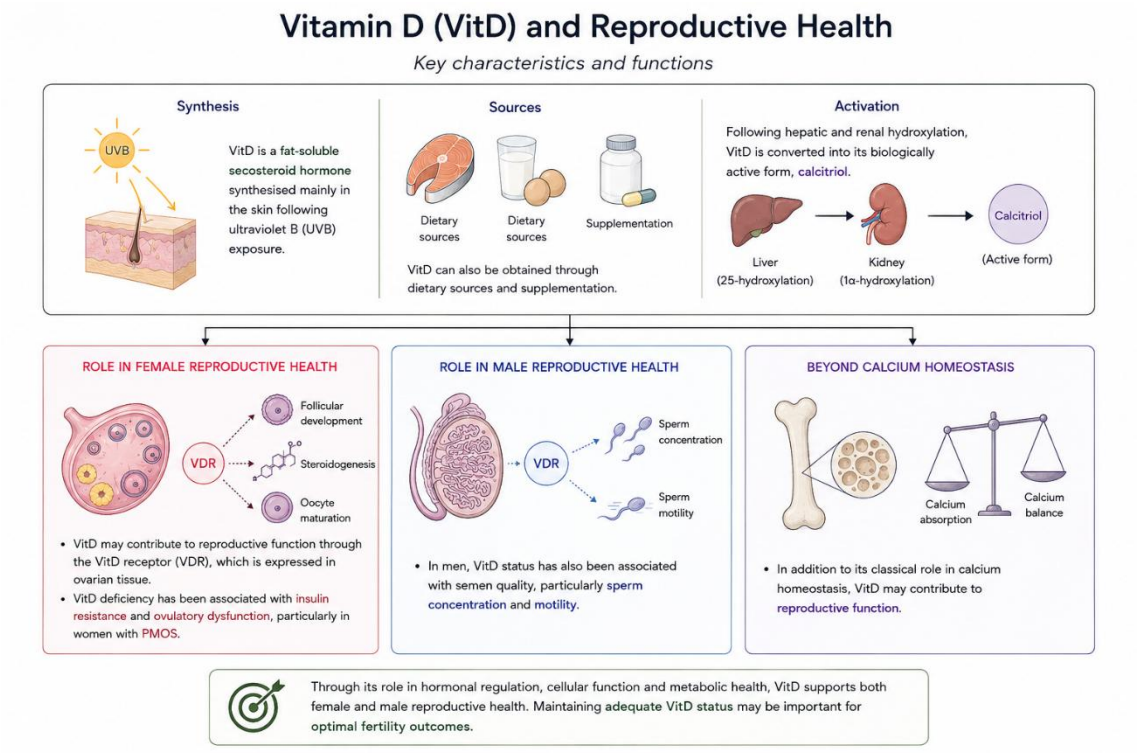


Figure 8 - Schematic representation of the proposed role of VitD in both female and male reproductive health. Created using artificial intelligence (AI).

4.1. Effects of Vitamin D supplementation in women

4.1.1. Fertilization rates and embryo quality

VitD is one of the most studied supplements when it comes to women fertility. In respect of embryo quality, there are various articles which study its impact [79], [80], [81], [82], [83], [84], [85], [86].

Regarding VitD supplementation, only in one article fertilization rates were reported to be improved, namely in women diagnosed with PMOS [79]. Christoforidis *et al.* evaluated the number of fertilized embryos, without proving supplementation's efficacy [80]. As for embryo quality, some research could not support VitD efficacy [79], [82].

Higher serum or follicular fluid VitD levels were stated to improve the number of good embryos obtained [81]. Contrarily, several articles could not correlate VitD levels to embryo quality [83], [84], [85], [86] and fertilization rates [82], [84], [85], [86] in different populations.

4.1.2. Implantation, clinical pregnancy rates and live birth rates

VitD has been linked to higher CPR [79], [87]. The supplementation of VitD has been reported to enhance CPR with regard to IVF, ICSI, fresh embryo transfer cycles, and frozen embryo transfer [87]. Nonetheless, the same cannot be iterated as for implantation rates. Abedi *et al.* reported enhanced CPR and biochemical pregnancy rates [82]. Another meta-analysis, focused on women with PMOS demonstrated that supplementation enhanced CPR [79]. Christoforidis *et al.* also found an insignificant result in LBR and CPR in frozen embryo transfer cycles [80].

As for blood, serum or follicular VitD levels, the evidence is more homogeneous. Various articles associate higher levels of VitD with increased CPR [83], [84], [86], [88], [91]. Wang *et al.* interestingly observed in IVF cycles that CPR were diminished in the advanced age group with VitD deficiency but were not altered in the young group [86]. Zhao *et al.* meta-analysis correlated deficient levels of VitD to a trend to lower CPR, but higher ongoing pregnancy [90]. Illiuta *et al.* observed a higher biochemical pregnancy and ongoing pregnancy in the sufficient VitD group, but only an insignificant trend towards higher CPR [91]. Moreover, some articles failed to prove CPR increase when VitD levels were higher in mixed population [83], [85], [92]. Enhanced LBR were also correlated with higher blood, serum or follicular VitD levels, either in a population with different patient's characteristics [88], [89], [90], [91]. However, some research could not

find any difference between control and treatment [81], [85]. Implantation rates were positively correlated to serum VitD levels in the study by Wang *et al*. [86]. Nonetheless, the observed inconsistent pattern in other outcomes was also verified, as several articles failed to prove that VitD levels influence implantation rates [88], [91], [92].

In a general way, VitD supplementation was more prominently associated with enhanced pregnancy rates, in mixed population or PMOS [79], [82], [87], as shown in Figure 5. However, one article reporting mixed population had conflicting findings, reporting no difference in CPR after VitD supplementation [80]. Regarding biochemical pregnancy rates, one article showed that VitD supplementation was correlated with higher levels in mixed population [82]. Contrarily, studies involving mixed population and PMOS, did not observe any statistically significant difference [79], [87]. Only in women with PMOS, MR, fertilization rates and MII oocytes rate were enhanced upon supplementation [79]. The dosage ranges are comparable among both groups.

As for VitD levels, there is considerably more evidence regarding the outcomes studied, in accordance with Figure 6. A total of five articles reported enhanced CPR in women from a diversified group with higher VitD levels [81], [84], [86], [88], [89]. Nonetheless, four articles could not show a positive correlation between VitD levels and CPR in mixed populations [83], [85], [90], [91], as well one considering NOR was also not verified to have any difference [92]. Increased live birth rates were also associated with higher VitD levels in mixed population [88], [89], [90], [91]. Nevertheless, the same pattern was observed, with research performed on mixed populations claiming that the difference was not significant [81], [85]. The same can be observed regarding biochemical pregnancy rates. Three studies reported higher rates in women with higher VitD levels, but one study did not [84], [88], [89], [91]. Although in one study, implantation rates were enhanced by increased levels in mixed population, in two studies in mixed population and in one study for NOR there was no difference [86], [88], [91], [92].

4.1.3. Miscarriage rates

Solely one article from the analysed literature stated that VitD supplementation had a positive influence on MR, namely in women with PMOS [79]. Taken this into account, VitD supplementation does not seem to influence MR in most cases [80], [87]. Moreover, VitD levels also did not affect MR in several groups with different characteristics [85], [86], [88], [89], [91], [92].

4.1.4. Overall interpretation

VitD supplementation seems to be associated with increased positive outcomes in PMOS patients when compared to mixed population. This is likely due to specific effects within this subgroup that are possibly diluted when observing general population. Notably, PMOS is often associated with VitD deficiency, which is a possible contributor to elevated parathyroid hormone, as well as impaired menstrual cycle, anovulation, insulin resistance and hyperandrogenism [79], [93]. Thus, VitD deficiency seems to worsen PMOS symptoms [93]. Further studies suggest that VitD supplementation is able to reduce both androgen and anti-müllerian hormone levels, improve menstrual regularity and folliculogenesis, and is positively associated with ovulation probability in women with PMOS [76].

VitD supplementation may be considered especially in the case of insufficiency or deficiency, and in women diagnosed with PMOS. As previously referred, VitD supplementation was more markedly associated with improved outcomes in women with PMOS when compared to other patient's characteristics. Notably, VitD supplementation in PMOS was associated with enhanced CPR, fertilization and MII oocytes rates and diminished miscarriage [79]. Although the results in PMOS were more relevant, beneficial effects from supplementation were also observed in other populations, such as enhanced CPR and biochemical pregnancy [82], [87]. The same can be said regarding VitD levels improvement, as LBR, CPR and biochemical pregnancy rates, embryo quality, fertilization rates, rate of MII oocytes and number of oocytes retrieved were ameliorated in some cases [81], [84], [86], [88], [89], [90], [91]. Nevertheless, these outcomes should be interpreted with caution, due to the heterogeneity of the performed research.

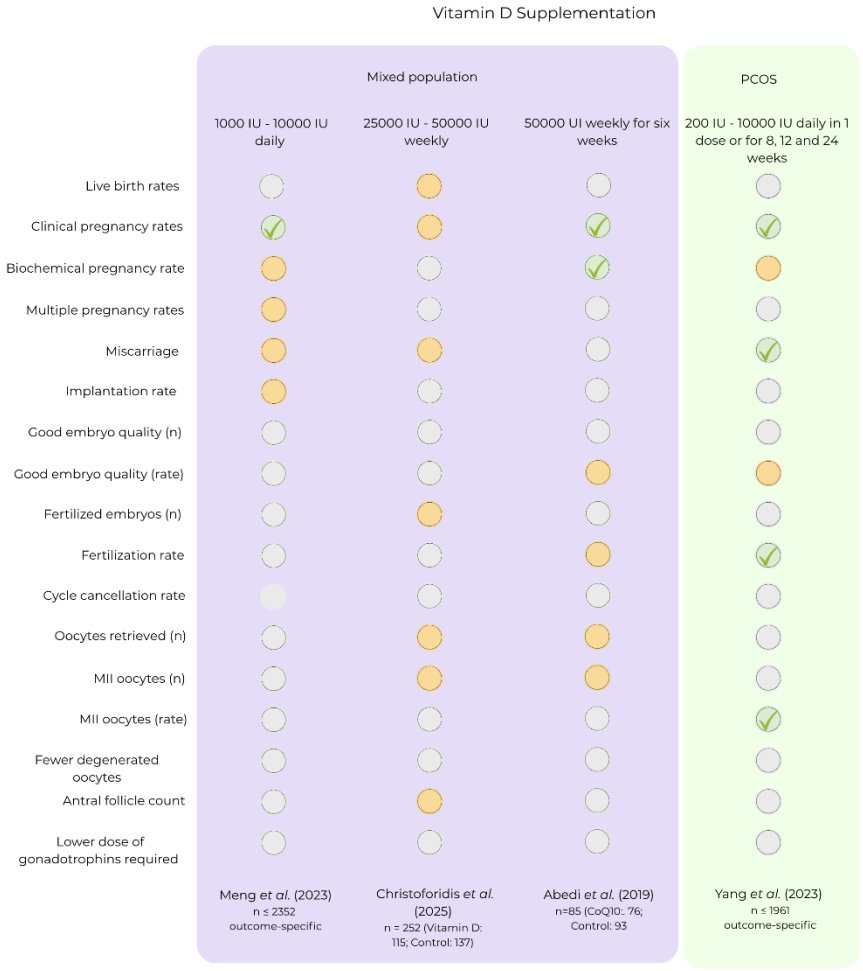
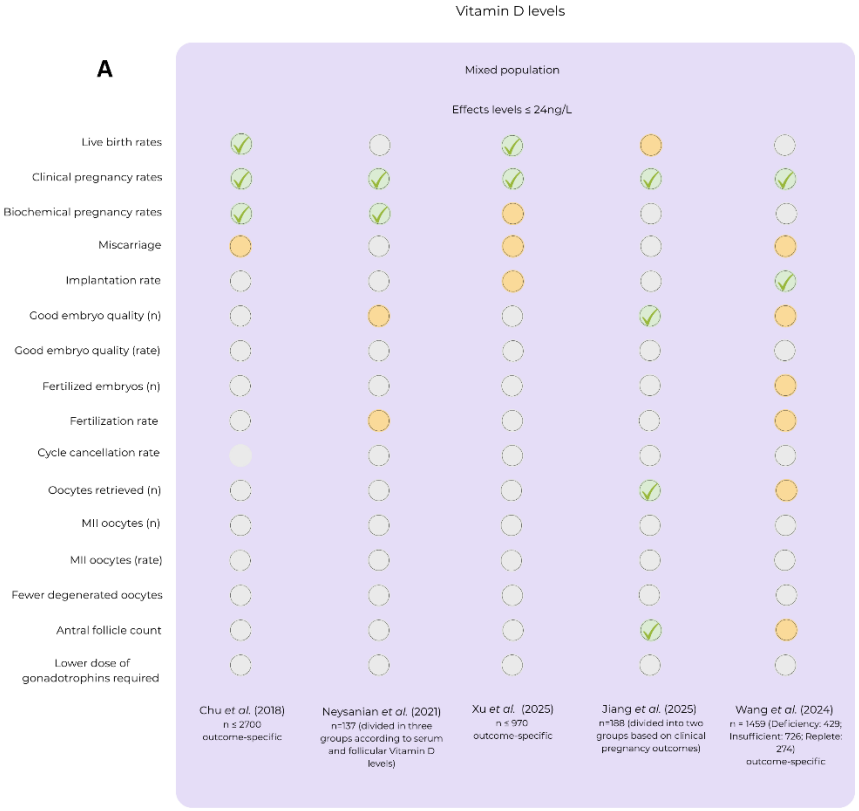
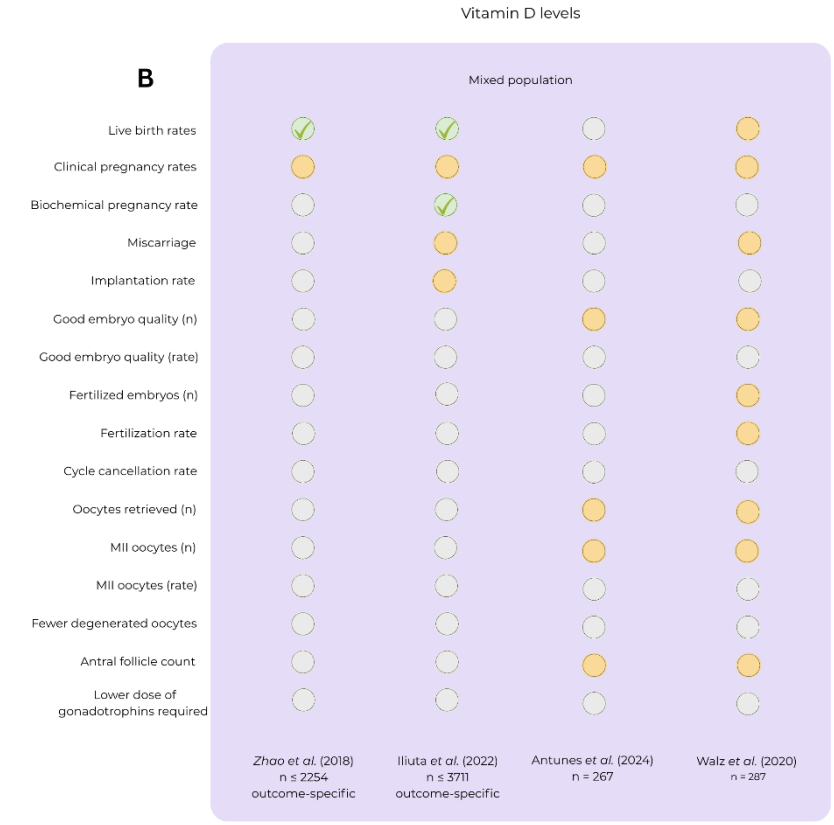


Figure 9 - Graphical representation summarizing the studied articles and the main outcomes of VitD supplementation in female patients on assisted reproductive outcomes across different patient populations (Mixed population in lilac and PMOS in light green). Columns represent group population and the respective doses and treatment duration in the case in which they were explicitly discriminated in the articles. The respective references and number of patients are below the columns. The articles which analysed for each outcome a different sample number, are indicated. Rows display main reproductive outcomes and the results for each one in the specific article. Green circles correspond to a statistically significant enhancement after treatment, orange circles to a non-statistically significant difference upon treatment and grey circles to not evaluated outcomes.


Vitamin D levels
A
Mixed population
Effects levels ≤ 24ng/L
Live birth rates
Clinical pregnancy rates
Biochemical pregnancy rates
Miscarriage
Implantation rate
Good embryo quality (n)
Good embryo quality (rate)
Fertilized embryos (n)
Fertilization rate
Cycle cancellation rate
Oocytes retrieved (n)
MII oocytes (n)
MII oocytes (rate)
Fewer degenerated oocytes
Antral follicle count
Lower dose of gonadotrophins required
Chu et al. (2018) n ≤ 2700 outcome-specific
Neysanian et al. (2021) n=137 (divided in three groups according to serum and follicular Vitamin D levels)
Xu et al. (2025) n ≤ 970 outcome-specific
Jiang et al. (2025) n=188 (divided into two groups based on clinical pregnancy outcomes)
Wang et al. (2024) n = 1459 (Deficiency: 429; Insufficient: 726; Replete: 274) outcome-specific


Vitamin D levels
B
Mixed population
Live birth rates
Clinical pregnancy rates
Biochemical pregnancy rate
Miscarriage
Implantation rate
Good embryo quality (n)
Good embryo quality (rate)
Fertilized embryos (n)
Fertilization rate
Cycle cancellation rate
Oocytes retrieved (n)
MII oocytes (n)
MII oocytes (rate)
Fewer degenerated oocytes
Antral follicle count
Lower dose of gonadotrophins required
Zhao et al. (2018) n ≤ 2254 outcome-specific
Iliuta et al. (2022) n ≤ 3711 outcome-specific
Antunes et al. (2024) n = 267
Walz et al. (2020) n = 287

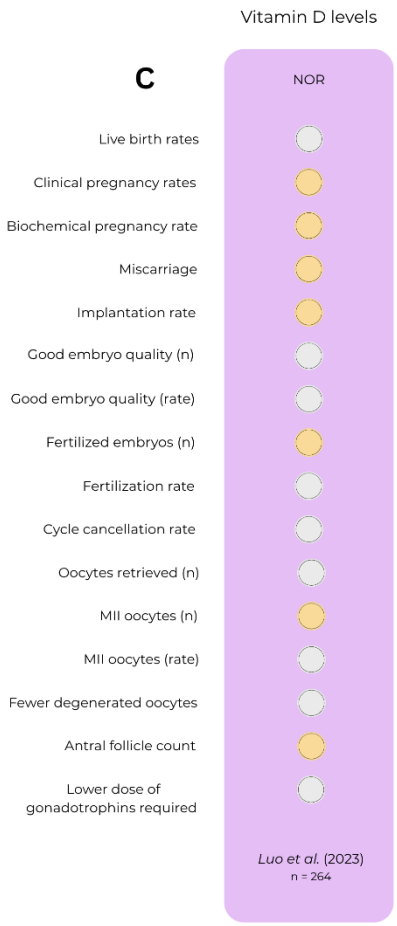


Figure 10 - Graphical representation summarizing the studied articles and the main outcomes of enhanced VitD levels in female patients on assisted reproductive outcomes across different patient populations.
A and **B:** Mixed population in lilac;
C: NOR in violet and PMOS in light green.
Columns represent group population and the levels that were effective in the case in which they were explicitly discriminated in the articles. The respective references and number of patients are below the columns. The articles which analysed for each outcome a different sample number, are indicated. Rows display main reproductive outcomes and the results for each one in the specific article. Green circles correspond to a statistically significant enhancement, orange circles to a non-statistically significant difference upon levels and grey circles to not evaluated outcomes.

4.2. Effects of Vitamin D supplementation in men

4.2.1. Sperm quality and reproductive outcomes

VitD has been studied in order to understand its effects on male fertility. Several studies reported that infertile men have lower serum levels of VitD when compared with fertile men [77], [78], [94], [95]. The most consistently observed outcomes were progressive sperm motility [94] and total motility [78], [94], [95], [96]. One study associated VitD levels with sperm concentration, morphology, and total sperm count [78]. A systematic review and meta-analysis correlated low levels of VitD to a decrease in sperm concentration,

total sperm count, motility, and progressive motility. However, no difference was observed regarding semen volume [77].

As for supplementation, studies have shown improvements in sperm motility with VitD treatment, especially in men with baseline problems [97], [98]. Additionally, some research revealed decreases in sperm apoptosis and oxidative stress indicators [97]. Nevertheless, despite supplementation, no appreciable changes in semen parameters were observed in other studies [99]. Notably, Jensen *et al.* noted that live birth rates were higher only in oligozoospermic men [99].

Regarding non-specific infertility or subfertility population, VitD supplementation improved total sperm count [98] and live birth rates per transfer [99], but only one article was analyzed for each outcome, as represented in Figure 7. Progressive motility was enhanced in one article [98], but another reported no difference [99]. No other outcome was influenced by VitD supplementation in patients with those characteristics, including normal morphology, total motility, sperm concentration and sperm volume [99]. As for asthenozoospermic men, progressive motility was increased in the only study which evaluated it [97]. Moreover, total motility was enhanced in one study [97], although not consistently [99]. Normal morphology, sperm concentration and volume were not significantly different in any article [97].

4.2.2. Overall interpretation

VitD levels were positively correlated with increased total motility most of the articles analyzed in unspecific infertility or subfertility [94], [95], [96], as illustrated in Figure 8. Progressive motility was consistently enhanced in one study [94]. As for total sperm count and concentration, the results were conflicting, but the majority of the research supported improved outcomes [94], [95], [96]. Normal morphology was higher in one study which evaluated it and had no significant differences in the other two [94], [95], [96]. Regarding sperm volume, only one article supports a significant positive difference [94], [96]. Regarding a broader population, with either fertile, subfertile or infertile men, progressive motility, sperm concentration and total sperm count were consistently improved in two articles, when higher VitD levels were considered [77], [78]. Total motility was also enhanced but was only reported in one article [78]. Normal morphology, sperm viability and sperm volume did not seem to be impacted by different VitD levels [77], [78].

These findings support supplementation of VitD in men with insufficiency or deficiency of this nutrient, but not in men with clinically adequate levels of VitD.

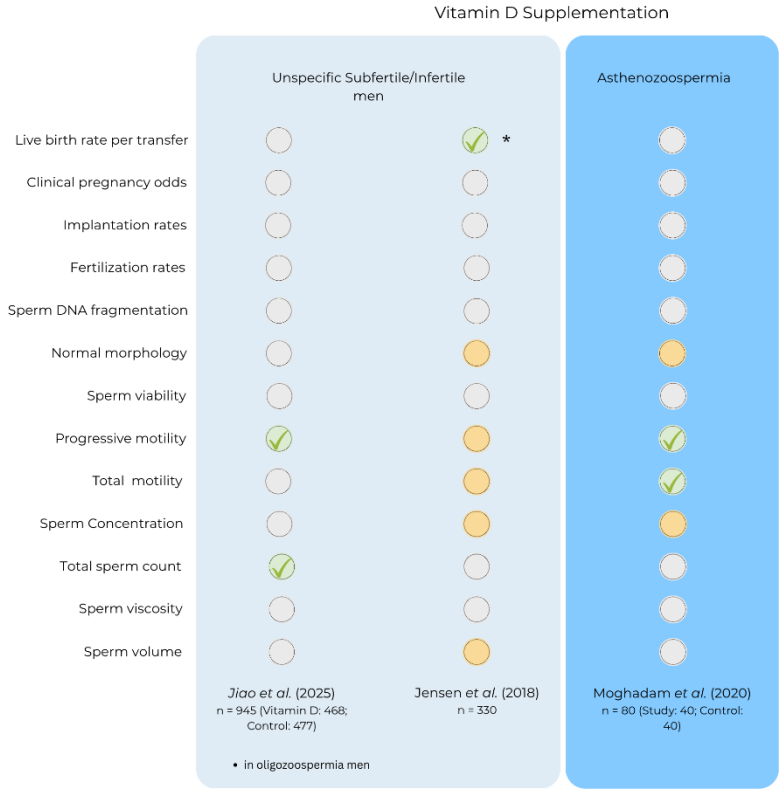


Figure 11 - Graphical representation summarizing the studied articles and the main outcomes of VitD supplementation in male patients on assisted reproductive outcomes across different patient populations (Unspecific subfertile/infertile male population in light blue and Asthenozoospermia in darker blue). Columns represent group population and the respective doses and treatment duration in the case in which they were explicitly discriminated in the articles. The respective references and number of patients are below the columns. The articles which analysed for each outcome a different sample number, are indicated. Rows display main reproductive outcomes and the results for each one in the specific article. Green circles correspond to a statistically significant enhancement after treatment, orange circles to a non-statistically significant difference upon treatment and grey circles to not evaluated outcomes.

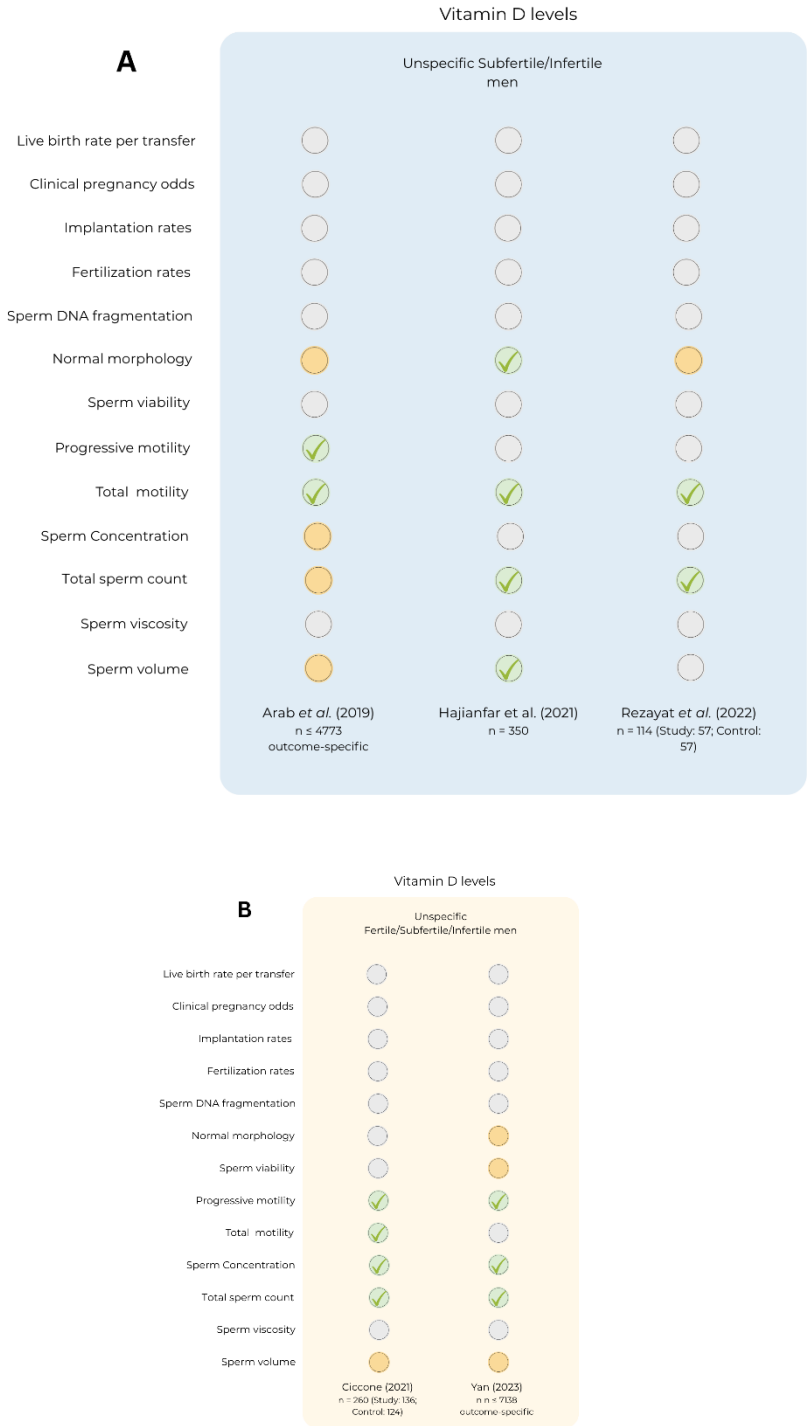


Figure 12 - Graphical representation summarizing the studied articles and the main outcomes of enhanced VitD levels in male patients on assisted reproductive outcomes across different patient populations.
A: Unspecified subfertile/infertile men population in light blue;
B: Unspecified fertile/subfertile/infertile men population in light beige.
Columns represent group population. The respective references and number of patients are below the columns. The articles which analysed for each outcome a different sample number, are indicated. Rows display main reproductive outcomes and the results for each one in the specific article. Green circles correspond to a statistically significant enhancement, orange circles to a non-statistically significant difference upon levels and grey circles to not evaluated outcomes.

5. L-Carnitine

LC is a naturally occurring compound involved in mitochondrial energy metabolism, where it facilitates the transport of long-chain fatty acids into mitochondria for β-oxidation and ATP production [100]. Because spermatozoa have high energy requirements for motility, LC is highly concentrated in the epididymis and may contribute to sperm maturation and function [100], [101] In addition to its metabolic role, LC has antioxidant properties that may help reduce oxidative stress and protect spermatozoa from oxidative damage [100]. Consistent with these biological functions, studies in men with idiopathic infertility suggest that LC supplementation may improve sperm motility, particularly progressive motility, and may have beneficial effects on other semen parameters [64], [101]. These effects are thought to reflect improved mitochondrial energy metabolism and reduced oxidative stress.

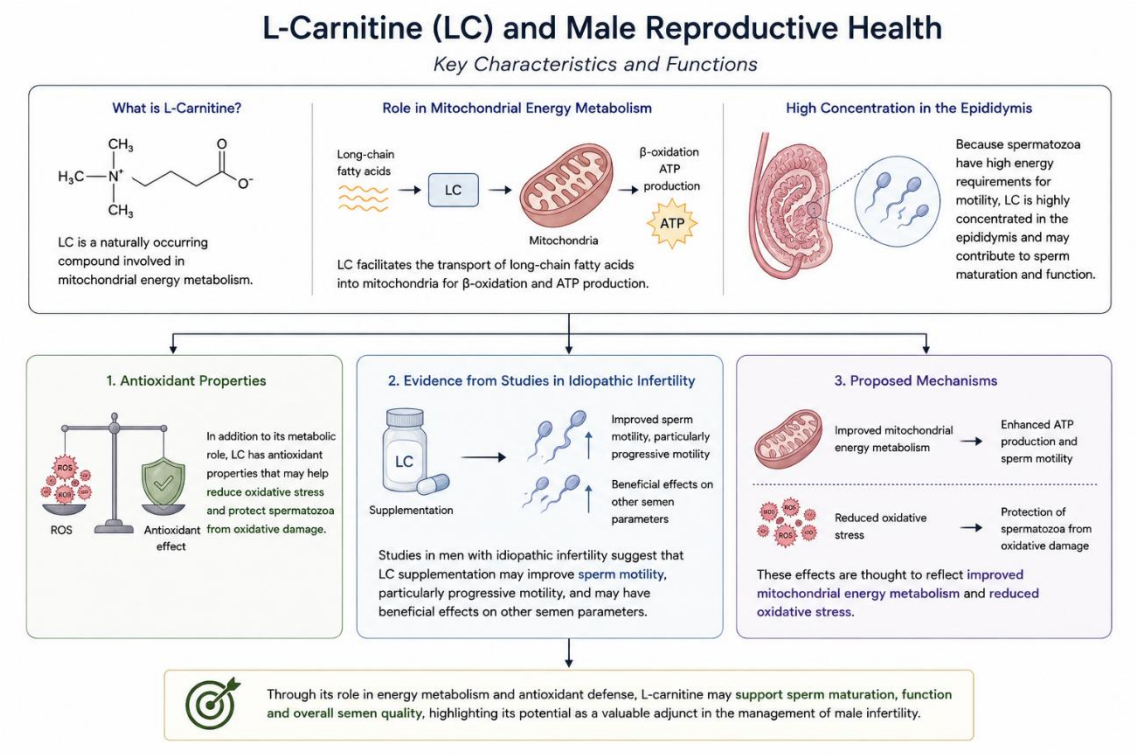


Figure 13 - Schematic representation of the proposed role of LC in male reproductive health. Created using artificial intelligence (AI).

5.1. Effects of L-Carnitine supplementation in men

5.1.1. Sperm quality and reproductive outcomes

LC supplementation has been widely investigated for its potential role in improving male reproductive function. Clinical studies indicate that daily doses ranging from one to three g administered over periods of three to six months lead to significant improvements in sperm motility, concentration, morphology, and total sperm count, with some evidence of enhanced pregnancy outcomes [100]. The randomized controlled trial by Ma *et al.*, in which patients received LC (15 g per bag, administered orally, twice daily) suggested that in addition to the enhancement of sperm motility, concentration and morphology, LC is associated with increased testosterone and luteinizing hormone [102]. Khaw *et al.* verified that the intake of LC and l-acetylcarnitine supplementation in men with idiopathic infertility, improved total sperm motility and morphology, but did not alter concentration or clinical pregnancy rates [101]. A systematic review and meta-analysis correlated supplementation with LC and l-acetyl carnitine to better motility and clinical pregnancy rates, but failed to prove an enhancement on sperm volume, concentration and percentage of atypical forms [103]. Moreover, the systematic review and network meta-analysis of randomized controlled trials by Su *et al.* did not show a statistically significant improvement in sperm morphology and concentration with LC compared with placebo. In contrast, LC significantly improved sperm motility and was ranked as the most effective antioxidant for enhancing motility in the analysis [53].

5.2.2. Overall interpretation

Taken together, LC increased total motility in all the articles studied, independently of the population [53], [101], [102], [103] (Figure 9). Moreover, progressive motility was enhanced in idiopathic OAT [103] and in one article in idiopathic male infertility [101]. Nonetheless, in the latter population, one article had conflicting results [102]. Normal morphology was improved in idiopathic male infertility [101], [102], but not in idiopathic OAT [103] or non-specific infertility [53]. Sperm concentration was higher in one article in idiopathic male infertility [102], but this result was not observed in any other article [53], [101], [103]. Clinical pregnancy rates were improved in idiopathic OAT [103], but not in idiopathic male infertility [103]. Sperm volume does not seem to be improved by LC supplementation [101], [103].

Based upon these findings, LC can be prescribed generally and especially in patients with diminished motility.



Figure 14 - Graphical representation summarizing the studied articles and the main outcomes of LC supplementation in male patients on assisted reproductive outcomes across different patient populations (Idiopathic male infertility population in darker blue, idiopathic OAT, and unspecified subfertile/infertile men population in light blue. Columns represent group population and the respective doses and treatment duration in the case in which they were explicitly discriminated in the articles. The respective references and number of patients are below the columns. The articles which analysed for each outcome a different sample number, are indicated. Rows display main reproductive outcomes and the results for each one in the specific article. Green circles correspond to a statistically significant enhancement after treatment, orange circles to a non-statistically significant difference upon treatment and grey circles to not evaluated outcomes.

6. Vitamin E

VitE is a fat-soluble antioxidant that protects cell membranes from oxidative damage by inhibiting lipid peroxidation caused by reactive oxygen species (ROS) [104], [105]. Because spermatozoa are particularly susceptible to oxidative stress due to the high content of polyunsaturated fatty acids in their plasma membranes and their limited endogenous antioxidant capacity, excessive ROS can impair sperm motility, viability and DNA integrity [6]. By neutralising free radicals and preserving membrane integrity, VitE contributes to the maintenance of sperm function and fertilising potential [104], [105]. Consistent with these biological functions, studies in men with idiopathic infertility suggest that VitE supplementation, either alone or in combination with other antioxidants, may improve sperm motility and reduce oxidative damage [104], [105].

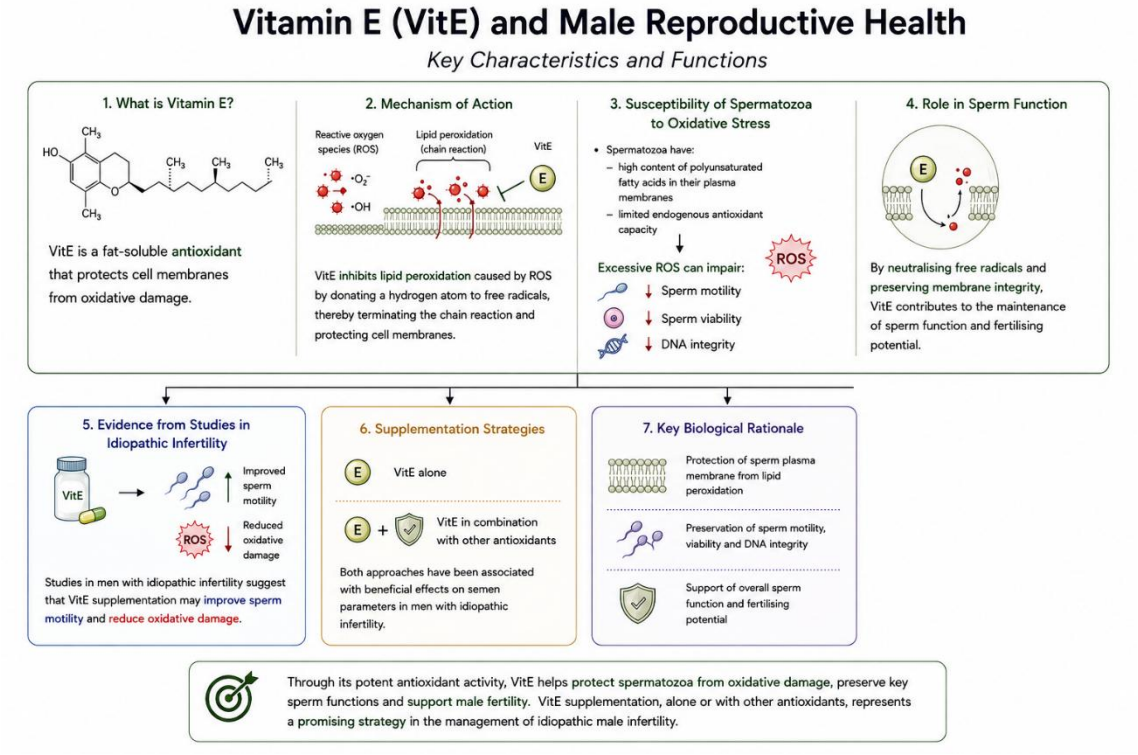


Figure 15 - Schematic representation of the proposed role of VitE in male reproductive health. Created using artificial intelligence (AI).

6.1. Effects of Vitamin E supplementation in men

6.1.1. Sperm quality and reproductive outcomes

Supplementatiom with VitE was solely reported in subfertile or infertile men [53], [104], [105] (Figure 10). It was observed that there was a higher live birth rate per transfer in the only article which analyzed that outcome [104]. The same can be said regarding total motility [53]. As for progressive motility, one article verified a positive correlation [105], but another did not [104]. The results for clinical pregnancy odds were also inconsistent [104], [105]. Total sperm counts followed the same pattern [104], [105]. Sperm concentration was found to be higher in one article upon VitE supplementation [105], but other two articles contradicted this result [53], [104]. Implantation rates, sperm volume and sperm viability were not different between groups [104], [105]. Nevertheless, more studies are needed, since only one study evaluated each outcome. In one study, fertilization rate and normal sperm morphology were significantly higher in the placebo group than in the VitE group [104]. However, in another two studies there was no difference between groups regarding normal morphology [53], [105].

6.2.2. Overall interpretation

Taken together, the available evidence regarding VitE supplementation is inconsistent. Although improvements in certain semen parameters and live birth per transfer have been reported, these findings were not consistently reproduced across studies. Therefore, current evidence is insufficient to support the routine use of VitE supplementation for improving male fertility, and further well-designed randomized controlled trials are needed.

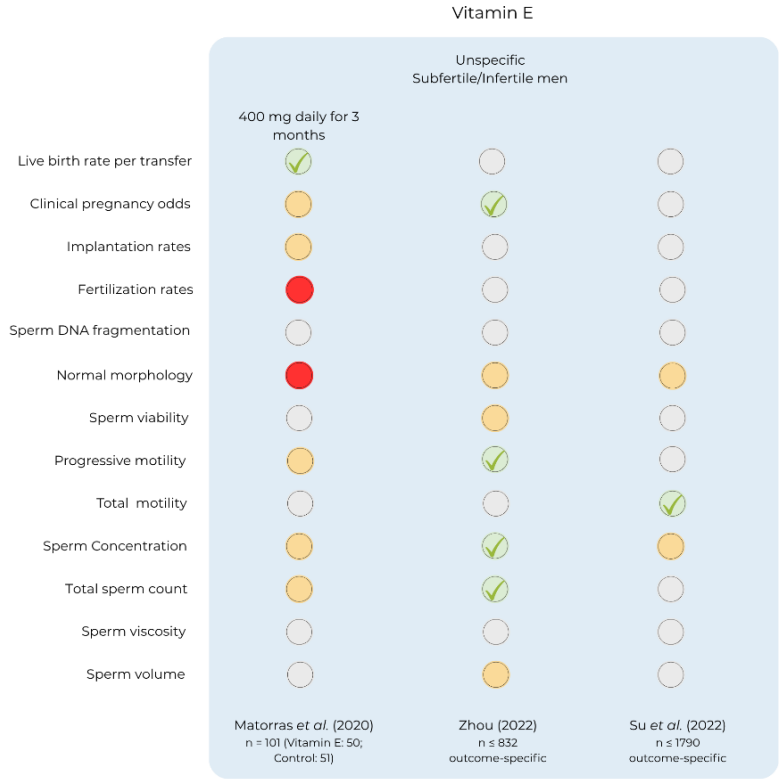


Figure 16 - Graphical representation summarizing the studied articles and the main outcomes of VitE supplementation in male patients on assisted reproductive outcomes in unspecified subfertile/infertile men population. Columns represent group population and the respective doses and treatment duration in the case in which they were explicitly discriminated in the articles. The respective references and number of patients are below the columns. The articles which analysed for each outcome a different sample number, are indicated. Rows display main reproductive outcomes and the results for each one in the specific article. Green circles correspond to a statistically significant enhancement after treatment, orange circles to a non-statistically significant difference upon treatment and grey circles to not evaluated outcomes.

Conclusion

The reviewed literature shown in this thesis highlights the potential of dietary supplementation protocols in the optimization and improvement of reproductive outcomes in both men and women. In the last decade, there has been increasing reliance on ART due to rising infertility rates. Nevertheless, the techniques have remained relatively unchanged over the years, leading to increased interest in alternative strategies, such as dietary nutrient supplementation. Despite the observed positive effects of such strategies in the general population groups evaluated, the benefits of adequate supplementation are particularly evident in individuals diagnosed with clinical conditions that diminish reproductive capabilities. In this context, CoQ10 shows consistent benefits in both women and men. MI appears particularly useful in women, while LC is more relevant for male reproductive outcomes. VitD is mainly effective when used to address a deficiency rather than as a general supplement. In contrast, MEL in women and VitE in men do not show clear evidence of improving reproductive outcomes. Overall, the evidence supports supplementation, but it should be individually considered based on patient characteristics and nutritional status.

References

[1] World Health Organization, *Guideline for the Prevention, Diagnosis and Treatment of Infertility*. Geneva, Switzerland: World Health Organization, 2025. ISBN: 978-92-4-011577-4.

[2] World Health Organization, *Infertility Prevalence Estimates, 1990–2021*. Geneva, Switzerland: World Health Organization, 2023. ISBN: 978-92-4-006831-5.

[3] S. A. Carson and A. N. Kallen, “Diagnosis and management of infertility: A review,” *JAMA*, Jul. 6, 2021, doi: 10.1001/jama.2021.4788.

[4] C. Wyns *et al*., “ART in Europe, 2018: Results generated from European registries by ESHRE,” *Hum. Reprod. Open*, vol. 2022, no. 3, 2022, Art. no. hoac022, doi: 10.1093/hropen/hoac022.

[5] A. J. Gaskins and J. E. Chavarro, “Diet and fertility: A review,” *Am. J. Obstet. Gynecol.*, vol. 218, no. 3, pp. 379–389, Mar. 2018, doi: 10.1016/j.ajog.2017.08.010.

[6] A. Agarwal, D. Durairajanayagam, and S. S. du Plessis, “Utility of antioxidants during assisted reproductive techniques: An evidence-based review,” *Reprod. Biol. Endocrinol.*, vol. 12, 2014, Art. no. 112, doi: 10.1186/1477-7827-12-112.

[7] A. Penzias *et al*., “Fertility evaluation of infertile women: A committee opinion,” *Fertil. Steril.*, vol. 116, no. 5, pp. 1255–1265, Nov. 2021, doi: 10.1016/j.fertnstert.2021.08.038.

[8] American Society for Reproductive Medicine, “Female age-related fertility decline,” *Fertil. Steril.*, vol. 101, no. 3, pp. 633–634, Mar. 2014, doi: 10.1016/j.fertnstert.2013.12.032.

[9] K. Itriyeva, “The normal menstrual cycle,” *Curr. Probl. Pediatr. Adolesc. Health Care*, vol. 52, no. 5, May 2022, Art. no. 101183, doi: 10.1016/j.cppeds.2022.101183.

[10] W. H. B. Wallace and T. W. Kelsey, “Human ovarian reserve from conception to the menopause,” *PLoS One*, vol. 5, no. 1, 2010, Art. no. e8772, doi: 10.1371/journal.pone.0008772.

[11] A. Sawal, K. B. Chaudhary, B. Dang, and P. Bokariya, “Comparative study of oocyte quality in young and advanced-aged women,” *J. Anat. Soc. India*, vol. 74, no. 4, pp. 338–342, Oct. 2025, doi: 10.4103/jasi.jasi_58_25.

[12] A. R. E. Moghadam, M. T. Moghadam, M. Hemadi, and G. Saki, “Oocyte quality and aging,” *JBRA Assist. Reprod.*, 2022, doi: 10.5935/1518-0557.20210026.

[13] M. A. J. Smits *et al*., “Human ovarian aging is characterized by oxidative damage and mitochondrial dysfunction,” *Hum. Reprod.*, vol. 38, no. 11, pp. 2208–2220, Nov. 2023, doi: 10.1093/humrep/dead177.

[14] J. M. Franasiak *et al*., “The nature of aneuploidy with increasing age of the female partner: A review of 15,169 consecutive trophectoderm biopsies evaluated with comprehensive chromosomal screening,” *Fertil. Steril.*, vol. 101, no. 3, pp. 656–663, Mar. 2014, doi: 10.1016/j.fertnstert.2013.11.004.

[15] A. K. Wesselink, K. J. Rothman, E. E. Hatch, E. M. Mikkelsen, H. T. Sørensen, and L. A. Wise, “Age and fecundability in a North American preconception cohort study,” *Am. J. Obstet. Gynecol.*, vol. 217, no. 6, pp. 667.e1–667.e8, Dec. 2017, doi: 10.1016/j.ajog.2017.09.002.

[16] A. J. Boxem *et al*., “Age among women and men, time to pregnancy and risk of miscarriage,” *BMC Med.*, vol. 23, no. 1, 2025, doi: 10.1186/s12916-025-04462-8.

[17] C. Castellini, G. Cordeschi, D. Tienforti, and A. Barbonetti, “Relationship between male aging and semen quality: A retrospective study on over 2500 men,” *Arch. Gynecol. Obstet.*, vol. 309, no. 6, pp. 2843–2852, Jun. 2024, doi: 10.1007/s00404-024-07448-8.

[18] R. Sharma, K. R. Biedenharn, J. M. Fedor, and A. Agarwal, “Lifestyle factors and reproductive health: Taking control of your fertility,” *Reprod. Biol. Endocrinol.*, vol. 11, 2013, Art. no. 66, doi: 10.1186/1477-7827-11-66.

[19] H. Seraj, M. A. Nazari, A. A. Atai, S. Amanpour, and M. Azadi, “A review: Biomechanical aspects of the fallopian tube relevant to its function in fertility,” Jun. 2024, doi: 10.1007/s43032-024-01479-x.

[20] E. Maman, E. Y. Adashi, M. Baum, and A. Hourvitz, “Prediction of ovulation: New insight into an old challenge,” *Sci. Rep.*, vol. 13, no. 1, 2023, doi: 10.1038/s41598-023-47241-2.

[21] A. Stachowska, A. M. Kicińska, A. Kotulak-Chrząszcz, and A. Babińska, “Usefulness of the sympto-thermal method with standardized cervical mucus assessment (InVivo method) for evaluating the monthly cycle in women with polycystic ovary syndrome (PCOS),” *Healthcare*, vol. 12, no. 11, 2024, Art. no. 1108, doi: 10.3390/healthcare12111108.

[22] L. Achinger *et al*., “The known and unknown about female reproductive tract mucus rheological properties,” *BioEssays*, 2025, doi: 10.1002/bies.70002.

[23] World Health Organization, *WHO Laboratory Manual for the Examination and Processing of Human Semen*, 6th ed. Geneva, Switzerland: World Health Organization, 2021, ISBN: 978-92-4-003078-7.

[24] World Health Organization, *WHO Laboratory Manual for the Examination and Processing of Human Semen*, 5th ed. Geneva, Switzerland: World Health Organization, 2010/2012.

[25] S. A. Yatsenko and A. Rajkovic, “Genetics of human female infertility,” *Biol. Reprod.*, vol. 101, no. 3, pp. 549–561, Sep. 2019, doi: 10.1093/biolre/ioz084.

[26] C. Krausz and A. Riera-Escamilla, “Genetics of male infertility,” *Nat. Rev. Urol.*, vol. 15, 2018, doi: 10.1038/s41585-018-0005-3.

[27] K. L. O’Flynn O’Brien, A. C. Varghese, and A. Agarwal, “The genetic causes of male factor infertility: A review,” *Fertil. Steril.*, vol. 93, no. 1, pp. 1–12, Jan. 2010, doi: 10.1016/j.fertnstert.2009.10.045.

[28] G. N. Allahbadia, “Intrauterine insemination: Fundamentals revisited,” *J. Obstet. Gynaecol. India*, vol. 67, no. 5, 2017, doi: 10.1007/s13224-017-1060-x.

[29] M. K. Mahmoud, D. Punukollu, and T. Mahmood, "In vitro fertilization," *Obstetrics, Gynaecology and Reproductive Medicine*, vol. 23, no. 8, pp. 238–246, Aug. 2013, doi: 10.1016/j.ogrm.2013.05.005.

[30] D. K. Gardner and C. Simón, Eds., *Handbook of In Vitro Fertilization*, 4th ed. Boca Raton, FL, USA: CRC Press, 2017.

[31] R. Sciorio and P. Rinaudo, “Culture conditions in the IVF laboratory: State of the ART and possible new directions,” *J. Assist. Reprod. Genet.*, vol. 40, no. 11, pp. 2591–2607, Nov. 2023, doi: 10.1007/s10815-023-02934-5.

[32] F. Zegers-Hochschild *et al*., “The International Glossary on Infertility and Fertility Care, 2025,” *Hum. Reprod.*, vol. 41, no. 6, pp. 892–909, Jun. 2026, doi: 10.1093/humrep/deag029.

[33] P. G. Garbhini *et al*., “Day-3 vs. day-5 fresh embryo transfer,” *JBRA Assist. Reprod.*, vol. 27, no. 2, pp. 163–168, 2023, doi: 10.5935/1518-0557.20220027.

[34] ESHRE Guideline Group on Ovarian Stimulation, “Guideline of the European Society of Human Reproduction and Embryology,” European Society of Human Reproduction and Embryology.

[35] Z. P. Nagy, D. Shapiro, and C. C. Chang, “Vitrification of the human embryo: A more efficient and safer in vitro fertilization treatment,” *Fertil. Steril.*, vol. 113, no. 2, pp. 241–247, Feb. 2020, doi: 10.1016/j.fertnstert.2019.12.009.

[36] A. Vaiarelli *et al*., “Pre-implantation genetic testing in ART: Who will benefit and what is the evidence?,” *J. Assist. Reprod. Genet.*, 2016, doi: 10.1007/s10815-016-0785-2.

[37] R. Q. Du *et al*., “A review of pre-implantation genetic testing technologies and applications,” *Reprod. Med. Biol.*, 2023, doi: 10.1097/RD9.0000000000000049.

[38] Takeuchi, "Pre-implantation genetic testing: Past, present, future," *Reproductive Medicine and Biology*, vol. 20, no. 1, pp. 27–40, Jan. 2021, doi: 10.1002/rmb2.12352.gz

[39] World Health Organization, *Introduction: Standards for Maternal and Neonatal Care*. Geneva, Switzerland: World Health Organization, 2003. [Online].

[40] X. Nie, X. Dong, Y. Hu, F. Xu, C. Hu, and C. Shu, “Coenzyme Q10 stimulate reproductive vitality,” *Drug Des. Devel. Ther.*, 2023, doi: 10.2147/DDDT.S386974.

[41] Y. Xu *et al*., “Pretreatment with coenzyme Q10 improves ovarian response and embryo quality in low-prognosis young women with decreased ovarian reserve: A randomized controlled trial,” *Reprod. Biol. Endocrinol.*, vol. 16, no. 1, 2018, doi: 10.1186/s12958-018-0343-0.

[42] A. T. Alahmar, A. E. Calogero, R. Singh, R. Cannarella, P. Sengupta, and S. Dutta, “Coenzyme Q10, oxidative stress, and male infertility: A review,” *Clin. Exp. Reprod. Med.*, vol. 48, no. 2, pp. 97–104, 2021, doi: 10.5653/cerm.2020.04175.

[43] A. T. Alahmar, P. Sengupta, S. Dutta, and A. E. Calogero, “Coenzyme Q10, oxidative stress markers, and sperm DNA damage in men with idiopathic oligoasthenoteratospermia,” *Clin. Exp. Reprod. Med.*, vol. 48, no. 2, pp. 150–155, 2021, doi: 10.5653/cerm.2020.04084.

[44] S. Bakri *et al*., “Efficacy and safety of coenzyme Q10 in idiopathic male infertility: A systematic review and meta-analysis of randomized trials,” *World J. Mens Health*, vol. 43, 2025, doi: 10.5534/wjmh.250159.

[45] A. Ben-Meir *et al*., “Coenzyme Q10 restores oocyte mitochondrial function and fertility during reproductive aging,” *Aging Cell*, vol. 14, no. 5, pp. 887–895, Oct. 2015, doi: 10.1111/acel.12368.

[46] Y. Bentov and R. F. Casper, “The aging oocyte—Can mitochondrial function be improved?,” *Fertil. Steril.*, vol. 99, no. 1, 2013, doi: 10.1016/j.fertnstert.2012.11.031.

[47] G. Lin, X. Li, S. L. Jin Yie, and L. Xu, “Clinical evidence of coenzyme Q10 pretreatment for women with diminished ovarian reserve undergoing IVF/ICSI: A systematic review and meta-analysis,” *Ann. Med.*, vol. 56, no. 1, 2024, doi: 10.1080/07853890.2024.2389469.

[48] S. Qi, Q. Liang, L. Yang, X. Zhou, K. Chen, and J. Wen, “Effect of coenzyme Q10 and transcutaneous electrical acupoint stimulation in assisted reproductive technology: A retrospective controlled study,” *Reprod. Biol. Endocrinol.*, vol. 20, no. 1, 2022, doi: 10.1186/s12958-022-01043-9.

[49] P. Florou, P. Anagnostis, P. Theocharis, M. Chourdakis, and D. G. Goulis, “Does coenzyme Q10 supplementation improve fertility outcomes in women undergoing assisted reproductive technology procedures? A systematic review and meta-analysis of randomized-controlled trials,” *J. Assist. Reprod. Genet.*, 2020, doi: 10.1007/s10815-020-01906-3.

[50] Y. Huang *et al*., “Oxidative stress and inflammatory markers in ovarian follicular fluid of women with diminished ovarian reserve during in vitro fertilization,” *J. Ovarian Res.*, vol. 16, no. 1, 2023, doi: 10.1186/s13048-023-01293-0.

[51] Q. Zhu *et al*., “Downregulation of glucose-energy metabolism via AMPK signaling pathway in granulosa cells of diminished ovarian reserve patients,” *Gene*, vol. 933, 2025, Art. no. 148979, doi: 10.1016/j.gene.2024.148979.

[52] T. M. Akhigbe *et al*., “Does coenzyme Q10 improve semen quality and circulating testosterone level? A systematic review and meta-analysis of randomized controlled trials,” *Front. Pharmacol.*, 2024, doi: 10.3389/fphar.2024.1497930.

[53] L. Su *et al*., “Effect of antioxidants on sperm quality parameters in subfertile men: A systematic review and network meta-analysis of randomized controlled trials,” *Adv. Nutr.*, 2022, doi: 10.1093/advances/nmab127.

[54] T. G. Bahar, S. Z. Masoumi, S. Pilehvari, F. Kazemi, S. Moradkhani, and S. Mahmoudi, “Effect of CoQ10 supplement on spermogram parameters and sexual function of infertile men referred to the Infertility Center of Fatemieh Hospital, Hamadan,

Iran, 2019: A randomized controlled trial study," *Int. J. Fertil. Steril.*, vol. 17, no. 2, pp. 99–106, Feb. 2023, doi: 10.22074/IJFS.2022.544330.1234.

[55] A. T. Alahmar, "The impact of two doses of coenzyme Q10 on semen parameters and antioxidant status in men with idiopathic oligoasthenoteratozoospermia," *Clin. Exp. Reprod. Med.*, vol. 46, no. 3, pp. 112–118, Sep. 2019, doi: 10.5653/cerm.2019.00136.

[56] A. T. Alahmar, "Coenzyme Q10 improves sperm motility and antioxidant status in infertile men with idiopathic oligoasthenospermia," *Clin. Exp. Reprod. Med.*, vol. 49, no. 4, pp. 277–284, 2022, doi: 10.5653/cerm.2022.05463.

[57] F. Facchinetti *et al*., "Breakthroughs in the use of inositols for assisted reproductive treatment (ART)," *Trends Endocrinol. Metab.*, 2020, doi: 10.1016/j.tem.2020.04.003.

[58] M. L. Croze and C. O. Soulage, "Potential role and therapeutic interests of myo-inositol in metabolic diseases," *Biochimie*, vol. 95, no. 10, pp. 1811–1827, 2013, doi: 10.1016/j.biochi.2013.05.011.

[59] A. Etrusco, A. S. Laganà, V. Chiantera, G. Buzzaccarini, and V. Unfer, "Myo-inositol in assisted reproductive technology from bench to bedside," *Trends Endocrinol. Metab.*, 2024, doi: 10.1016/j.tem.2023.09.005.

[60] P. Merviel *et al*., "Impact of myo-inositol treatment in women with polycystic ovary syndrome in assisted reproductive technologies," *Reprod. Health*, vol. 18, no. 1, 2021, doi: 10.1186/s12978-021-01073-3.

[61] F. Seyedoshohadaei *et al*., "Myo-inositol effect on pregnancy outcomes in infertile women undergoing in vitro fertilization/intracytoplasmic sperm injection: A double-blind RCT," *Int. J. Reprod. Biomed.*, vol. 20, no. 8, pp. 643–650, Aug. 2022, doi: 10.18502/ijrm.v20i8.11753.

[62] X. Zheng *et al*., "Inositol supplement improves clinical pregnancy rate in infertile women undergoing ovulation induction for ICSI or IVF-ET," *Medicine*, vol. 96, no. 49, 2017, Art. no. e8842, doi: 10.1097/MD.0000000000008842.

[63] M. Tabatabaie *et al*., "The effect of myo-inositol supplement on molecular regulation of folliculogenesis, steroidogenesis, and assisted reproductive technique outcomes in patients with polycystic ovarian syndrome," *Mol. Biol. Rep.*, vol. 49, no. 2, pp. 875–884, Feb. 2022, doi: 10.1007/s11033-021-06833-9.

[64] J. Zhang, H. Zhang, W. Zhou, M. Jiang, and X. Lin, “Effect of myo-inositol supplementation in mixed ovarian response IVF cohort: A systematic review and meta-analysis,” *Front. Endocrinol.*, 2025, doi: 10.3389/fendo.2025.1520362.

[65] L. Nazari *et al.*, “Effect of myo-inositol supplementation on ICSI outcomes among poor ovarian responder patients: A randomized controlled trial,” *J. Gynecol. Obstet. Hum. Reprod.*, vol. 49, no. 5, 2020, Art. no. 101698, doi: 10.1016/j.jogoh.2020.101698.

[66] S. Mohammadi, F. Eini, F. Bazarganipour, S. A. Taghavi, and M. A. Kutenaee, “The effect of myo-inositol on fertility rates in poor ovarian responder women undergoing assisted reproductive technique: A randomized clinical trial,” *Reprod. Biol. Endocrinol.*, vol. 19, no. 1, 2021, doi: 10.1186/s12958-021-00741-0.

[67] R. J. Reiter *et al.*, “Aging-related ovarian failure and infertility: Melatonin to the rescue,” *Antioxidants*, vol. 12, no. 3, 2023, Art. no. 695, doi: 10.3390/antiox12030695.

[68] H. Tamura *et al.*, “Importance of melatonin in assisted reproductive technology and ovarian aging,” *Int. J. Mol. Sci.*, vol. 21, no. 3, 2020, Art. no. 1135, doi: 10.3390/ijms21031135.

[69] J. Espino *et al.*, “Impact of melatonin supplementation in women with unexplained infertility undergoing fertility treatment,” *Antioxidants*, vol. 8, no. 9, 2019, Art. no. 338, doi: 10.3390/antiox8090338.

[70] H. Tang, J. Hao, B. Xu, Y. Wang, Y. Li, and J. Zhao, “Melatonin supplementation and outcomes of assisted reproductive technology: A systematic review and meta-analysis,” *BMC Pregnancy Childbirth*, 2026, doi: 10.1186/s12884-025-08503-1.

[71] M. A. B. Mejlhede, J. B. Jepsen, and U. B. Knudsen, “Oral melatonin supplementation during in vitro fertilization treatment: A systematic PRISMA review and meta-analysis of randomized controlled trials,” 2021, doi: 10.1080/09513590.2021.1974378.

[72] S. Fernando *et al.*, “Melatonin in assisted reproductive technology: A pilot double-blind randomized placebo-controlled clinical trial,” *Front. Endocrinol.*, vol. 9, 2018, Art. no. 545, doi: 10.3389/fendo.2018.00545.

[73] B. N. Jahromi, S. Sadeghi, S. Alipour, M. E. Parsanezhad, and S. M. Alamdarloo, “Effect of melatonin on the outcome of assisted reproductive technique cycles in women with diminished ovarian reserve: A double-blinded randomized clinical trial,” *Iranian Journal of Medical Sciences*, vol. 42, no. 1, pp. 73–78, 2017.

[74] K. L. Hu, X. Ye, S. Wang, and D. Zhang, “Melatonin application in assisted reproductive technology: A systematic review and meta-analysis of randomized trials,” *Front. Endocrinol.*, vol. 11, 2020, Art. no. 160, doi: 10.3389/fendo.2020.00160.

[75] M. F. Holick, “Vitamin D deficiency,” *N. Engl. J. Med.*, vol. 357, no. 3, pp. 266–281, Jul. 2007.

[76] S. Várbíró *et al.*, “Effects of vitamin D on fertility, pregnancy and polycystic ovary syndrome—A review,” *Nutrients*, vol. 14, no. 8, 2022, Art. no. 1649, doi: 10.3390/nu14081649.

[77] T. F. Yan, J. J. Qi, L. X. Li, and F. Li, “The association between serum vitamin D levels and male fertility: A systematic review and meta-analysis,” 2023, Art. no. 9002938, doi: 10.1155/2023/9002938.

[78] I. Ciccone *et al.*, “Serum vitamin D content is associated with semen parameters and serum testosterone levels in men,” *Asian J. Androl.*, vol. 23, no. 1, pp. 52–58, Jan. 2021, doi: 10.4103/aja.aja_9_20.

[79] M. Yang *et al*., “Effects of vitamin D supplementation on ovulation and pregnancy in women with polycystic ovary syndrome: A systematic review and meta-analysis,” *Front. Endocrinol.*, 2023, doi: 10.3389/fendo.2023.1148556.

[80] N. Christoforidis, M. Papapanou, D. Michalakis, M. Dimitraki, A. Chatziparasidou, and C. Siristatidis, “Effect of vitamin D supplementation on frozen embryo transfer cycle outcomes,” *Hum. Fertil.*, vol. 28, no. 1, 2025, doi: 10.1080/14647273.2025.2493251.

[81] S. Jiang, Z. Chen, and L. Li, “Assessing vitamin D’s impact on pregnancy success: A predictive model for assisted reproductive technology outcomes,” *Front. Reprod. Health*, vol. 7, 2025, doi: 10.3389/frph.2025.1510484.

[82] S. Abedi, M. Taebi, and M. H. N. Esfahani, “Effect of vitamin D supplementation on intracytoplasmic sperm injection outcomes: A randomized double-blind placebo-controlled trial,” *Int. J. Fertil. Steril.*, vol. 13, no. 1, pp. 18–23, Apr. 2019, doi: 10.22074/ijfs.2019.5470.

[83] R. de A. Antunes *et al.*, “Vitamin D levels in couples undergoing in vitro fertilization treatment: lack of association with embryo quality or pregnancy rates,” *Fertil. Steril.*, vol. 122, no. 5, pp. 866–874, Nov. 2024, doi: 10.1016/j.fertnstert.2024.06.023.

[84] G. Neysanian, M. Taebi, A. Rezaeian, M. H. Nasr-Esfahani, and M. Jahangirifar, “The effects of serum and follicular fluid vitamin D levels on assisted reproductive

techniques: A prospective cohort study," *Int. J. Fertil. Steril.*, vol. 15, no. 4, pp. 280–285, Sep. 2021, doi: 10.22074/ijfs.2021.138605.1033.

[85] N. L. Walz *et al.*, "Serum Vitamin D status is associated with increased blastocyst development rate in women undergoing IVF," *Reprod. Biomed. Online*, vol. 41, pp. 1101–1111, 2020

[86] K. Wang, F. Dong, S. Ma, and Z. Bu, "The association between vitamin D deficiency and clinical pregnancy rate in IVF patients with different age," *Front. Endocrinol.*, vol. 15, 2024, doi: 10.3389/fendo.2024.1485238.

[87] X. Meng *et al.*, "Influence of vitamin D supplementation on reproductive outcomes of infertile patients: A systematic review and meta-analysis," *Reprod. Biol. Endocrinol.*, 2023, doi: 10.1186/s12958-023-01068-8.

[88] C. Xu *et al.*, "Association between vitamin D level and clinical outcomes of assisted reproductive treatment: A systematic review and dose-response meta-analysis," 2025, doi: 10.1007/s43032-024-01578-9.

[89] J. Chu, I. Gallos, A. Tobias, B. Tan, A. Eapen, and A. Coomarasamy, "Vitamin D and assisted reproductive treatment outcome: A systematic review and meta-analysis," *Hum. Reprod.*, vol. 33, no. 1, pp. 65–80, Jan. 2018, doi: 10.1093/humrep/dex326.

[90] J. Zhao, X. Huang, B. Xu, Y. Yan, Q. Zhang, and Y. Li, "Whether vitamin D was associated with clinical outcome after IVF/ICSI: A systematic review and meta-analysis," *Reprod. Biol. Endocrinol.*, vol. 16, no. 1, 2018, doi: 10.1186/s12958-018-0324-3.

[91] F. Iliuta, J. I. Pijoan, L. Lainz, A. Exposito, and R. Matorras, "Women's vitamin D levels and IVF results: A systematic review of the literature and meta-analysis, considering three categories of vitamin status (replete, insufficient and deficient)," *Hum. Fertil.*, vol. 25, no. 2, pp. 228–246, 2022, doi: 10.1080/14647273.2020.1807618.

[92] R. Luo *et al.*, "The role of serum vitamin D in patients with normal ovarian reserve undergoing the first IVF/ICSI cycle," *Front. Endocrinol.*, vol. 14, 2023, doi: 10.3389/fendo.2023.1249445.

[93] Z. M. Alawad, "Level of follicular fluid vitamin D and embryo quality in a sample of Iraqi women undergoing IVF," *J. Fac. Med. Baghdad*, vol. 60, no. 4, pp. 215–221, 2019, doi: 10.32007/med.1936/jfacmedbagdad.v60i4.7.

[94] A. Arab, A. Hadi, S. P. Moosavian, G. Askari, and M. Nasirian, “The association between serum vitamin D, fertility and semen quality: A systematic review and meta-analysis,” *Int. J. Surg.*, 2019, doi: 10.1016/j.ijsu.2019.09.025.

[95] A. A. Rezayat, A. A. Asadpour, A. Yarahmadi, H. Ahmadnia, A. M. Hakkak, and S. Soltani, “Association between serum vitamin D concentration with spermiogram parameters and reproductive hormones among infertile Iranian males: A cross-sectional study,” *Reprod. Sci.*, vol. 29, no. 1, pp. 270–276, Jan. 2022, doi: 10.1007/s43032-021-00771-4.

[96] H. Hajianfar, E. Karimi, N. Mollaghasemi, S. Rezaei, and A. Arab, “Is there a relationship between serum vitamin D and semen parameters? A cross-sectional sample of the Iranian infertile men,” *Basic Clin. Androl.*, vol. 31, no. 1, 2021, doi: 10.1186/s12610-021-00147-3.

[97] M. T. Moghadam, G. Hosseini, F. Absalan, M. H. Tabar, and R. Nikbakht, “Effects of vitamin D on apoptosis and quality of sperm in asthenozoospermia,” *JBRA Assist. Reprod.*, vol. 24, no. 3, pp. 316–323, 2020, doi: 10.5935/1518-0557.20200009.

[98] Y. Jiao, Z. Feng, T. Zhang, and X. Chen, “Impact of vitamin D3 supplementation on androgens and semen parameters in infertile men: A systematic review and meta-analysis based on prospective randomised controlled trials,” *Arch. Esp. Urol.*, vol. 78, no. 6, pp. 772–781, Jul. 2025, doi: 10.56434/j.arch.esp.urol.20257806.103.

[99] M. Blomberg Jensen, J. Gerner Lawaetz, J. H. Petersen, A. Juul, and N. Jørgensen, “Effects of vitamin D supplementation on semen quality, reproductive hormones, and live birth rate: A randomized clinical trial,” *J. Clin. Endocrinol. Metab.*, vol. 103, no. 3, pp. 870–881, 2018, doi: 10.1210/jc.2017-01656.

[100] F. G. Mateus, S. Moreira, A. D. Martins, P. F. Oliveira, M. G. Alves, and M. de L. Pereira, “L-carnitine and male fertility: Is supplementation beneficial?,” *J. Clin. Med.*, vol. 12, no. 18, 2023, Art. no. 5796, doi: 10.3390/jcm12185796.

[101] S. C. Khaw, Z. Z. Wong, R. A. Anderson, and S. M. da Silva, “L-carnitine and L-acetylcarnitine supplementation for idiopathic male infertility,” *Reprod. Fertil.*, 2020, doi: 10.1530/RAF-20-0037.

[102] L. Ma and Y. Sun, “Comparison of L-carnitine vs. CoQ10 and vitamin E for idiopathic male infertility: A randomized controlled trial,” *Eur. Rev. Med. Pharmacol. Sci.*, vol. 26, no. 13, pp. 4698–4704, 2022, doi: 10.26355/eurrev_202207_29194.

[103] X. Zhang, Y. Cui, L. Dong, M. Sun, and Y. Zhang, “The efficacy of combined L-carnitine and L-acetylcarnitine in men with idiopathic oligoasthenoteratozoospermia: A systematic review and meta-analysis,” *Andrologia*, vol. 52, no. 2, 2020, Art. no. e13470, doi: 10.1111/and.13470.

[104] R. Matorras *et al*., “Effect of vitamin E administered to men in infertile couples on sperm and assisted reproduction outcomes: A double-blind randomized study,” *F&S Rep.*, vol. 1, no. 3, pp. 219–226, Dec. 2020, doi: 10.1016/j.xfre.2020.09.006.

[105] X. Zhou, H. Shi, S. Zhu, H. Wang, and S. Sun, “Effects of vitamin E and vitamin C on male infertility: A meta-analysis,” *Int. Urol. Nephrol.*, vol. 54, no. 8, pp. 1793–1805, Aug. 2022, doi: 10.1007/s11255-022-03237-x.

Attachments

Attachment 1

Supplementary Table 1 - Table summarizing the characteristics of the analysed articles for female patients. [+] favourable increase/improvement; [-] favourable reduction for outcomes where lower values are desirable; [=] no significant/favourable difference.

Supplement	Reference	Study design	Population	n	Intervention / exposure	Corrected outcomes from evidence map
Coenzyme Q10	Florou et al. (2020)	Systematic review and meta-analysis	Mixed population undergoing ART	n = 449; CoQ10: 215; control: 234	CoQ10 supplementation; variable dose and duration	[+] Clinical pregnancy rate; [=] live birth rate and miscarriage rate.
Coenzyme Q10	Xu et al. (2018)	Randomized controlled trial	Young women with poor ovarian reserve; age <35 years	n = 169; CoQ10: 76; control: 93	CoQ10 200 mg three times daily for 2 months	[+] Good embryo quality (n), fertilization rate and oocytes retrieved (n); [-] gonadotrophin dose required; [=] live birth rate, clinical pregnancy rate, miscarriage rate, cycle cancellation rate and antral follicle count.
Coenzyme Q10	Qi et al. (2022)	Retrospective controlled study	Women with poor ovarian response; age ≥35 years	n = 330; CoQ10: 110; CoQ10 + TEAS: 110; control: 110	CoQ10 10 mg three times daily for 2 months, with or without TEAS	[+] Implantation rate, good embryo quality (n), oocytes retrieved (n), MII oocytes (n) and antral follicle count; [=] live birth rate, clinical pregnancy rate, miscarriage rate, good embryo quality rate, fertilization rate, cycle cancellation rate, MII oocyte rate and gonadotrophin dose required.
Coenzyme Q10	Lin et al. (2024)	Systematic review and meta-analysis	Women with diminished ovarian reserve	n = 1529; CoQ10: 761; control: 768	CoQ10 supplementation; variable dose and duration	[+] Clinical pregnancy rate, good embryo quality (n) and oocytes retrieved (n); [-] miscarriage rate, cycle cancellation rate and gonadotrophin dose required.
Myo-inositol	Seyedoshohadaei et al. (2021)	Clinical study	Mixed population undergoing ART	n = 70; myo-inositol: 36; control: 34	2000 mg myo-inositol + 200 µg folic acid twice daily for 2 months	[+] Live birth rate, clinical pregnancy rate, fertilized embryos (n), oocytes retrieved (n) and MII oocytes (n); [=] miscarriage rate, good embryo quality (n) and fewer degenerated oocytes.
Myo-inositol	Zheng et al. (2017)	Systematic review and meta-analysis	Mixed population undergoing IVF/ICSI	n ≤ 935; myo-inositol: 482; outcome-specific control groups	Myo-inositol supplementation; variable dose and duration	[+] Clinical pregnancy rate, good embryo quality rate, [-] miscarriage rate, degenerated oocytes and dose of gonadotrophins required; [=] live birth rate, cycle cancellation rate, oocytes retrieved (n) and MII oocyte rate.
Myo-inositol	Zhang et al. (2025)	Systematic review and meta-analysis	Infertile women, including women with PMOS and poor ovarian response	n ≤ 981; myo-inositol: 478; control: 503; outcome-specific	Myo-inositol supplementation; variable dose and duration	Mixed/POR analysis: [+] live birth rate and fertilization rate; [=] clinical pregnancy rate, good embryo quality rate, oocytes retrieved (n) and MII oocyte rate. PMOS subgroup: [+] fertilization rate and MII oocyte rate; [-] gonadotrophin dose required; [=] clinical pregnancy rate and oocytes retrieved (n).
Myo-inositol	Nazari et al. (2020)	Randomized controlled trial	Poor ovarian responder women	n = 112; myo-inositol + folic acid: 56; folic acid: 56	4 g myo-inositol + 400 µg folic acid daily for 1 month	[+] Good embryo quality (n) and fertilization rate; [=] clinical pregnancy rate, miscarriage, oocytes retrieved (n) and MII oocytes (n).

Supplement	Reference	Study design	Population	n	Intervention / exposure	Corrected outcomes from evidence map
Myo-inositol	Mohammadi et al. (2021)	Randomized clinical trial	Poor ovarian responder women	n = 60; myo-inositol + folic acid: 30; folic acid: 30	4 g myo-inositol + 400 µg folic acid daily for 3 months	[+] Fertilization rate; [-] gonadotrophin dose required; [=] clinical pregnancy rate, miscarriage rate, oocytes retrieved (n) and MII oocytes (n).
Myo-inositol	Tabatabaie et al. (2022)	Experimental clinical study	Women with PMOS, aged 20-35 years	n = 90; three groups of 30 participants	4 g myo-inositol + 400 µg folic acid daily for 1 month	[+] Good embryo quality rate, fertilization rate and MII oocyte rate; [=] oocytes retrieved (n).
Melatonin	Hu et al. (2018)	Systematic review and meta-analysis of randomized trials	Women undergoing IVF	n = 1203; melatonin: 645; control: 558	Melatonin ≥3 mg daily	[+] Clinical pregnancy rate, good embryo quality (n), oocytes retrieved (n) and MII oocytes (n); [=] live birth rate and miscarriage rate.
Melatonin	Tang et al. (2025)	Systematic review and meta-analysis	Women undergoing ART	n = 1241	Melatonin 2-8 mg daily	[+] Clinical pregnancy rate, good embryo quality (n), fertilization rate and MII oocytes (n); [=] live birth rate, miscarriage rate and oocytes retrieved (n).
Melatonin	Melhjede et al. (2021)	PRISMA systematic review and meta-analysis	Women undergoing IVF	n = 889; melatonin: 535; control: 354	Melatonin 3 mg daily to 8 mg twice daily	[+] Oocytes retrieved (n); [=] live birth rate, clinical pregnancy rate, miscarriage rate, good embryo quality (n) and MII oocytes (n).
Melatonin	Fernando et al. (2018)	Double-blind randomized placebo-controlled trial	Women undergoing ART	n = 140; 2 mg: 41; 4 mg: 49; 8 mg: 10; control: 40	Melatonin 2, 4 or 8 mg twice daily during ovarian stimulation	[+] Fertilization rate; [=] live birth rate, clinical pregnancy rate, good embryo quality (n), fertilized embryos (n), oocytes retrieved (n) and MII oocytes (n).
Melatonin	Espino et al. (2018)	Randomized pilot study	Women with unexplained infertility undergoing ART	n = 160; subgroup allocation unclear	Melatonin 3-6 mg daily from first appointment/controlled ovarian stimulation until oocyte retrieval	[+] Good embryo quality (n), fertilization rate, oocytes retrieved (n) and MII oocyte rate; [=] clinical pregnancy rate.
Melatonin	Jahromi et al. (2018)	Double-blind randomized clinical trial	Women with diminished ovarian reserve	n = 66; melatonin: 32; control: 34	Melatonin 3 mg daily from day 5 of the cycle prior to stimulation until oocyte retrieval	[+] Good embryo quality (n); [=] clinical pregnancy rate, miscarriage rate, MII oocytes (n) and antral follicle count.
Vitamin D supplementation	Meng et al. (2023)	Systematic review and meta-analysis	Infertile patients	n = 325; outcome-specific	Vitamin D 1000-10,000 IU daily	[+] Clinical pregnancy rate; [=] biochemical pregnancy rate, multiple pregnancy rate, miscarriage rate and implantation rate.
Vitamin D supplementation	Christoforidis et al. (2025)	Clinical study	Women undergoing frozen embryo transfer	n = 252; vitamin D: 115; control: 137	Vitamin D 25,000-50,000 IU weekly	[=] Live birth rate, clinical pregnancy rate, miscarriage rate, fertilized embryos (n), oocytes retrieved (n), MII oocytes (n) and antral follicle count.
Vitamin D supplementation	Abedi et al. (2024)	Randomized double-blind placebo-controlled trial	Women undergoing ICSI	n = 85; vitamin D: 70; control: 15	Vitamin D 50,000 IU weekly for 6 weeks	[+] Clinical pregnancy rate and biochemical pregnancy rate; [=] good embryo quality rate, fertilization rate, oocytes retrieved (n) and MII oocytes (n).
Vitamin D supplementation	Yang et al. (2023)	Systematic review and meta-analysis	Women with PMOS	n = 196; outcome-specific	Vitamin D 200-10,000 IU daily, one dose or for 8, 12 and 24 weeks	[+] Clinical pregnancy rate, fertilization rate and MII oocyte rate; [-] miscarriage rate; [=] biochemical pregnancy rate and good embryo quality rate.
Vitamin D status / levels	Chu et al. (2018)	Systematic review and meta-analysis	Women undergoing ART	Outcome-specific n	Serum vitamin D status	[+] Live birth rate, clinical pregnancy rate and biochemical pregnancy rate; [=] miscarriage rate.
Vitamin D status / levels	Neysanian et al. (2021)	Prospective cohort study	Women undergoing ART, grouped according to serum and follicular fluid vitamin D levels	n = 137	Serum and follicular fluid vitamin D assessment	[+] Clinical pregnancy rate and biochemical pregnancy rate; [=] good embryo quality (n) and fertilization rate.

Supplement	Reference	Study design	Population	n	Intervention / exposure	Corrected outcomes from evidence map
Vitamin D status / levels	Xu et al. (2025)	Systematic review and dose-response meta-analysis	Women undergoing ART	n = 970; outcome-specific	Vitamin D status assessment; effects reported at ≤24 ng/L	[+] Live birth rate and clinical pregnancy rate; [=] biochemical pregnancy rate, miscarriage rate and implantation rate.
Vitamin D status / levels	Jiang et al. (2025)	Predictive modelling study	Women undergoing ART, grouped according to clinical pregnancy outcome	n = 188; divided into two groups based on clinical pregnancy outcomes	Vitamin D status included in ART success prediction	[+] Clinical pregnancy rate, good embryo quality rate, oocytes retrieved (n) and antral follicle count; [=] live birth rate.
Vitamin D status / levels	Wang et al. (2018)	Clinical observational study	Women undergoing IVF; deficient, insufficient and replete groups	n = 149; deficient: 49; insufficient: 72; replete: 28	Serum vitamin D assessment	[+] Clinical pregnancy rate and implantation rate; [=] miscarriage rate, good embryo quality (n), fertilized embryos (n), fertilization rate, oocytes retrieved (n) and antral follicle count.
Vitamin D status / levels	Zhao et al. (2024)	Systematic review and meta-analysis	Women undergoing IVF/ICSI	n = 2254; outcome-specific	Vitamin D status assessment	[+] Live birth rate; [=] clinical pregnancy rate.
Vitamin D status / levels	Iliuta et al. (2025)	Systematic review and meta-analysis	Women undergoing IVF	n < 371; outcome-specific	Vitamin D level assessment	[+] Live birth rate and biochemical pregnancy rate; [=] clinical pregnancy rate, miscarriage rate and implantation rate.
Vitamin D status / levels	Antunes et al. (2018)	Observational cohort study	Couples undergoing IVF treatment	n = 267	Vitamin D level assessment	[=] Clinical pregnancy rate, good embryo quality (n), oocytes retrieved (n), MII oocytes (n) and antral follicle count.
Vitamin D status / levels	Walz et al. (2020)	Cohort study	Women undergoing IVF	n = 287	Serum vitamin D assessment	[=] Live birth rate, clinical pregnancy rate, miscarriage rate, good embryo quality (n), fertilized embryos (n), fertilization rate, oocytes retrieved (n), MII oocytes (n) and antral follicle count.
Vitamin D status / levels	Luo et al. (2023)	Cohort study	Women with normal ovarian reserve undergoing first IVF/ICSI cycle	n = 264	Serum vitamin D assessment	[=] Clinical pregnancy rate, biochemical pregnancy rate, miscarriage rate, implantation rate, fertilized embryos (n), MII oocytes (n) and antral follicle count.

Supplementary Table 2 - Table summarizing the characteristics of the analysed articles for male patients. [+] favourable increase/improvement; [-] favourable reduction for outcomes where lower values are desirable; [=] no significant/favourable difference.

Supplement	Reference	Study design	Population	n	Intervention / exposure	Corrected outcomes from evidence map
Coenzyme Q10	Akhigbe et al. (2025)	Systematic review and meta-analysis	Men with infertility included in CoQ10 intervention studies	Outcome-specific n; up to 877	CoQ10 supplementation; variable dose and duration	[+] Normal morphology, progressive motility, total motility and total sperm count; [=] sperm concentration and sperm volume.
Coenzyme Q10	Su et al. (2022)	Systematic review and network meta-analysis	Subfertile men included in antioxidant trials	Outcome-specific n; up to 1790	CoQ10 supplementation; variable dose and duration	[+] Normal morphology, total motility and sperm concentration.
Coenzyme Q10	Bahar et al. (2023)	Double-blind placebo-controlled randomized clinical trial	Idiopathic infertile men referred to an infertility center	n = 60; CoQ10: 30; control: 30	CoQ10 100 mg daily for 12 weeks	[+] Normal morphology; [=] progressive motility, total sperm count, sperm viscosity and sperm volume.
Coenzyme Q10	Bakri et al. (2025)	Systematic review and meta-analysis of randomized trials	Men with idiopathic male infertility	n = 781; CoQ10: 430; control: 351	CoQ10 supplementation; variable dose and duration	[+] Clinical pregnancy odds, normal morphology, total motility, sperm concentration and sperm volume; [=] total sperm count and sperm viscosity.
Coenzyme Q10	Alahmar et al. (2019)	Randomized controlled trial	Idiopathic oligoasthenoteratozoospermia (OAT)	n = 85; CoQ10: 50; control: 35	CoQ10 200 or 400 mg daily for 3 months	[+] Progressive motility, total motility and sperm concentration; [=] normal morphology and sperm volume.
Coenzyme Q10	Alahmar et al. (2020)	Randomized controlled trial	Idiopathic oligoasthenoteratozoospermia (OAT)	n = 100; CoQ10: 50; control: 50	CoQ10 200 mg daily for 3 months	[+] Progressive motility, total motility and sperm concentration; [=] normal morphology and sperm volume.
Coenzyme Q10	Alahmar et al. (2022)	Clinical intervention study	Idiopathic oligoasthenozoospermia (OA)	n = 65; CoQ10: 30; control: 35	CoQ10 300 mg daily for 3 months	[+] Progressive motility, total motility and sperm concentration; [=] normal morphology and sperm volume.
L-carnitine	Ma et al. (2022)	Randomized controlled trial	Idiopathic male infertility	n = 143; study: 73; control: 70	L-carnitine 15 g/bag orally, twice daily	[+] Normal morphology, total motility and sperm concentration; [=] progressive motility.
L-carnitine	Khaw et al. (2020)	Systematic review and meta-analysis of randomized controlled trials	Idiopathic male infertility	Outcome-specific n; up to 49	L-carnitine and/or L-acetyl-carnitine supplementation; variable dose and duration	[+] Normal morphology, progressive motility and total motility; [=] clinical pregnancy odds, sperm concentration and sperm volume.

Supplement	Reference	Study design	Population	n	Intervention / exposure	Corrected outcomes from evidence map
L-carnitine	Zhang et al. (2019)	Systematic review and meta-analysis	Idiopathic oligoasthenoteratozoospermia (OAT)	n = 693; outcome-specific	L-carnitine and/or L-acetyl-carnitine supplementation; variable dose and duration	[+] Clinical pregnancy odds, progressive motility and total motility; [=] normal morphology, sperm concentration and sperm volume.
L-carnitine	Su et al. (2022)	Systematic review and network meta-analysis	Subfertile men included in antioxidant trials	Outcome-specific n; up to 1790	L-carnitine supplementation; variable dose and duration	[+] Total motility; [=] normal morphology and sperm concentration.
Vitamin E	Matorras et al. (2020)	Double-blind randomized controlled study	Men from infertile couples undergoing IVF/ICSI	n = 101; vitamin E: 50; control: 51	Vitamin E 400 mg daily for 3 months	[+] Live birth rate per transfer; [-] fertilization rate and normal morphology; [=] clinical pregnancy odds, implantation rate, progressive motility, sperm concentration and total sperm count.
Vitamin E	Zhou et al. (2022)	Systematic review and meta-analysis of randomized controlled trials	Infertile men included in vitamin E trials	Outcome-specific n; up to 832	Vitamin E supplementation; variable dose and duration	[+] Clinical pregnancy odds, progressive motility, sperm concentration and total sperm count; [=] normal morphology, sperm viability and sperm volume.
Vitamin E	Su et al. (2022)	Systematic review and network meta-analysis	Subfertile men included in antioxidant trials	Outcome-specific n; up to 1790	Vitamin E supplementation; variable dose and duration	[+] Total motility; [=] normal morphology and sperm concentration.
Vitamin D supplementation	Jiao et al. (2025)	Systematic review and meta-analysis of prospective randomized controlled trials	Infertile men included in vitamin D3 supplementation trials	n = 945; outcome-specific	Vitamin D3 supplementation; variable dose and duration	[+] Progressive motility and total sperm count.
Vitamin D supplementation	Jensen et al. (2018)	Single-centre triple-blinded randomized clinical trial	Vitamin D-insufficient infertile men	n = 330	Initial cholecalciferol loading dose followed by vitamin D and calcium supplementation for 150 days	[+] Live birth rate per transfer; [=] normal morphology, progressive motility, total motility, sperm concentration and sperm volume.
Vitamin D supplementation	Moghadam et al. (2020)	Experimental clinical study	Asthenozoospermia	n = 80; study: 40; control: 40	Vitamin D exposure/intervention protocol in asthenozoospermic semen samples	[+] Progressive motility and total motility; [=] normal morphology and sperm concentration.
Vitamin D status / levels	Arab et al. (2019)	Systematic review and meta-analysis of observational studies	Fertile and infertile adult men	n = 4773; outcome-specific	Serum vitamin D status assessment	[+] Progressive motility and total motility; [=] normal morphology, sperm concentration, total sperm count and sperm volume.
Vitamin D status / levels	Hajianfar et al. (2021)	Cross-sectional study	Iranian infertile men	n = 350	Serum vitamin D status assessment	[+] Normal morphology, total motility, total sperm count and sperm volume.
Vitamin D status / levels	Rezayat et al. (2022)	Case-control study	Infertile men and fertile controls	n = 114; study: 57; control: 57	Serum vitamin D status assessment	[+] Total motility and total sperm count; [=] normal morphology.

Supplement	Reference	Study design	Population	n	Intervention / exposure	Corrected outcomes from evidence map
Vitamin D status / levels	Ciccone et al. (2021)	Cross-sectional study	Men selected for semen and hormone assessment	n = 260; study: 136; control: 124	Serum vitamin D status assessment	[+] Progressive motility, total motility, sperm concentration and total sperm count; [=] sperm volume.
Vitamin D status / levels	Yan et al. (2023)	Systematic review and meta-analysis	Fertile and infertile men included in vitamin D status studies	Outcome-specific n; up to 7138	Serum vitamin D status assessment	[+] Progressive motility, sperm concentration and total sperm count; [=] normal morphology, sperm viability and sperm volume.