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Male reproductive health and infertility

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Utility of Antioxidants in the Treatment of Male Infertility: Clinical Guidelines Based on a Systematic Review and Analysis of Evidence

Ashok Agarwal[1], Kristian Leisegang[2], Ahmad Majzoub[1,3,4], Ralf Henkel[1,5,6], Renata Finelli[1], Manesh Kumar Panner Selvam[1], Nicholas Tadros[7], Neel Parekh[8], Edmund Y. Ko[9], Chak-Lam Cho[10,11], Mohamed Arafa[1,3,12], Marco G. Alves[13], Pedro Fontes Oliveira[14], Juan G. Alvarez[15], Rupin Shah[16]

[1]American Center for Reproductive Medicine, Cleveland Clinic, Cleveland, OH, USA, [2]School of Natural Medicine, Faculty of Community and Health Sciences, University of the Western Cape, Bellville, South Africa, [3]Department of Urology, Hamad Medical Corporation, [4]Department of Urology, Weill Cornell Medicine - Qatar, Doha, Qatar, [5]Department of Metabolism, Digestion and Reproduction, Imperial College London, London, UK, [6]Department of Medical Bioscience, University of the Western Cape, Bellville, South Africa, [7]Division of Urology, Southern Illinois University School of Medicine, Springfield, IL, [8]Department of Urology, Cleveland Clinic, Cleveland, OH, [9]Department of Urology, Loma Linda University, Loma Linda, CA, USA, [10]Department of Surgery, Union Hospital, [11]S. H. Ho Urology Centre, Department of Surgery, The Chinese University of Hong Kong, Hong Kong, [12]Andrology Department, Cairo University, Giza, Egypt, [13]Department of Anatomy & Unit for Multidisciplinary Research in Biomedicine (UMIB), Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Porto, [14]QOPNA & LAQV, Department of Chemistry, University of Aveiro, Aveiro, Portugal, [15]Centro Androgen, La Coruña, Spain and Harvard Medical School, Boston, MA, USA, [16]Department of Urology, Lilavati Hospital and Research Centre, Mumbai, India

It is widely accepted that oxidative stress plays an important role in the pathophysiology of male infertility and that antioxidants could have a significant role in the treatment of male infertility. The main objectives of this study are: 1) to systematically review the current evidence for the utility of antioxidants in the treatment of male infertility; and 2) propose evidence-based clinical guidelines for the use of antioxidants in the treatment of male infertility. A systematic review of the available clinical evidence was performed, with articles published on Scopus being manually screened. Data extracted included the type of antioxidant used, the clinical conditions under investigation, the evaluation of semen parameters and reproductive outcomes. The adherence to the Cambridge Quality Checklist, Cochrane Risk of Bias for randomized controlled trials (RCTs), CONSORT guidelines and JADAD score were analyzed for each included study. Further, we provided a Strength Weakness Opportunity Threat (SWOT) analysis to analyze the current and future value of antioxidants in male infertility. Of the 1,978 articles identified, 97 articles were included in the study. Of these, 52 (53.6%) were uncontrolled (open label), 12 (12.4%) unblinded RCTs, and 33 (34.0%) blinded RCTs, whereas 44 (45.4%) articles tested individual antioxidants, 31 (32.0%) a combination of several products in variable dosages, and 22 (22.6%) registered antioxidant products. Based on the published evidence, we 1) critically examined the necessity of additional double-blind, randomized, placebo-controlled trials, and 2) proposed updated evidence-based clinical guidelines for antioxidant therapy in male infertility. The current systematic review on antioxidants and male infertility clearly shows that antioxidant supplementation improves semen parameters. In addition, it provides the indications for antioxidant treatment in specific clinical conditions, including varicocele, unexplained and idiopathic male infertility, as well as in cases of altered semen quality.

Keywords: Antioxidants; Oxidative stress; Practice guideline; Semen analysis; Sperm maturation

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Correspondence to: Ashok Agarwal https://orcid.org/0000-0003-0585-1026
Andrology Center and American Center for Reproductive Medicine, Cleveland Clinic, Mail Code X-11, 10681 Carnegie Avenue, Cleveland, OH 44195, USA.
Tel: +1-216-444-9485, **Fax:** +1-216-445-6049, **E-mail:** agarwaa@ccf.org, **Website:** www.Clevelandclinic.org/ReproductiveResearchCenter

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INTRODUCTION

Infertility affects approximately 15% of couples globally, with 2.5%–12% believed to be solely due to male factors. The incidence of male factor infertility varies according to the geographical location, ranging from 20% to 70% [1]. The causes of male infertility are diverse, including genetic causes, varicocele, reproductive tract infections, obstructive or non-obstructive azoospermia, male hypogonadism, and anti-sperm antibodies. However, a large proportion of cases remain unexplained (unexplained male infertility, UMI) (±15%) or idiopathic (idiopathic male infertility, IMI) (30%–50%) in the absence of identifiable female factors [2,3]. In addition, numerous environmental and lifestyle factors have been associated with poor reproductive outcomes in males [4]. Importantly, oxidative stress has been established as a significant mediator in many known causes and risk factors of male infertility, and has further been associated with 30% to 80% of IMI cases in a condition termed male oxidative stress infertility (MOSI) [2,5]. Therefore, the use of antioxidants to reduce oxidative stress across a range of etiologies and risk factors of male infertility has gained increasing attention. This is supported by the wide availability of oral antioxidants, excellent safety and bioavailability profiles, and that antioxidants are considered relatively cost effective [5,6]. Therefore, there is a growing trend of prescribing antioxidants to all males with infertility, even without complete evaluation or relevant guidelines [7].

Exogenous administration of antioxidants has been explored for decades, and the effects of several antioxidants on male fertility have been extensively reported. Numerous trials of different qualities using various antioxidants as mono- or poly-formulations, which may include pharmacologically-active herbal extracts, have been reported [8-11]. The topic was first summarized by a Cochrane meta-analysis in 2011, with updated reviews in 2014 and 2019 [8,9,11]. These reviews investigated the therapeutic benefit of male antioxidant treatment for couples undergoing assisted reproductive technology (ART). Based on limited randomized controlled trials (RCTs), the reviews concluded that low level evidence supports antioxidant therapy in infertile males to increase pregnancy and live birth rates, with no evidence for increased risk of miscarriage [8,9,11]. Majzoub and Agarwal (2018) [10] conducted a systematic review on antioxidant treatment in infertile men, concluding that antioxidants have a positive effect on male fertility, including semen parameters and advanced sperm function, ART outcomes and live birth rates. Antioxidants that are commonly used clinically and investigated scientifically as either an individual application or in combination include vitamin A, vitamin C, vitamin E, carnitine, N-acetyl cysteine, coenzyme Q10 and lycopene, along with important antioxidant co-factors zinc, selenium, and folic acid, as these compounds are significantly involved in essential sperm functions (Fig. 1) [12-15]. However, the outcomes of clinical trials included in the systematic reviews are not consistent, ranging from clear benefit to no clinical effect of the treatment, or even having significant detrimental effects [16-20]. The reasons for this inconsistency are multifactorial and include: small numbers of participants in the studies, variable treatment regimens, dosages, treatment duration, and the lack of placebo-controlled studies. In addition, many of the trials did not evaluate final reproductive outcomes, such as live birth rate, but only certain specific aspects such as seminal volume, sperm concentration and motility, morphology, seminal levels of reactive oxygen species (ROS) or oxidative stress. Furthermore, these antioxidants were

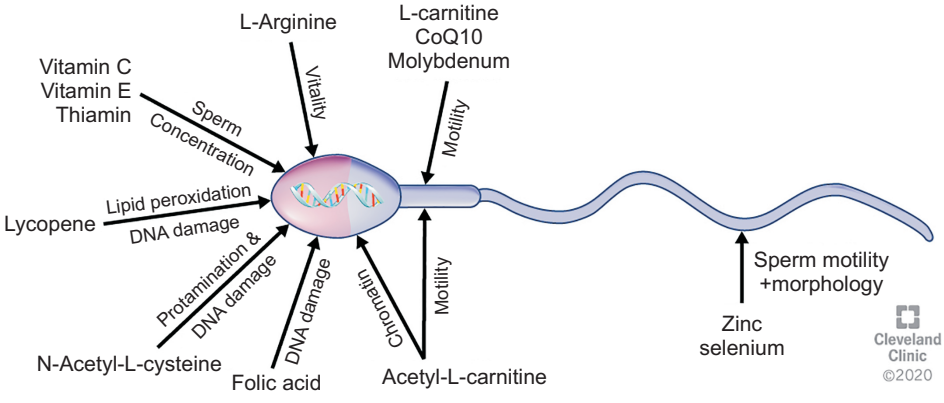

Fig. 1. Individual antioxidant compounds that have significant effects on sperm functions. CoQ: coenzyme Q.

often given in non-proven dosages, thus neglecting the fine bodily redox balance that is necessary for normal physiology, including reproductive functions [21]. If the dosage is too low, the treatment might be ineffective; if it is too high, it can result in an excess of antioxidants causing 'reductive stress', which is as detrimental as oxidative stress [22,23]. Reductive stress due to inappropriate antioxidant dosage may lead to infertility [23,24]. In this regard, high dosages of vitamin E have been shown to have adverse effects [20]. Recently more balanced antioxidant formulations have shown promising results whereby seminal oxidative stress was reduced, sperm function improved and pregnancies achieved [25,26].

With the current rationale and increased use of antioxidants to counteract male infertility, and the heterogeneous and inconsistent data currently available, this study aims to: 1) systematically review the current evidence for antioxidant use to ameliorate male infertility; and 2) propose updated evidence-based clinical guidelines for the use of antioxidants in male infertility.

MATERIALS AND METHODS

1. Literature search strategy

In order to support the development of clinical guidelines for antioxidant use in male infertility, a systematic review of the available clinical evidence was performed to systematically identify relevant clinical trials investigating the impact of antioxidant therapy on semen quality. A literature search was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [27]. The Scopus database was chosen as it currently includes over 1.4 billion cited references, with over 70,000 indexed articles (https://www.elsevier.com/solutions/scopus/how-scopus-works/content). The search was conducted on July 15th, 2020 to identify studies investigating the use of antioxidants in the treatment of male infertility, with no restriction on publication date.

The following keyword strings and Boolean Operators were used: TITLE-ABS-KEY ("antioxidant*") AND TITLE-ABS-KEY ("male infertil*" OR "infertile male*" OR "infertile men" OR "male subfertil*" OR "male steril*" OR "sperm*" OR "seminal" OR "semen"). Further specifications of the search are presented in Supplementary Table 1. Automatic filters were used in the database to specifically include only English original articles and exclude other types of publication such as book chapters, conference papers, editorials, notes, letters, short surveys, erratum, and books.

The articles identified through the keyword search were subsequently screened manually by title, keywords and abstract for eligibility. This screening was independently done by three researchers (RF; KL; MKPS), and the number of articles excluded through screening was recorded. Full text articles were then reviewed for eligibility using the inclusion and exclusion criteria provided in Table 1 , and the number of articles excluded based on these criteria was recorded.

Data was subsequently extracted from the eligible articles, including the clinical trial design, the type of antioxidant or antioxidant formulation used, the clinical condition under investigation, the evaluation of semen parameters and/or sperm function tests (*i.e.,* sperm DNA fragmentation [SDF], oxidative stress markers, capacitation/acrosome reaction, and zona binding test) as well as reproductive outcomes (*i.e.*, fertilization, implantation, pregnancy, miscarriage, and live birth rates).

2. Evaluation of study quality

The quality of all studies included was evaluated by applying the Cambridge Quality Checklist [28]. More-

Table 1. Proposed inclusion and exclusion for article selection

Inclusion	Exclusion
Human participants	Animal and *in vitro* studies
Antioxidants used as intervention individually or combined	Intervention not clearly reported as an antioxidant
Open or controlled clinical trials	Abstracts only, conference abstracts, book chapters, case series, review articles
At least one semen parameter (sperm concentration, motility, morphology) and/or sperm function parameters (sperm DNA fragmentation, seminal oxidative stress markers, mitochondrial membrane integrity) reported after antioxidant treatment	Non-english studies

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over, the quality of RCT was further evaluated by using the Cochrane Risk of Bias [29] and the JADAD score [30], as well as by evaluating the adherence to CONSORT guidelines [31]. Based on a combination of these quality evaluation tools, the studies were categorized into "low" (0) and "high" (1) quality. All uncontrolled studies were considered "low-quality", in comparison with controlled studies, which were evaluated according to the criteria reported in Supplementary Table 2. We have created this scoring system, as no such method was previously reported in the literature.

In addition, the most recent clinical trials reporting the effect of antioxidant treatment on male infertility, published from January 2019 to July 2020, were further ranked based on study design, the sample size analysed, the inclusion/exclusion criteria used for selecting the population, the antioxidant regimen used, the length of treatment, the assessment of oxidative stress markers, pregnancy and live birth rates. This range of time was specifically selected to gain an understanding of the most recent evidence on the antioxidant therapy and the quality of the studies currently conducted. The selection of these criteria was achieved through a consensus among the male infertility experts involved in this study. The system provides a total score of a maximum of 12 points and a classification of the articles in "low" (<6 points) and "high" (≥6 points) quality, as reported in Supplementary Table 3.

Clinical recommendations were proposed based on the quality of the evidence, classified as A, B, C, D (Oxford Centre for Evidence-Based Medicine 2011 Levels of Evidence; https://www.cebm.net/wp-content/uploads/2014/06/CEBM-Levels-of-Evidence-Introduction-2.1.pdf and https://www.cebm.net/wp-content/uploads/2014/06/CEBM-Levels-of-Evidence-2.1.pdf).

3. Statistical analysis

Statistical analysis was performed using MedCalc statistical software version 19.5.3. (MedCalc Software bv, Ostend, Belgium). Chi-square test was used to evaluate the association between the quality of the study and the outcomes (positive or no/negative effect) due to antioxidant treatment on semen parameters and sperm function such as oxidative stress and SDF are presented in Table 2 [25,26,32-121]. A p-value <0.05 was considered statistically significant. When the p-value was ≥0.05, a sample size calculation was carried out to predict the required sample size to attain a statistical

Table 2. Articles investigating the impact of antioxidant treatment on reproductive outcomes

Clinical condition	SN	Reference	Clinical trial design	Antioxidant formulation dosage and length of treatment	Study population	Reproductive outcomes after antioxidant treatment	Cambridge Quality Checklist			Cochrane Risk of Bias for RCT	CONSORT Guidelines (out of 25)	JADAD (Oxford Quality) (out of 5)	Quality of evidence published
							Checklist for correlate (out of 5)	Checklist for risk factors (out of 3)	Checklist for causal risk factors (out of 7)				
Infertile men	1	Kessopoulou et al (1995) [19]	RCT blinded	α-tocopheryl acetate (Ephynal, F. Hoffman-La Roche ltd) 300 mg/daily for 3 months	30 infertile men	No difference in semen parameters before and after treatment No difference in ROS levels Improved zona binding	2	3	7	Unclear risk of bias for random sequence generation, allocation concealment, selective reporting, other sources and blinding (outcome assessment)	20	4	1

Table 2. Continued 1

Clinical condition	SN	Reference	Clinical trial design	Antioxidant formulation dosage and length of treatment	Study population	Reproductive outcomes after antioxidant treatment	Cambridge Quality Checklist			Cochrane Risk of Bias for RCT	CONSORT Guidelines (out of 25)	JADAD (Oxford Quality) (out of 5)	Quality of evidence published
							Checklist for correlate (out of 5)	Checklist for risk factors (out of 3)	Checklist for causal risk factors (out of 7)				
	2	Roseff (2002) [32]	Uncontrolled (open label)	Pycnogenol (Horphag Research, Geneve, Switzerland) 200 mg daily for 3 months	19 infertile men	No difference in semen parameters Improved sperm binding capacity	2	3	3	N/A	N/A	N/A	0
	3	Keskes-Ammar et al (2003) [18]	RCT unblinded	Vitamin E (400 mg) (Ephynal 100 mg, 2 tablets) or selenium (225 µg) for 3 months	54 infertile men	Improved sperm motility Reduced MDA levels	1	3	4	No risk of bias identified	12	3	0
	4	Tremellen et al (2007) [33]	RCT blinded	Menevit (Bayer, Sydney, Australia) 1 capsule/day for 3 months	Men with evidence of seminal oxidative stress and SDF>25% by TUNEL. The total number of patients is not clearly reported.	No differences between treated and placebo for fertilization, implantation, pregnancy, and miscarriage rates Live pregnancy rate higher in treated patients	0	3	7	No risk of bias identified	19	3	0
	5	Ménézo et al (2007) [20]	Uncontrolled (open label)	Vitamins C and E (400 mg each), ß-carotene (18 mg), zinc (500 µmoL), selenium (1 µmoL) for 3 months	58 patients experiencing 2 previous failures of IVF or ICSI, and DFI and chromatin decondensation>15%	Reduced SDF but higher sperm decondensation	0	3	3	N/A	N/A	N/A	0
	6	Tunc et al (2009) [34]	Uncontrolled (open label)	Menevit (Bayer Australia Ltd, Sydney, Australia) 1 capsule/daily for 3 months for a maximum of 3 months	50 infertile men with high OS	No difference in semen parameters Reduced SDF, ROS and apoptotic markers Improved DNA protamination	3	3	3	N/A	N/A	N/A	0

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Table 2. Continued 2

Clinical condition	SN	Reference	Clinical trial design	Antioxidant formulation dosage and length of treatment	Study population	Reproductive outcomes after antioxidant treatment	Cambridge Quality Checklist			Cochrane Risk of Bias for RCT	CONSORT Guidelines (out of 25)	JADAD (Oxford Quality) (out of 5)	Quality of evidence published
							Checklist for correlate (out of 5)	Checklist for risk factors (out of 3)	Checklist for causal risk factors (out of 7)				
	7	Shukla et al (2010) [35]	Uncontrolled (open label)	Mucuna pruriens seed powder 5 g/daily for 3 months	120 infertile men	Improved sperm count and motility, seminal plasma lipid peroxide levels, SOD, catalase, GSH and ascorbic acid	1	3	6	N/A	N/A	N/A	0
	8	da Silva et al (2013) [17]	RCT blinded	Folic acid 5 mg/daily for 3 months	70 infertile men	No difference in semen parameters	3	3	7	No risk of bias identified	15	5	1
	9	Bejarano et al (2014) [36]	Uncontrolled (open label)	Melatonin 6 mg/daily for 45 days	30 infertile men	Improved semen parameters, urinary and semen TAC Reduced SDF Improved embryo quality	3	3	3	N/A	N/A	N/A	0
	10	Martínez-Soto et al (2016) [37]	Uncontrolled (open label)	1.5 g capsules of docosahexaenoic acid oil/ daily for 10 weeks	57 infertile men	No changes in semen parameters Increased seminal TAC, reduced DNA damage	0	3	3	N/A	N/A	N/A	0
	11	Chattopadhyay et al (2016) [16]	Uncontrolled (open label)	L-Carnitine, Acetyl-L-Carnitine, CoQ10, Lycopene, Zinc, Folic acid, Vitamin B12, Selenium, Fructose, and citric acid (dosage not reported) for 6 months	115 infertile men	Increased sperm count, motility, TAC Reduced ROS levels	0	3	3	N/A	N/A	N/A	0

Table 2. Continued 3

Clinical condition	SN	Reference	Clinical trial design	Antioxidant formulation dosage and length of treatment	Study population	Reproductive outcomes after antioxidant treatment	Cambridge Quality Checklist			Cochrane Risk of Bias for RCT	CONSORT Guidelines (out of 25)	JADAD (Oxford Quality) (out of 5)	Quality of evidence published
							Checklist for correlate (out of 5)	Checklist for risk factors (out of 3)	Checklist for causal risk factors (out of 7)				
	12	Hosseini et al (2016) [38]	RCT blinded	Ginger powder 250 mg/daily for 3 months	100 patients with SDF≥15%	No difference in semen parameters Decreased SDF	2	3	7	Unclear risk of bias for other sources	23	5	1
	13	Stenqvist et al (2018) [39]	RCT blinded	Vitamin C (30 mg), vitamin E (5 mg), vitamin B12 (0.5 ug), l-carnitine (750 mg), coenzyme Q10 (10 mg), folic acid (100 ug), zinc (5 mg), selenium (25 ug) with malto-dextrin, calcium carbonate, citric acid, ste-viol glycoside, flavours, beta-carotene, silicon dioxide/daily for 6 months	77 infertile men with DFI ≥25%	Improved sperm concentration, no change in DNA damage	4	3	7	No risk of bias identified	19	5	1
	14	Ahmad et al (2008) [40]	Uncontrolled (open label)	Mucuna pruriens seed powder 5 g/daily for 3 months	60 infertile men	Improved volume, sperm concen-tration, count, motility Reduced MDA levels	0	3	5	N/A	N/A	N/A	0
	15	Alizadeh et al (2018) [41]	RCT blinded	Curcumin 80 mg/daily for 10 weeks	60 infertile men	Increased sperm count, concen-tration, total mo-tility and vitality, TAC Reduced MDA and inflammatory biomarkers	0	3	7	Unclear risk of bias for other sources	19	4	0

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Table 2. Continued 4

Clinical condition	SN	Reference	Clinical trial design	Antioxidant formulation dosage and length of treatment	Study population	Reproductive outcomes after antioxidant treatment	Cambridge Quality Checklist			Cochrane Risk of Bias for RCT	CONSORT Guidelines (out of 25)	JADAD (Oxford Quality) (out of 5)	Quality of evidence published
							Checklist for correlate (out of 5)	Checklist for risk factors (out of 3)	Checklist for causal risk factors (out of 7)				
	16	Salehi et al (2019) [42]	Uncontrolled (open label)	Vitamin E (50 mg), vitamin C (500 mg) and CoQ10 (100 mg) for 3 months	485 infertile men with DFI>27% by SCSA	Improved semen parameters Reduced DNA damage Pregnancy rate=16.8%	5	3	3	N/A	N/A	N/A	0
	17	Hasoon (2019) [43]	Uncontrolled (open label)	L-arginine (1 g) and CoQ10 (200 mg) for 8 months	24 infertile men	Improved volume, sperm count, motility, and normal morphology	2	3	3	N/A	N/A	N/A	0
	18	Nurmawati et al (2020) [44]	RCT blinded	Astaxanthin 8 mg/daily for 1 month	25 infertile men	Improved sperm concentration, motility, and morphology Reduced MDA and 8-OHdG levels	4	3	7	Unclear risk of bias for selective reporting and other sources; high risk of bias for random sequence generation, allocation concealment and blinding (outcome assessment)	15	3	0
	19	Hadi et al (2020) [45]	Uncontrolled (open label)	L-carnitine 2 g/daily for 3 months	58 infertile men	Improved sperm count, total motility, and normal morphology In serum: reduced FSH and LH levels, increased testosterone, and inhibin levels	2	3	3	N/A	N/A	N/A	0

Table 2. Continued 5

Clinical condition	SN	Reference	Clinical trial design	Antioxidant formulation dosage and length of treatment	Study population	Reproductive outcomes after antioxidant treatment	Cambridge Quality Checklist			Cochrane Risk of Bias for RCT	CONSORT Guidelines (out of 25)	JADAD (Oxford Quality) (out of 5)	Quality of evidence published
							Checklist for correlate (out of 5)	Checklist for risk factors (out of 3)	Checklist for causal risk factors (out of 7)				
	20	Schisterman et al (2020) [46]	RCT blinded	Folic acid 5 mg/daily and 30 mg zinc for 6 months	1,185 male partners of couples planning IVF for infertility treatment	No changes in semen parameters; improved SDF by Comet assay; no significant differences in β-HCG–detected pregnancy, clinical intrauterine pregnancy, ectopic pregnancy, pregnancy with multiple fetuses, live birth rate	2	3	7	Unclear risk of bias for random sequence generation, allocation concealment, other sources, selective reporting and blinding	14	3	0
Varicocele	21	Comhaire et al (2000) [47]	Uncontrolled (open label)	Acetylcysteine (600 mg) or capsules providing a daily amount of β-carotene (30 mg) and α-tocopherol (180 mg)/daily. In addition, capsules containing essential fatty acids for a daily intake of docosahexaenoic acid (1 g), gammalinolenic acid (0.25 g) and arachidonic acid (0.10 g) for 6 months	7 idiopathic patients 11 varicocele patients History of cryptorchidism (n=2), patients with male accessory gland infection (n=7), immunological infertility (n=4), endocrine cause (n=1)	Improved sperm concentration and acrosome reaction Reduced ROS levels and 8-OHdG levels	2	3	3	N/A	N/A	N/A	0

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Table 2. Continued 6

Clinical condition	SN	Reference	Clinical trial design	Antioxidant formulation dosage and length of treatment	Study population	Reproductive outcomes after antioxidant treatment	Cambridge Quality Checklist			Cochrane Risk of Bias for RCT	CONSORT Guidelines (out of 25)	JADAD (Oxford Quality) (out of 5)	Quality of evidence published
							Checklist for correlate (out of 5)	Checklist for risk factors (out of 3)	Checklist for causal risk factors (out of 7)				
	22	Paradiso Galatioto et al (2008) [48]	RCT unblinded	Ccommercial preparation of: NAC (10 mg/kg/die), vitamin C (3 mg/kg/die), vitamin E (0.2 mg/kg/die), vitamin A (0.06 IU/kg/die), thiamine (0.4 mg/kg/die), riboXavin (0.1 mg/kg/die), piridoxin (0.2 mg/kg/die), nicotinamide (1 mg/kg/die), pantothenate (0.2 mg/kg/die), biotin (0.04 mg/kg/die), cyanocobalamin (0.1 mg/kg/die), ergocalciferol (8 IU/kg/die), calcium (1 mg/kg/die), magnesium (0.35 mg/kg/die), phosphate (0.45 mg/kg/die), iron (0.2 mg/kg/ die), manganese (0.01 mg/kg/die), copper (0.02 mg/kg/die), zinc (0.01 mg/kg/die) for a minimum of 90 days	42 varicocele patients with persistent oligospermia 6 months after retrograde embolization	Improved semen parameters No change in pregnancy rate	3	3	7	No risk of bias identified	16	5	1

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Table 2. Continued 7

Clinical condition	SN	Reference	Clinical trial design	Antioxidant formulation dosage and length of treatment	Study population	Reproductive outcomes after antioxidant treatment	Cambridge Quality Checklist			Cochrane Risk of Bias for RCT	CONSORT Guidelines (out of 25)	JADAD (Oxford Quality) (out of 5)	Quality of evidence published
							Checklist for correlate (out of 5)	Checklist for risk factors (out of 3)	Checklist for causal risk factors (out of 7)				
	23	Oliva et al (2009) [49]	Uncontrolled (open label)	Pentoxifylline (1.2 g), folic acid (5 mg) and zinc sulfate (66 mg) for 3 months	36 varicocele patients	Improved semen parameters	2	3	3	N/A	N/A	N/A	0
	24	Festa et al (2014) [50]	Uncontrolled (open label)	CoQ10 100 mg/daily for 3 months	38 varicocele patients	Sperm concentration, progressive motility, and TAC	0	3	4	N/A	N/A	N/A	0
	25	Pourmand et al (2014) [51]	RCT unblinded	L-carnitine 750 mg/daily for 6 months	100 varicocele patients	No changes in semen parameters, SDF and protamine damage assay	0	3	7	Unclear risk of bias for random sequence generation, allocation concealment, and incomplete outcome data	14	2	0
	26	Nematollahi-Mahani et al (2014) [52]	RCT unblinded	A) Zinc sulphate/ folic acid B) Folic acid (5 mg/daily) C) Zinc sulphate (66 mg/daily) D) Placebo For 6 months	160 varicocele patients	No difference in TAC between the groups Increased SOD activity in group A and C	0	3	7	Unclear risk of bias for random sequence generation, allocation concealment, selective reporting, other sources, and incomplete outcome data	12	2	0

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Table 2. Continued 8

Clinical condition	SN	Reference	Clinical trial design	Antioxidant formulation dosage and length of treatment	Study population	Reproductive outcomes after antioxidant treatment	Cambridge Quality Checklist			Cochrane Risk of Bias for RCT	CONSORT Guidelines (out of 25)	JADAD (Oxford Quality) (out of 5)	Quality of evidence published
							Checklist for correlate (out of 5)	Checklist for risk factors (out of 3)	Checklist for causal risk factors (out of 7)				
	27	Cyrus et al (2015) [53]	RCT blinded	Vitamin C 250 mg/daily for 3 months	115 varicocele patients	Improved semen parameters	2	3	6	Unclear risk of bias for random sequence generation, allocation concealment, selective reporting, other sources, blinding (participants and personnel, outcome assessment), and incomplete outcome data	18	5	0
	28	Gual-Frau et al (2015) [54]	Uncontrolled (open label)	L-Carnitine (1,500 mg), vitamin C (60 mg), CoQ10 (20 mg), vitamin E (10 mg), vitamin B9 (200 μg), vitamin B12 (1 μg), zinc (10 mg), selenium (50 μg) for 3 months	20 varicocele patients	Improved total sperm count and reduced SDF	2	3	3	N/A	N/A	N/A	0

Table 2. Continued 9

Clinical condition	SN	Reference	Clinical trial design	Antioxidant formulation dosage and length of treatment	Study population	Reproductive outcomes after antioxidant treatment	Cambridge Quality Checklist			Cochrane Risk of Bias for RCT	CONSORT Guidelines (out of 25)	JADAD (Oxford Quality) (out of 5)	Quality of evidence published
							Checklist for correlate (out of 5)	Checklist for risk factors (out of 3)	Checklist for causal risk factors (out of 7)				
	29	Barekat et al (2016) [55]	RCT blinded	N-acetyl-L-cysteine (NAC) 200 mg/daily for 3 months	35 varicocele patients	No changes for sperm concentration, motility, morphology, % of ROS negative sperm and intensity of sperm ROS Improved normal protamine content and DNA integrity Pregnancy rate: NAC group=33.4%, control group=10%. No p-value reported	1	3	4	Unclear risk of bias for random sequence generation, allocation concealment, selective reporting, other sources and blinding (participants and personnel as well as outcome assessment)	12	2	0
	30	Kızılay and Altay (2019) [56]	RCT unblinded	L-carnitine fumarate (2 g), Acetyl-L- carnitine HCl (1 g), fructose (2 g), citric acid (100 mg), vitamin C (180 mg), zinc (20 mg), folic acid (400 mg), selenium (100 mg), coenzyme Q-10 (40 mg), vitamin B12 (3 mg)/daily for 6 months	90 varicocele patients	Improved semen parameters Higher pregnancy rate	3	3	7	High risk of bias for random sequence generation, allocation concealment, other sources, blinding (participants and personnel, outcome assessment), incomplete outcome data	19	2	0

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Table 2. Continued 10

Clinical condition	SN	Reference	Clinical trial design	Antioxidant formulation dosage and length of treatment	Study population	Reproductive outcomes after antioxidant treatment	Cambridge Quality Checklist			Cochrane Risk of Bias for RCT	CONSORT Guidelines (out of 25)	JADAD (Oxford Quality) (out of 5)	Quality of evidence published
							Checklist for correlate (out of 5)	Checklist for risk factors (out of 3)	Checklist for causal risk factors (out of 7)				
	31	Ardestani Zadeh et al (2019) [57]	RCT unblinded	Folic acid (5 mg), Selenium (200 μg) and vitamin E (400 IU)/daily for 6 months	60 varicocele patients	Improved sperm count and motility	2	3	7	Unclear risk of bias for allocation concealment, other sources; high risk of bias for blinding (participants and personnel, outcome assessment)	24	4	1
Abnormal semen quality	32	Suleiman et al (1996) [58]	RCT blinded	Vitamin E 300 mg/daily for 6 months	Oligoasthenо- (n=74), azoospermic (n=38), asthenospermic (n=94), oligospermic (n=30) patients High viscosity (n=22); oligospermic with high viscosity (n=6); asthenospermic with high viscosity (n=12); oligoasthenospermic with high viscosity (n=10)	Improved sperm motility Reduced MDA levels Higher pregnancy and live birth rates	4	3	7	Unclear risk of bias for random sequence generation, allocation concealment, other sources, blinding (participants and personnel, outcome assessment), incomplete outcome data	12	3	0
	33	Rolf et al (1999) [59]	RCT blinded	Vitamin C (1,000 mg) and Vitamin E (800 mg)/daily for 56 days	31 asthenozoospermic patients	No changes in semen parameters	0	3	7	No risk of bias identified	21	5	1

Table 2. Continued 11

Clinical condition	SN	Reference	Clinical trial design	Antioxidant formulation dosage and length of treatment	Study population	Reproductive outcomes after antioxidant treatment	Cambridge Quality Checklist			Cochrane Risk of Bias for RCT	CONSORT Guidelines (out of 25)	JADAD (Oxford Quality) (out of 5)	Quality of evidence published
							Checklist for correlate (out of 5)	Checklist for risk factors (out of 3)	Checklist for causal risk factors (out of 7)				
	34	Vicari and Calogero (2001) [60]	Uncontrolled (open label)	Carnitene® (Sigma-Tau, Pomezia-Rome, Italy) Twice/day for3 months, followed by a treatment-free period of 3 months	54 OAT patients with prostato-vesiculo-epididy-mitis	Improved sperm progressive motility and viability Reduced ROS production Higher pregnancy rate	3	3	3	N/A	N/A	N/A	0
	35	Suzuki et al (2003) [61]	Uncontrolled (open label)	Sairei-to 9.0 g/daily for 3 months	16 healthy men 47 non-nor-mozoospermic patients	Improved sperm concentration, and total motility No change in SOD activity	3	3	3	N/A	N/A	N/A	0
	36	Balercia et al (2004) [62]	Uncontrolled (open label)	CoQ10 (Phar-maNord, Veyle, Denmark) 400 mg/daily for 6 months	22 asthenozoosper-mic patients	Improved progres-sive motility after treatment, which reduced after 6 months of wash-out Pregnancy rate=2.4%, with 3 out of 22 pa-tients achieving a spontaneous pregnancy	4	3	4	N/A	N/A	N/A	0
	37	Piomboni et al (2008) [63]	Uncontrolled (open label)	Fattore M (Progine, Flor-ence, Italy) 2 tables/day for 3 months	51 asthenotera-tozoospermic patients	Improved semen parameters and leukocytosper-mia Reduced SDF	1	3	6	N/A	N/A	N/A	0

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Table 2. Continued 12

Clinical condition	SN	Reference	Clinical trial design	Antioxidant formulation dosage and length of treatment	Study population	Reproductive outcomes after antioxidant treatment	Cambridge Quality Checklist			Cochrane Risk of Bias for RCT	CONSORT Guidelines (out of 25)	JADAD (Oxford Quality) (out of 5)	Quality of evidence published
							Checklist for correlate (out of 5)	Checklist for risk factors (out of 3)	Checklist for causal risk factors (out of 7)				
	38	Ghanem et al (2010) [64]	RCT blinded	Clomiphene citrate (25 mg/day) and vitamin E (400 mg/day) for 6 months	60 oligoasthenozoospermic patients	Increased sperm concentration and motility Higher pregnancy rate	0	3	7	Unclear risk of bias for other sources and blinding (participants and personnel, outcome assessment)	17	4	0
	39	Ahmad et al (2010) [65]	Uncontrolled (open label)	Withania somnifera 5 g/daily for 3 months	Oligo- (n=25), astheno- (n=25) and normozoospermic (n=25) patients	Improved sperm count and motility, SOD, catalase, and glutathione levels Decreased MDA and Protein Carbonyl levels	2	3	4	N/A	N/A	N/A	0
	40	Nadjarzadeh et al (2011) [66]	RCT blinded	CoQ10 capsules (Nutraceutical Science Institute, NC, USA) 200 mg/daily for 3 months	60 OAT patients	No changes in semen parameters Reduced MDA and improved TAC	4	3	7	Unclear risk of bias for random sequence generation, allocation concealment, other sources and blinding (participants and personnel); high risk of bias for incomplete outcome data	20	4	1

Table 2. Continued 13

Clinical condition	SN	Reference	Clinical trial design	Antioxidant formulation dosage and length of treatment	Study population	Reproductive outcomes after antioxidant treatment	Cambridge Quality Checklist			Cochrane Risk of Bias for RCT	CONSORT Guidelines (out of 25)	JADAD (Oxford Quality) (out of 5)	Quality of evidence published
							Checklist for correlate (out of 5)	Checklist for risk factors (out of 3)	Checklist for causal risk factors (out of 7)				
	41	Shukla et al (2011) [67]	Uncontrolled (open label)	Withania somnifera 5 g/daily for 3 months	Oligo- (n=25), astheno- (n=25) and normozoospermic (n=25) patients	Decreased intracellular ROS and apoptosis; increased levels of Cu2+, Zn2+, Fe2+ and Au2+	2	3	4	N/A	N/A	N/A	0
	42	Safarinejad (2011) [68]	RCT blinded	Eicosapentanoic (1.12 g) and docosahexaenoic (0.72 g) acid/daily for 8 months	211 OAT patients	Improved total sperm count, concentration, motility, normal morphology, seminal SOD, and catalase	3	3	7	Unclear risk of bias for other sources and incomplete outcome data	21	5	1
	43	Safarinejad (2011) [69]	RCT blinded	Pentoxifylline 800 mg/daily for 6 months	278 OAT patients	No changes in semen parameters, seminal SOD, catalase, and reproductive hormones	3	3	7	Unclear risk of bias for other sources and incomplete outcome data	19	5	0
	44	Moslemi and Tavanbakhsh (2011) [70]	Uncontrolled (open label)	Selenium (200 µg), vitamin E (400 units)/daily for 100 days	690 asthenoteratospermic patients	Improved semen parameters Higher spontaneous pregnancy	1	3	3	N/A	N/A	N/A	0
	45	Safarinejad et al (2011) [71]	RCT blinded	Crocus sativus 60 mg/daily for 26 weeks	260 OAT patients	No changes in semen parameters, SOD and catalase-like activity, LH, FSH, PRL, TSH, testicular volume	2	3	7	Unclear risk of bias for random sequence generation, selective reporting	20	5	1
	46	Safarinejad (2012) [72]	Uncontrolled (open label)	CoQ10 300 mg/daily for 12 months	287 OAT patients	Improved semen parameters No change in pregnancy and miscarriage rates	2	3	4	N/A	N/A	N/A	0

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Table 2. Continued 14

Clinical condition	SN	Reference	Clinical trial design	Antioxidant formulation dosage and length of treatment	Study population	Reproductive outcomes after antioxidant treatment	Cambridge Quality Checklist			Cochrane Risk of Bias for RCT	CONSORT Guidelines (out of 25)	JADAD (Oxford Quality) (out of 5)	Quality of evidence published
							Checklist for correlate (out of 5)	Checklist for risk factors (out of 3)	Checklist for causal risk factors (out of 7)				
	47	Abad et al (2013) [73]	Uncontrolled (open label)	Androferti (Q Pharma Laboratories, Alicante, Spain) 1 capsule/daily for 3 months	20 asthenoteratozoospermic patients	Improved sperm concentration, motility, vitality, morphology, DNA integrity Pregnancy rate=5%	2	3	3	N/A	N/A	N/A	0
	48	Ajayi et al (2013) [74]	Uncontrolled (open label)	Vitamin C (200 mg), vitamin E (200 mg), folic acid (1 mg), zinc (50 mg), selenium (200 μg), n-acetyl-L-cysteine (100 mg), L-carnitine (600 mg), citrulline (600 mg), glutathione red. (100 mg), lycopene (8 mg), CoQ10 (30 mg)/daily for at least 2 months	Oligo- (n=20), astheno- (n=33), OAT (n=42) patients 65 healthy men	Improved semen parameters	3	3	3	N/A	N/A	N/A	0

Table 2. Continued 15

Clinical condition	SN	Reference	Clinical trial design	Antioxidant formulation dosage and length of treatment	Study population	Reproductive outcomes after antioxidant treatment	Cambridge Quality Checklist			Cochrane Risk of Bias for RCT	CONSORT Guidelines (out of 25)	JADAD (Oxford Quality) (out of 5)	Quality of evidence published
							Checklist for correlate (out of 5)	Checklist for risk factors (out of 3)	Checklist for causal risk factors (out of 7)				
	49	Nadjarzadeh et al (2014) [75]	RCT blinded	CoQ10 (Nutraceutical Science Institute, NC, USA) 200 mg/daily for 3 months	60 OAT patients	No changes in semen parameters Increased seminal level of CoQ10, catalase and SOD activity; reduced level of seminal plasma 8-iso-prostane	4	3	7	Unclear risk of bias for random sequence generation, allocation concealment, selective reporting, other sources, blinding (participants and personnel, outcome assessment), and incomplete outcome data	18	3	0
	50	Raigani et al (2014) [76]	RCT blinded	Folic acid (5 mg) and zinc sulphate (220 mg)/daily for 4 months	83 OAT patients	No difference in semen parameters Increased sperm chromatin integrity	2	3	7	Unclear risk of bias for allocation concealment, other sources	20	4	1
	51	Kobori et al (2014) [77]	Uncontrolled (open label)	CoQ10 (120 mg), vitamin C (80 mg), vitamin E (40 mg)/daily for 6 months	169 OAT patients	Improved sperm concentration and motility 48 (28.4%) pregnancies achieved; of those, 16 were spontaneous and 32 by using ART	0	3	3	N/A	N/A	N/A	0

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Table 2. Continued 16

Clinical condition	SN	Reference	Clinical trial design	Antioxidant formulation dosage and length of treatment	Study population	Reproductive outcomes after antioxidant treatment	Cambridge Quality Checklist			Cochrane Risk of Bias for RCT	CONSORT Guidelines (out of 25)	JADAD (Oxford Quality) (out of 5)	Quality of evidence published
							Checklist for correlate (out of 5)	Checklist for risk factors (out of 3)	Checklist for causal risk factors (out of 7)				
	52	Thakur et al (2015) [78]	Uncontrolled (open label)	Ubiquinol 150 mg/daily for 6 months	60 OAT patients	Improved sperm concentration, total and progressive motility Testosterone unchanged	0	3	3	N/A	N/A	N/A	0
	53	Kobori et al (2015) [79]	Uncontrolled (open label)	Edicare (KOBAYASHI Pharmaceutical Co., Ltd, Japan) 6 pills/daily for 3 months	47 OAT patients	Improved sperm concentration	3	3	3	N/A	N/A	N/A	0
	54	Hadwan et al (2015) [80]	Uncontrolled (open label)	Zinc sulphate 440 mg/daily for 3 months	60 asthenozoospermic patients 60 healthy men	Improved volume, progressive motility, total sperm count, and catalase activity	3	3	3	N/A	N/A	N/A	0
	55	Al-Hilli et al (2009) [81]	Uncontrolled (open label)	Simvastation tablet 40 mg/daily for 3 months	Astheno- (n=1), oligoastheno- (n=2), OAT (n=7), terato- (n=7), asthenonecro- (n=2), asthenoterato- (n=20), asthenoteratonecro- (n=4), 0ligo-asthenoteratonecrozoospermic (n=2) patients	Improved sperm motility and normal sperm morphology Decreased MDA level	2	3	4	N/A	N/A	N/A	0

Table 2. Continued 17

Clinical condition	SN	Reference	Clinical trial design	Antioxidant formulation dosage and length of treatment	Study population	Reproductive outcomes after antioxidant treatment	Cambridge Quality Checklist			Cochrane Risk of Bias for RCT	CONSORT Guidelines (out of 25)	JADAD (Oxford Quality) (out of 5)	Quality of evidence published
							Checklist for correlate (out of 5)	Checklist for risk factors (out of 3)	Checklist for causal risk factors (out of 7)				
	56	Martinez et al (2015) [82]	RCT blinded	Resveratrol 25 mg/daily SG1002 (Nuevas Alternativas Naturales Thermafat, S.A. de C.V., Monterrey, Mexico) 750 mg/daily for 75 days	54 oligoasthenozoospermic patients	Improved sperm concentration and motility	0	3	7	Unclear risk of bias for allocation concealment, other sources; high risk of bias for incomplete outcome data	18	5	0
	57	Gvozdjáková et al (2015) [83]	Uncontrolled (open label)	Carni-Q-Nol (Tishcon Corp., Westbury, NY, USA) 2 softsules for the first 3 months, 3 softsules for the last 3 months	40 oligoasthenozoospermic patients	Improved sperm concentration Reduced concentrations of α-tocopherol and γ-tocopherol in seminal fluid, as well as TBARS, a marker of lipid peroxidation Pregnancy in 45% of couples	0	3	3	N/A	N/A	N/A	0
	58	ElSheikh et al (2015) [84]	RCT unblinded	A) Vitamin E (400 mg/daily) B) Clomiphene citrate (25 mg/daily) C) Vitamin E+clomiphene citrate for 6 months	90 oligoasthenozoospermic patients	Improved sperm concentration in group B and C, while total sperm motility improved in all groups	0	3	7	Unclear risk of bias for other sources	15	3	0
	59	Montanino Oliva et al (2016) [85]	Uncontrolled (open label)	(Andrositol, Lo.Li. Pharma s.r.l., Rome, Italy) 2 capsules/daily for 3 months	45 asthenozoospermic patients	Improved concentration, motility, normal morphology	0	3	3	N/A	N/A	N/A	0

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Table 2. Continued 18

Clinical condition	SN	Reference	Clinical trial design	Antioxidant formulation dosage and length of treatment	Study population	Reproductive outcomes after antioxidant treatment	Cambridge Quality Checklist			Cochrane Risk of Bias for RCT	CONSORT Guidelines (out of 25)	JADAD (Oxford Quality) (out of 5)	Quality of evidence published
							Checklist for correlate (out of 5)	Checklist for risk factors (out of 3)	Checklist for causal risk factors (out of 7)				
	60	Singh et al (2016) [86]	Uncontrolled (open label)	Tablet Fertisure M (Sun Pharma) Twice/day for 3 months	7 oligozoospermic patients 31 oligoasthenozoospermic patients 2 OAT patients	Improved sperm count and motility, glutathione level Reduced MDA level	2	3	3	N/A	N/A	N/A	0
	61	Alahmar (2017) [87]	Uncontrolled (open label)	Hansal A–Z Vital (Hansal Pharm GmbH, Germany) for 3 months	32 oligoasthenozoospermic patients	Improved sperm concentration, total and progressive motility	3	3	3	N/A	N/A	N/A	0
	62	Yamamoto et al (2017) [88]	RCT unblinded	A) Natsushibori (Kagome Co., Ltd., Japan) B) CINAL Combination Tablet (600 mg/day, Shionogi Pharmaceutical Co., Japan), Juvela N Soft Capsule (200 mg/day, Tanabe Seiyaku Hanbai Co., Japan), and Tathion Tablet (300 mg/day, Eisai Co., Japan) For 3 months	54 oligoasthenozoospermic patients	Improved sperm motility	2	3	7	Unclear risk of bias for allocation concealment, other sources; high risk of bias for blinding (participants and personnel, outcome assessment)	16	2	0
	63	Magdi et al (2017) [89]	Uncontrolled (open label)	Vitamin C (1 g), vitamin E (400 mg) and L-carnitine (2 g)/daily for 6 months	210 OAT patients	Improved sperm count, total and progressive motility, normal morphology after treatment	0	3	3	N/A	N/A	N/A	0

Table 2. Continued 19

Clinical condition	SN	Reference	Clinical trial design	Antioxidant formulation dosage and length of treatment	Study population	Reproductive outcomes after antioxidant treatment	Cambridge Quality Checklist			Cochrane Risk of Bias for RCT	CONSORT Guidelines (out of 25)	JADAD (Oxford Quality) (out of 5)	Quality of evidence published
							Checklist for correlate (out of 5)	Checklist for risk factors (out of 3)	Checklist for causal risk factors (out of 7)				
	64	Alsalman et al (2018) [90]	Uncontrolled (open label)	Zinc 220 mg/daily for 3 months	60 asthenozoospermic patients	Improved volume, progressive motility, normal morphology, total thiol concentration, total disulfide linkage concentration, GPx levels	3	3	6	N/A	N/A	N/A	0
	65	Busetto et al 2018 [26]	RCT blinded	Proxeed Plus (Sigma-Tau HealthScience, Utrecht, the Netherlands) 2 sachets/daily for 6 months	104 patients with semen abnormalities (of those, 52 with varicocele)	Increased semen parameters, except sperm morphology 10 spontaneous pregnancy in treated couples *vs*. 2 in the placebo	0	3	7	Unclear risk of bias for other sources	20	4	1
	66	Lu et al (2018) [91]	RCT blinded	Melatonin 400 mg/daily for 3 months	54 oligozoospermic patients	Improved semen parameters Improved TAC	1	3	6	Unclear risk of bias for random sequence generation, allocation concealment, selective reporting, other sources, blinding (participants and personnel, outcome assessment), and incomplete outcome data	15	5	0

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Table 2. Continued 20

Clinical condition	SN	Reference	Clinical trial design	Antioxidant formulation dosage and length of treatment	Study population	Reproductive outcomes after antioxidant treatment	Cambridge Quality Checklist			Cochrane Risk of Bias for RCT	CONSORT Guidelines (out of 25)	JADAD (Oxford Quality) (out of 5)	Quality of evidence published
							Checklist for correlate (out of 5)	Checklist for risk factors (out of 3)	Checklist for causal risk factors (out of 7)				
	67	Jannatifar et al (2019) [92]	Uncontrolled (open label)	N-acetylcysteine 600 mg/daily for 3 months	50 asthenozoospermic patients	Improved volume, sperm concentration, total and progressive motility, normal morphology Reduced MDA, SDF and protamine deficiency; improved TAC	3	3	3	N/A	N/A	N/A	0
	68	Gambera et al (2019) [93]	Uncontrolled (open label)	Arginine (3 g), CoQ10 (200 mg), vitamin C (240 mg), vitamin B3 (27 mg), Tribulus terrestris (60 mg), ginseng (12 mg), inositol (100 mg), vitamin E (36 mg) for 2 months	32 OAT patients	Improved sperm concentration, sperm count, progressive motility, normal morphology and vitality after therapy Oxisperm; reduced seminal oxidative stress after therapy Unclear capacitation check	0	3	2	N/A	N/A	N/A	0
	69	Micic et al (2019) [94]	RCT blinded	Proxeed Plus, consisting of 1 g LC, 0.5 g ALC, 0.725 g fumarate, 1 g fructose, 50 mg citric acid, 10 mg zinc, 20 mg coenzyme Q10, 50 μg selenium, 90 mg vitamin C, 200 μg folic acid and 1.5 μg vitamin B12 for 6 months	175 oligoasthenozoospermic patients	Improved semen parameters; increased seminal carnitine and α-glucosidase activity; reduced SDF	3	3	7	Unclear risk of bias for selection, other sources of bias, blinding of participants and personnel, and outcome assessment	16	3	0

Table 2. Continued 21

Clinical condition	SN	Reference	Clinical trial design	Antioxidant formulation dosage and length of treatment	Study population	Reproductive outcomes after antioxidant treatment	Cambridge Quality Checklist			Cochrane Risk of Bias for RCT	CONSORT Guidelines (out of 25)	JADAD (Oxford Quality) (out of 5)	Quality of evidence published
							Checklist for correlate (out of 5)	Checklist for risk factors (out of 3)	Checklist for causal risk factors (out of 7)				
	70	Nouri et al (2019) [95]	RCT blinded	Lycopene 25 mg/daily for 3 months	44 oligozoospermic patients	Improved volume, total sperm count, concentration, total motility, TAC	2	3	7	Unclear risk of bias for allocation concealment, other sources	18	4	0
	71	Busetto et al (2020) [96]	RCT blinded	L-carnitine (1 g), fumarate (725 mg), acetyl-L-carnitine (500 mg), fructose (1 g), citric acid (50 mg), selenium (50 μg), coenzyme Q10 (20 mg), vitamin C (90 mg), zinc (10 mg), folic acid (200 μg), vitamin B12 (1.5 μg)/daily for 6 months	104 patients with altered semen quality. Of those, 52 showed grade I-III varicoceles	Improved total sperm count, total and progressive motility Higher pregnancy rate	4	3	7	No risk of bias identified	22	5	1
	72	Alahmar et al (2020) [97]	Uncontrolled (open label)	CoQ10 200 mg/daily for 3 months	65 oligoasthenozoospermic patients	Improved sperm concentration, progressive and total motility, CoQ 10 level, TAC and GPx Reduced ROS levels and SDF	4	2	4	N/A	N/A	N/A	0

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Table 2. Continued 22

Clinical condition	SN	Reference	Clinical trial design	Antioxidant formulation dosage and length of treatment	Study population	Reproductive outcomes after antioxidant treatment	Cambridge Quality Checklist			Cochrane Risk of Bias for RCT	CONSORT Guidelines (out of 25)	JADAD (Oxford Quality) (out of 5)	Quality of evidence published
							Checklist for correlate (out of 5)	Checklist for risk factors (out of 3)	Checklist for causal risk factors (out of 7)				
	73	Terai et al (2020) [98]	RCT unblinded	A) L-carnitine (750.1 mg), zinc (30 mg), astaxanthin (16.05 mg), CoQ10 (90.26 mg), vitamin C (1 g), vitamin B12 (60.1 μg), vitamin E (150 mg) B) Hochu-ekki-to (dosage not reported) For 3 months	31 oligoasthenozoospermic patients	Increased total motile sperm count after treatment in group A	0	3	3	Unclear risk of allocation concealment, selective reporting, other sources; no blindness of participants and personnel	16	3	0
	74	Steiner et al (2020) [99]	RCT blinded	Vitamin C (500 mg), vitamin E (400 mg), selenium (0.20 mg), L-carnitine (1 g), zinc (20 mg), folic acid (1 g), lycopene (10 mg), and vitamin D (2,000 IU)/daily for a maximum of 6 months	174 oligozoospermic patients	Improved sperm concentration No change in SDF No change in pregnancy and live birth rates	2	3	7	No risk of bias identified	20	5	1
	75	Alkumait et al (2020) [100]	RCT unblinded	A) Glutathione (250 mg sachets) B) CoQ10 (200 mg sachets) For 6 months	51 OAT patients	Improved semen parameters	2	3	7	Unclear risk of bias for allocation concealment, other sources; high risk of bias for blinding (participants and personnel, outcome assessment)	13	3	0

Table 2. Continued 23

Clinical condition	SN	Reference	Clinical trial design	Antioxidant formulation dosage and length of treatment	Study population	Reproductive outcomes after antioxidant treatment	Cambridge Quality Checklist			Cochrane Risk of Bias for RCT	CONSORT Guidelines (out of 25)	JADAD (Oxford Quality) (out of 5)	Quality of evidence published
							Checklist for correlate (out of 5)	Checklist for risk factors (out of 3)	Checklist for causal risk factors (out of 7)				
	76	Nazari et al (2020) [101]	Prospective study	Androferti supplement (Daru Darman Parmida, Iran) twice daily for 3 months	59 patients with idiopathic OAT	Improved semen parameters	2	3	3	N/A	N/A	N/A	0
Healthy men	77	Goyal et al (2007) [102]	Uncontrolled (open label)	Lycopene 22.8 mg/daily for 2 weeks	6 healthy men	Increased seminal lycopene. No increase in TAC levels	2	3	3	N/A	N/A	N/A	0
	78	Tartibian and Maleki (2012) [103]	RCT blinded	Honey dissolved in water (70 g)	39 healthy men	Decreased ROS, MDA Increased SOD, Catalase, TAC and decreased IL-1b, IL-6, IL-8, TNF-a	2	3	7	Unclear risk of bias for allocation concealment, other sources	18	4	0
	79	Williams et al (2020) [104]	RCT blinded	Lactolycopene 14 mg/daily for 3 months	60 healthy men	Improved % of fast progressive and normal morphology No difference in SDF%	0	3	7	No risk of bias identified	25	5	1
Urogenital inflammation	80	Vicari et al (2002) [105]	RCT unblinded	A) Carnitines (Carnitene 2 g/daily+Nicetile 1 g/daily) B) Nonsteroidal anti-inflammatory drugs (NSAID) (nimesulide 200 mg/daily+ serratiopeptidase 10 mg/daily) C) NSAID+ carnitines (2 months each) D) Carnitines+ NSAID (2 months each) For 4 months	98 patients with abacterial prostatovesiculoepididymitis and high seminal leukocytes (>1 million cells/ml)	Group C showed increased forward motility and vitality Reduced leukocyte count in all groups Groups B and C showed reduced ROS Spontaneous pregnancy rate=8.2% (6 in group C, 1 in groups B and D)	0	3	4	Unclear risk of bias for allocation concealment, other sources; high risk of bias for selective reporting and incomplete outcome data	16	3	0

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Table 2. Continued 24

Clinical condition	SN	Reference	Clinical trial design	Antioxidant formulation dosage and length of treatment	Study population	Reproductive outcomes after antioxidant treatment	Cambridge Quality Checklist			Cochrane Risk of Bias for RCT	CONSORT Guidelines (out of 25)	JADAD (Oxford Quality) (out of 5)	Quality of evidence published
							Checklist for correlate (out of 5)	Checklist for risk factors (out of 3)	Checklist for causal risk factors (out of 7)				
	81	Yang et al (2003) [106]	Uncontrolled (open label)	A) Dang Gui (Angelica Sinensis), Chuan Xiong (Ligusticum Chuanxiong Hort), Chi Shao (Paeonia Veitchii Lynch), Wu Ling Zhi (Trogopterus Xanthipes Milne- Edwards), Pu Huang (Typha Angustata Linne), My Yao (Commiphora Molmol Engler), Yuan Hu (Corydalis Yanhusuo), Gan Jiang (Zingiber Officinale Rosecoe), Guan Gui (Cinnamomum Cassia Presl), and Hui Xiang (Foenicunum Vulgare Miller) B) Shao-Fu-Zhu-Yu-Tang, Sun-Ten Pharmaceutical Company, Taichung, Taiwan For 2 months	Chronic prostatitis (n=36)	Improved semen parameters and acrosin activity	3	3	3	N/A	N/A	N/A	0
	82	Chayachinda et al (2020) [107]	RCT blinded	CoQ10 200 mg/day for 1 month	Leukocytospermia (n=84)	No difference in sperm concentration, motility, normal morphology	0	3	3	No risk of bias identified	22	5	1

Table 2. Continued 25

Clinical condition	SN	Reference	Clinical trial design	Antioxidant formulation dosage and length of treatment	Study population	Reproductive outcomes after antioxidant treatment	Cambridge Quality Checklist			Cochrane Risk of Bias for RCT	CONSORT Guidelines (out of 25)	JADAD (Oxford Quality) (out of 5)	Quality of evidence published
							Checklist for correlate (out of 5)	Checklist for risk factors (out of 3)	Checklist for causal risk factors (out of 7)				
IMI	83	Comhaire et al (2000) [47]	Uncontrolled (open label)	Acetylcysteine (600 mg) or capsules providing a daily amount of β-carotene (30 mg) and α-tocopherol (180 mg)/daily. In addition, capsules containing essential fatty acids for a daily intake of docosahexaenoic acid (1 g), gammalinolenic acid (0.25 g) and arachidonic acid (0.10 g) for 6 months	7 idiopathic patients 11 varicocele patients History of cryptorchidism (n=2), patients with male accessory gland infection (n=7), immunological infertility (n=4), endocrine cause (n=1)	Improved sperm concentration and acrosome reaction Reduced ROS levels and 8-OH-dG levels	2	3	3	N/A	N/A	N/A	0
	84	Gupta and Kumar (2002) [108]	Uncontrolled (open label)	Lycopene 4 mg/daily for 3 months	30 idiopathic patients	Improved sperm concentration and motility Higher pregnancy rate	3	3	3	N/A	N/A	N/A	0
	85	Balercia et al (2005) [109]	RCT blinded	A) Carnitene (Sigma Tau, Pomezia, Italy) B) Zibren (Sigma Tau) C) A combination of carnitene and zibren For 6 months	60 idiopathic patients	Improved sperm motility, total oxyradical scavenging capacity of the semen	2	3	7	No risk of bias identified	18	5	1
	86	Heidary et al (2008) [110]	Uncontrolled (open label)	Saffron 50 mg, 3 times weekly for 3 months	52 idiopathic patients	Improved normal morphology, total and progressive motility	0	3	3	N/A	N/A	N/A	0

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Table 2. Continued 26

Clinical condition	SN	Reference	Clinical trial design	Antioxidant formulation dosage and length of treatment	Study population	Reproductive outcomes after antioxidant treatment	Cambridge Quality Checklist			Cochrane Risk of Bias for RCT	CONSORT Guidelines (out of 25)	JADAD (Oxford Quality) (out of 5)	Quality of evidence published
							Checklist for correlate (out of 5)	Checklist for risk factors (out of 3)	Checklist for causal risk factors (out of 7)				
	87	Ciftci et al (2009) [111]	RCT unblinded	N-acetylcysteine 600 mg/daily for 3 months	120 idiopathic patients	Improved volume, motility, semen viscosity, semen and serum oxidative stress (TAC, total peroxide, oxidative stress index)	2	3	6	Unclear risk of bias for blinding (participants and personnel); high risk of bias for other sources	12	3	0
	88	Haghighian et al (2015) [112]	RCT blinded	α-lipoic acid 600 mg/daily for 3 months	44 idiopathic patients	Improved sperm concentration and motility, TAC; reduced MDA levels	2	3	7	No risk of bias identified	20	5	1
	89	Soleimani and Masoumi (2017) [113]	Uncontrolled (open label)	Grape seed extract 600 mg/daily for 3 months	29 idiopathic patients	Increased catalase, reduced MDA	2	1	3	N/A	N/A	N/A	0
	90	Negri et al (2017) [114]	Uncontrolled (open label)	FertiPlus SOD (α-lipoic acid, glutathione, folic acid, zinc, and vitamins B2, B3, B6, B12) Dosage not specified for single component, length of treatment not reported	55 idiopathic patients	No changes in semen parameters and SDF	0	2	3	N/A	N/A	N/A	0

Table 2. Continued 27

Clinical condition	SN	Reference	Clinical trial design	Antioxidant formulation dosage and length of treatment	Study population	Reproductive outcomes after antioxidant treatment	Cambridge Quality Checklist			Cochrane Risk of Bias for RCT	CONSORT Guidelines (out of 25)	JADAD (Oxford Quality) (out of 5)	Quality of evidence published
							Checklist for correlate (out of 5)	Checklist for risk factors (out of 3)	Checklist for causal risk factors (out of 7)				
	91	Kopets et al (2020) [115]	RCT blinded	L-carnitine/l-acetyl-carnitine (1,990 mg), l-arginine (250 mg), glutathione (100 mg), co-enzyme Q10 (40 mg), zinc (7.5 mg), vitamin B9 (234 mcg), vitamin B12 (2 mcg), selenium (50 mcg)/daily for 6 months	83 idiopathic patients	Increased % of normozoospermia in treated patients after 2 and 4 months in comparison with placebo Higher pregnancy rate	0	3	7	No risk of bias identified	24	5	1
	92	Arafa et al (2020) [25]	Uncontrolled (open label)	FH PRO for Men (Fairhaven Health LLC, Bellingham, WA, USA) Twice/day for 3 months	119 idiopathic patients 29 unexplained infertile men	Improved progressive motility and seminal oxidation reduction potential Reduced SDF	3	3	3	N/A	N/A	N/A	0
UMI	93	Greco et al (2005) [116]	Uncontrolled (open label)	Vitamin C (1 g) and vitamin E (1 g)/daily for 2 months	Oligoterato- (n=6), OAT (n=26) patients, 6 unexplained infertile men	Improved semen parameters and SDF No change in fertilization and cleavage rates after treatment Higher implantation and pregnancy rates	2	3	3	N/A	N/A	N/A	0
	94	Greco et al (2005) [117]	Uncontrolled (open label)	Vitamin C and E 1 g/daily for 2 months	64 unexplained infertile men	No difference in semen parameters Reduced SDF	1	3	7	N/A	N/A	N/A	0

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Table 2. Continued 28

Clinical condition	SN	Reference	Clinical trial design	Antioxidant formulation dosage and length of treatment	Study population	Reproductive outcomes after antioxidant treatment	Cambridge Quality Checklist			Cochrane Risk of Bias for RCT	CONSORT Guidelines (out of 25)	JADAD (Oxford Quality) (out of 5)	Quality of evidence published
							Checklist for correlate (out of 5)	Checklist for risk factors (out of 3)	Checklist for causal risk factors (out of 7)				
	95	Safarinejad et al (2012) [118]	RCT blinded	CoQ10 200 mg/daily for 26 week, followed by a treatment-free period of 12-week	228 unexplained infertile men	Improved semen parameters, seminal catalase, and SOD	4	3	7	No risk of bias identified	18	5	1
	96	Khani et al (2013) [119]	Uncontrolled (open label)	Sesame 0.5 mg/kg body weight for 3 months	25 unexplained infertile men	Improved sperm concentration, motility Pregnancy: 3 out of 25 patients Live birth rate: 3 out of 25 patients	0	3	3	N/A	N/A	N/A	0
	97	Arafa et al (2020) [25]	Uncontrolled (open label)	FH PRO for Men (Fairhaven Health LLC, Bellingham, WA, USA) Twice/day for 3 months	119 idiopathic patients 29 unexplained infertile men	Improved progressive motility and seminal oxidation reduction potential Reduced SDF	3	3	3	N/A	N/A	N/A	0
Hyperinsulinaemic male patients	98	Bosman et al (2015) [120]	Uncontrolled (open label)	A) metformin (500–2,000 mg daily B) Metformin+ StaminoGro (Georen Pharmaceuticals PTY LTD, Fontainebleau, South Africa) For 3 months	34 hyperinsulinaemic male patients	Improved sperm morphology in both groups Decreased CMA3 assay results in both groups after treatment	0	3	6	N/A	N/A	N/A	0

Table 2. Continued 29

Clinical condition	SN	Reference	Clinical trial design	Antioxidant formulation dosage and length of treatment	Study population	Reproductive outcomes after antioxidant treatment	Cambridge Quality Checklist			Cochrane Risk of Bias for RCT	CONSORT Guidelines (out of 25)	JADAD (Oxford Quality) (out of 5)	Quality of evidence published
							Checklist for correlate (out of 5)	Checklist for risk factors (out of 3)	Checklist for causal risk factors (out of 7)				
RPL	99	Hamidian et al (2020) [121]	Uncontrolled (open label)	Vitamin C 250 mg/daily for 3 months	20 patients with recurrent pregnancy loss	Improved sperm morphology Reduced SDF Changes in mRNA levels of PRM1, PRM2, and the PRM1/PRM2 ratio after treatment	2	3	4	N/A	N/A	N/A	0

Data is summarized based on the clinical trial design, the antioxidant formulation and the study population tested as well as the impact on reproductive outcomes. The quality and the risk of bias have been determined for each study by applying the Cambridge Quality Checklist, the Cochrane Risk of Bias for RCTs, CONSORT guidelines, and JADAD score.

SN: serial number, RCT: randomized controlled trial, 8-OHdG: 8-hydroxy-2' -deoxyguanosine, IMI: iopathic male infertility, UMI: unexplained male infertility, RPL: recurrent pregnancy loss, ROS, reactive oxygen species, N/A: not available, MDA: malondialdehyde, TUNEL: terminal deoxynucleotidyl transferase dUTP nick end labelling, SDF: sperm DNA fragmentation, ICSI: intracytoplasmic sperm injection, DFI: DNA fragmentation index, OS: oxidative stress, SOD: superoxide dismutase, GSH: gluthatione, TAC: total antioxidant capacity, SCSA: sperm chromatin structure assay, CoQ: coenzyme Q, 8-OHdG: 8-hydroxy-2' -deoxyguanosine, FSH: follicle-stimulating hormone, LH: luteinizing hormone, IVF: *in vitro* fertilization, β-HCG: beta-uman chorionic gonadotropin, OAT: oliasthenoteratozoospermia, PRL: prolactin, TSH: thyroid-stimulating hormone, ART: assisted reproductive techniques, TBARS: thiobarbituric acid reactive substances, GPx: glutathione peroxidase, PRM: protamine, CMA3: chromomycin A3.

significance of p<0.05.

RESULTS

A total number of 1,978 articles were identified through the application of the keyword search strategy. Through manual screening of the title, keywords and abstract, non-relevant articles (n=1,864) were excluded (Fig. 2).

Following full-text review for eligibility using the inclusion and exclusion criteria, 17 articles were further excluded, resulting in 97 articles that were eligible for inclusion (Fig. 2). Two of the studies are each repeated as they included both IMI and UMI participants respectively, resulting in a total of 99 studies included in Table 2. Relevant data were extracted from the articles and summarized in Table 2, including the studied population, reported impact of treatment on reproductive outcomes, evaluation of quality, and risk of bias.

Of the 97 articles collected, 52 (53.6%) were uncontrolled (open label) clinical trials, 12 (12.4%) were unblinded RCTs and 33 (34.0%) were blinded RCTs. Based on the type of antioxidants investigated, 44 (45.4%) of the articles tested individual antioxidants, 31 (32.0%) tested a combination of several products in variable dosages, and 22 (22.7%) used registered antioxidants products. Semen parameters were evaluated after antioxidant treatment in 92.8% (n=90 out of 97) of the included publications, while the remaining 7 studies evaluated markers of sperm function.

Based on the statistical analysis, it is reported that 85.7% and 89.6% of the low-quality studies showed significant improvement (p<0.0001) in semen and sperm function parameters, respectively, in infertile men after antioxidant supplementation, whereas 65.0% and 58.3% of the high-quality studies, respectively, reported positive effect of antioxidant treatment on semen and sperm function parameters (Table 3). However, these results were not significant due to the availability of a small number of studies in the literature reporting semen parameters (n=20) and those reporting sperm functions (n=12) and this has led to the underpowering of statistical analysis. Sample size calculation predicted that a total number of 95 and 292 studies reporting the outcome of semen parameters and sperm functions, respectively, will allow it to gain a statistical significance of p<0.05. Furthermore, statistical analysis revealed that 78.6% (p=0.0733) and 60% (p=0.6949) of low and high-quality studies, respectively, reported a positive effect of antioxidant treatment on reproductive outcomes. However, these values were not significant (p≥0.05) due to the availability of very few studies (n=14 for low-quality and n=5 for high-quality) in the literature. Sample size calculation predicted that a total number of 33 low and 202 high-quality studies are required to attain a statistical significance of p<0.05 for reproductive outcomes.

1. Varicocele

A total of 11 studies investigated a male population affected by varicocele (Table 2). Of those, semen parameters after antioxidant treatment were reported

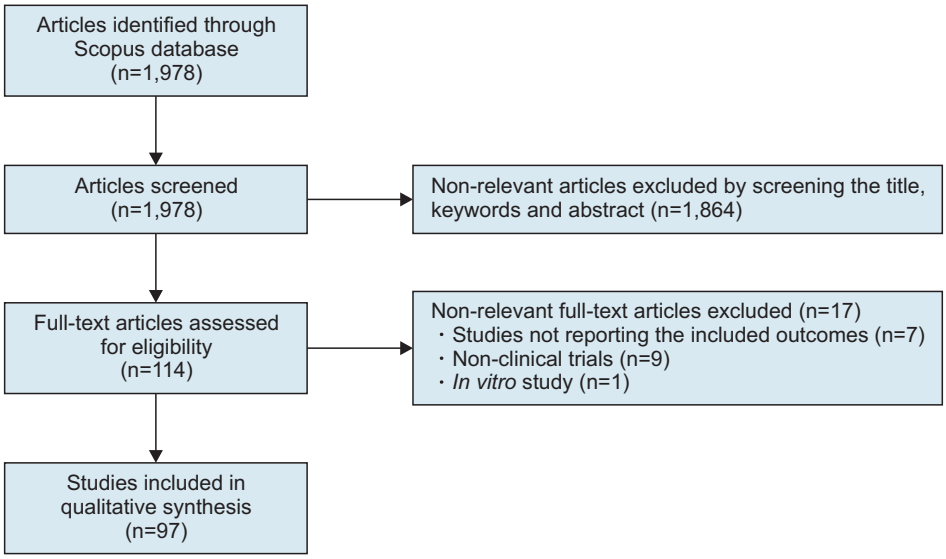

Fig. 2. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) workflow reporting the literature search strategy.

Table 3. Number of low and high-quality studies analysing semen parameters and/or sperm function after antioxidant treatment, overall as well as in each clinical condition

Group	Category	Report of semen parameters		Report of sperm function	
		Number of articles on the total of studies	% of studies reporting an improvement after AOX treatment	Number of articles on the total of studies	% of studies reporting an improvement after AOX treatment
Overall (n=97)	Low quality	70/90 (77.8)	85.7***	50/60 (83.3)	89.6***
	High quality	20/90 (22.2)	65.0	12/60 (20.0)	58.3
Varicocele (n=11)	Low quality	9/11 (81.8)	75.0	6/11 (54.5)	83.0
	High quality	2/11 (18.2)	-	0/11 (0)	-
Abnormal semen quality (n=45)	Low quality	36/44 (81.8)	94.4***	20/25 (80.0)	90.0**
	High quality	8/44 (18.2)	50.0	5/25 (20.0)	60.0
Idiopathic male infertility (n=10)	Low quality	6/9 (66.7)	83.0	5/7 (71.4)	80.0
	High quality	3/9 (33.3)	100***	2/7 (28.6)	100***
Unexplained male infertility (n=5)	Low quality	4/5 (80.0)	83.3	3/4 (75.0)	100***
	High quality	1/5 (20.0)	100***	1/4 (25.0)	100***

Values are presented as number (%) or percentage only.
AOX: antioxidant, -: not available.
Chi-square test: **p<0.01, ***p<0.0001.

in 90.9% (n=10 out of 11) of the included publications. Based on these studies, antioxidant supplementation seems to be beneficial in varicocele patients as 75.0% and 83.0% of low-quality studies, respectively, available in the literature, reported positive effect of antioxidant treatment on semen and sperm function parameters (Table 3). However, these values were not significant. Sample size calculation predicted that a total number of 41 and 24 studies reporting the outcome of semen parameters and sperm function, respectively are needed to reach a statistical significance of p<0.05.

2. Abnormal semen quality

A total of 45 studies investigated a male population with abnormal semen quality (Table 2). Of those, semen parameters after antioxidant treatment were reported in 97.8% (n=44 out of 45) of the included publications, whereas sperm function biomarkers were reported in 25 out of 45 studies (55.6%) (Table 3). The majority of the studies showed significant improvement in semen and sperm function parameters of men with abnormal semen quality after antioxidant supplementation, although these results were not statistically significant in case of the high-quality studies (Table 3). Sample size calculation predicted that a total number of 204 studies reporting the outcome of sperm function are required to reach a statistical significance of p<0.05.

3. Idiopathic male infertility

A total of 10 studies investigated idiopathic infertile men (Table 2). Of those, semen parameters after antioxidant treatment were reported in 90.0% (n=9 out of 10) of the included publications, whereas sperm function biomarkers were reported in 7 out of 10 studies (70.0%) (Table 3). Our statistical analysis showed that all the high-quality studies reported improvement in the semen and sperm function parameters (p<0.0001) after antioxidant treatment in men with IMI. Although a high percentage of low-quality studies showed improvement in semen and sperm function parameters in men with IMI after antioxidant supplementation, these values were not significant. Sample size calculation revealed that a total number of 24 and 30 studies, respectively, reporting the outcome of semen parameters and sperm function, may allow to reach statistical significance.

4. Unexplained male infertility

A total of 5 studies investigated the effect of antioxidant therapy in unexplained infertile men (Table 2). All of those studies reported semen parameters after antioxidant treatment (100%), whereas sperm function biomarkers were reported in 4 out of 5 studies (80.0%) (Table 3). All the low-quality studies showed improvement in sperm function, while sample size calculation predicted that a total number of 41 low-quality studies reporting the outcome of semen parameters would allow to attain a statistical significance of p<0.05. Further-

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Table 4. Articles published between January 2019 and July 2020 investigating the impact of antioxidant treatment on reproductive outcomes

SN	Reference	Study design	Study population/ sample size	Inclusion criteria	Exclusion criteria	Strict male inclusion/ exclusion	Female factor	Main outcomes reported	Power of statistical analysis	Study quality score (out of 4)	Study outcome (out of 3)
1	Terai et al (2020) [98]	RCT unblinded	31 oligoasthenozoospermic patients	Age: 20–60 years old; presence of oligozoospermia and/or asthenozoospermia	Azoospermia Sperm concentration $<5\times10^6$/mL Sperm motility<5% TMSC>30×10^6 Clinical conditions resulting in infertility History of cancer, chemotherapy, drug abuse Administration of androgens, anti-androgens, and immunosuppressants	0	N/A	Improved TMSC (p=0.04)	N/A	0	1
2	Schisterman et al (2020) [46]	Double-blind RCT	Treatment (n=1,185) *vs.* placebo (n=1,185)	Male partners of couples planning IVF for infertility treatment	Planning of donor sperm use or a gestational surrogate Pregnancy at enrollment Obstructive azoospermia Chronic diseases	0	N/A	No difference in semen parameters between both groups. Increase in SDF by Comet assay in treatment group *vs.* placebo group (Adjusted MD 2.4, 95% CI 0.5–4.4) No significant differences in β-HCG–detected pregnancy, clinical intrauterine pregnancy, ectopic pregnancy, pregnancy with multiple fetuses LBR: Treatment group 404 (34%) *vs.* placebo group 416 (35%) (ns)	90% power at a 2-sided α level of 0.05 to detect a risk difference of 7% in LBR (implying a risk ratio of 1.10), with continuity correction and allowing for a dropout rate of 15% Esteem of risk differences and risk ratios Sequential approach of Lan and DeMets with Bonferroni adjustment to distribute the 1-sided type I error rate among 3 continuous semen quality parameters Post hoc sensitivity analyses	2	0

Table 4. Continued 1

SN	Reference	Study design	Study population/ sample size	Inclusion criteria	Exclusion criteria	Strict male inclusion/ exclusion	Female factor	Main outcomes reported	Power of statistical analysis	Study quality score (out of 4)	Study outcome (out of 3)
3	Steiner et al (2020) [99]	Double-blind RCT	Treated (n=85) *vs.* placebo (n=86)	Infertile men with abnormal semen analysis in the last 6 months or DFI≥25%	Sperm concentration $<5\times10^6$/mL Consumption of fertility medication or testosterone	0	Yes	No difference in semen parameters, DFI by SCSA and PR LBR: 15% AOX *vs.* 24% placebo (ns) LBR=35% in the treated group and 25% in the placebo group with a 17% dropout	Sample size calculation, assuming a 20% dropout rate, ≥80% power at α=0.05	3	0
4	Kopets et al (2020) [115]	Double-blind RCT	Treated (n=42) *vs.* placebo (n=41)	Age: 21–50 years, with IMI	Allergy to any component Any clinical cause of male or female infertility Alcohol or drug addiction Use of any investigational product within the previous 3 months	1	Yes	Significant difference between both groups as regards normalization of semen parameters at 2 months (26/42 [61.9%]) males in treatment group *vs.* 8/41 [19.5%] males in placebo group) and at 4 months (29/42 [69.0%] *vs.* 9/41 [22.0%]). Significant change from baseline in mean values for all main semen parameters at 2 and 4 months, except for sperm morphology At 6 months higher PR in treatment than placebo group (10/42 [23.8%] *vs.* 2/41 [4.9%])	Sample size calculation assuming 1-beta error 0.80 and type I error alpha 5% Control for confounders by ANCOVA analysis	2	1

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Table 4. Continued 2

SN	Reference	Study design	Study population/ sample size	Inclusion criteria	Exclusion criteria	Strict male inclusion/ exclusion	Female factor	Main outcomes reported	Power of statistical analysis	Study quality score (out of 4)	Study outcome (out of 3)
5	Arafa et al (2020) [25]	Prospective study	Idiopathic (n=119) and unexplained male infertility (n=29)	Infertile men (20–50 years) with unknown etiology and female infertility factor	Azoospermia Sperm concentration $<1\times10^6$/mL Leucocytospermia Any cause for infertility Chemotherapy Clinical endocrinopathy Abnormal hormonal profile AOXs in the past 6 months Dietary, social habits or medical conditions which may impact on oxidative stress Use of drugs	1	Yes	IMI: significant improvement in sperm concentration ($p<0.001$), total motility ($p=0.001$), normal morphology ($p<0.001$), ORP ($p<0.001$), SDF ($p=0.001$) by Halosperm UMI: significant improvement in progressive motility ($p=0.002$), ORP ($p=0.03$), SDF ($p=0.02$)	N/A	3	3
6	Nazari et al (2020) [101]	Prospective study	59 patients with idiopathic OAT	Infertile patients with at least 1 abnormal semen parameter; age<45 years, BMI<30	Azoospermia Prostatitis Any clinical condition causing infertility History of hormonal therapy, drug addition, alcohol abuse, smoking, exposure to potential reproductive toxins	1	No	Significant improvements in sperm concentration ($p=0.004$) and normal morphology ($p=0.01$)	N/A	1	1
7	Nurmawati et al (2020) [44]	Single-blinded RCT	25 infertile men	Inclusion criteria not clearly stated	Exclusion criteria not clearly stated	0	No	Improved sperm concentration, motility, and morphology ($p<0.05$) Reduced levels of 8-OHdG levels ($p<0.01$) and MDA, with the value<1.98 being able to predict 100% of the normal sperm motility level (>40)	Sample size calculation assuming that the prevalence of male infertile couples with idiopathic causes in the world is 15% and in Indonesia 1.11%	2	2

Table 4. Continued 3

SN	Reference	Study design	Study population/ sample size	Inclusion criteria	Exclusion criteria	Strict male inclusion/ exclusion	Female factor	Main outcomes reported	Power of statistical analysis	Study quality score (out of 4)	Study outcome (out of 3)
8	Hadi et al (2020) [45]	Uncontrolled (open label)	58 infertile men	Inclusion criteria not clearly stated	Presence of varicocele, orchitis, cryptorchidism Consumption of herbals or medications that might affect seminal parameters in the last 3 months prior to the study	0	No	Improved sperm volume, count, total motility, and normal morphology ($p<0.05$)	N/A	1	1
9	Busetto et al (2020) [96]	Double-blinded RCT	104 patients with altered semen quality. Of those, 52 showed grade I–III varicoceles	Oligo- and/or astheno- and/or teratozoospermia, with or without varicocele (not surgically treated) and men from infertile couples	Known hypersensitivity to any of the compound History of undescended testes or cancer, endocrine disorders, post-pubertal mumps, genitourinary surgery, obstructive azoospermia or obstructive pathology of the urogenital system, autoimmune disease, cystic fibrosis History of taking any therapy affecting fertility, alcohol or drug abuse Subjects following any special diet or taking AOXs Involvement in any other clinical trials	0	Yes	Improved total sperm count ($p<0.0001$), total ($p<0.0001$) and progressive motility ($p=0.0012$) Higher PR in treated group *vs.* placebo (10 *vs.* 2 pregnancies, respectively; $p=0.0141$)	Sample size calculation assuming $\alpha=0.05$ (significance), $\beta=0.20$ (power of 80%), and up to 15% of patients dropping out of the study esteemed	3	1

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Table 4. Continued 4

SN	Reference	Study design	Study population/ sample size	Inclusion criteria	Exclusion criteria	Strict male inclusion/ exclusion	Female factor	Main outcomes reported	Power of statistical analysis	Study quality score (out of 4)	Study outcome (out of 3)
10	Alahmar et al (2020) [97]	Uncontrolled (open label)	65 oligoasthenozoospermic patients	Infertile patients showing oligoasthenozoospermia	Azoospermia Anatomical abnormalities of genital tract, varicocele, genital infection, scrotal surgery, systemic diseases Smoking Female factor Consumption of ntioxidant and selective serotonin reuptake inhibitors intake in the last 6 months	1	No	Improved sperm concentration, progressive and total motility ($p<0.05$), levels of CoQ 10 ($p<0.001$), TAC ($p<0.01$) and GPx ($p<0.001$) Reduced ROS levels ($p<0.05$) and SDF by SCD assay ($p<0.01$)	N/A	2	2
11	Alkumait et al (2020) [100]	RCT unblinded	51 OAT patients	Normal female factor with idiopathic OAT	Presence of chronic diseases, neoplasm, trauma, hypospadias, vas deference obstruction, varicocele, and genital tract infection Receiving treatment recently	1	No	Improved sperm concentration, motility ($p=0.01$) and morphology ($p=0.03$)	N/A	1	1
12	Williams et al (2020) [104]	Double-blinded RCT	60 healthy men	Healthy male volunteers, aged 18–30 years, lived within 1 h of the clinic or planning to live in the region for the duration of the study	Previous testicular surgery Existing or previous cancer Allergy to tomato, whey protein or soy derivatives	0	No	Improved % of fast progressive ($p=0.006$) and normal morphology ($p<0.001$) No difference in SDF by TUNEL	N/A	3	1

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Table 4. Continued 5

SN	Reference	Study design	Study population/ sample size	Inclusion criteria	Exclusion criteria	Strict male inclusion/ exclusion	Female factor	Main outcomes reported	Power of statistical analysis	Study quality score (out of 4)	Study outcome (out of 3)
13	Hamidian et al (2020) [121]	Uncontrolled (open label)	20 patients with recurrent pregnancy loss	Recurrency of pregnancy loss, age<40 years, no history of alcohol/drug abuse or smoking, altered semen quality	Obesity, diabetes, and varicocele Previous treatments with AOXs or other medications For the female partners, the presence of hormonal imbalance, chromosomal alterations, tubal obstruction, and bacterial or viral infections	1	Yes	Improved sperm morphology (p=0.000) Reduced SDF by TUNEL (p=0.00) Reduced sperm protamine deficiency assessed by CMA3-based assay (p=0.00)	N/A	2	3
14	Salehi et al (2019) [42]	Uncontrolled (open label)	485 infertile men with DFI>27% by SCSA	Aged 20–40 years	History of varicocele, surgery, and inflammation	1	No	Improved sperm concentration (p=0.003), total motility (p=0.001). Reduced DFI by SCSA (p=0.001) PR=16.8% for AOX treated patients	N/A	2	2
15	Hasoon (2019) [43]	Uncontrolled (open label)	24 infertile men	Unexplained subfertility	Presence of organic or obstructive infertility	1	No	Improved volume, sperm count, motility, and normal morphology (p<0.005)	N/A	0	1
16	Ardestani Zadeh et al (2019) [57]	Single blind RCT	60 varicocele patients	Varicocele patients who underwent sub-inguinal varicocelectomy	Usage of supplements Alcohol and/or drug addiction, smoking Diabetes mellitus, hormonal disorders, chronic or active infections Presenting side effects, and delayed complications of varicocelectomy	0	No	Improved sperm count (p=0.021) and motility (p=0.003)	N/A	2	1

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Table 4. Continued 6

SN	Reference	Study design	Study population/ sample size	Inclusion criteria	Exclusion criteria	Strict male inclusion/ exclusion	Female factor	Main outcomes reported	Power of statistical analysis	Study quality score (out of 4)	Study outcome (out of 3)
17	Kızılay and Altay (2019) [56]	RCT unblinded	90 varicocele patients	Varicocele patients treated with varicocelectomy, with spouses<35 years old, regular hormone profiles and menstrual cycles and no identified cause of infertility	Previous genitourinary system and/or varicocele surgery IMI Any clinical condition affecting fertility for the previous 3 months Patients following a fertility specific diet Alcohol or drug abuse, smoking	0	Yes	Improved TSC, sperm concentration, sperm count in normal morphology, and total and progressive motile sperm count ($p<0.05$) Higher PR in AOX treated patients than placebo group (29% *vs*. 17.9%, respectively; $p=0.029$)	Study powered to detect an effect size of $d\geq0.70$ as statistically significant in a two-tailed test with $\alpha=0.05$ and power of 0.80 with n=24 per condition.	3	1
18	Gambera et al (2019) [93]	Uncontrolled (open label)	32 OAT patients	Infertile patients with normal sexual development, medical history, serum hormone levels and physical examination	Azoospermia and infertility due to the female factor	0	Yes	Improved sperm concentration, sperm count, progressive motility, normal morphology, and vitality Oxisperm test: reduced seminal oxidative stress after therapy (no p-values reported)	N/A	2	2
19	Jannatifar et al (2019) [92]	Uncontrolled (open label)	50 asthenozoospermic patients	Infertile couples with no previous report of pregnancy, normal female and male partners	Varicocele, leukospermia, hormonal abnormalities, and/or obstruction, cryptorchidism, vasectomy, abnormal liver function Smoking, alcohol consumption Anatomical disorders, Klinefelter's syndrome, cancer, fever in the 90 days prior to sperm analysis, seminal sperm antibodies	1	No	Improved sperm concentration ($p=0.02$), total ($p=0.01$) and progressive motility ($p=0.001$), normal morphology ($p=0.001$), TAC ($p=0.01$) Reduced levels of MDA ($p=0.01$), SDF by TUNEL ($p=0.001$), % of sperm showing protamine deficiency by CMA3-based assay ($p=0.009$)	N/A	1	3

Table 4. Continued 7

SN	Reference	Study design	Study population/ sample size	Inclusion criteria	Exclusion criteria	Strict male inclusion/ exclusion	Female factor	Main outcomes reported	Power of statistical analysis	Study quality score (out of 4)	Study outcome (out of 3)
20	Nouri et al (2019) [95]	Double-blind RCT	44 oligozoospermic patients	Infertile men (25–45 years), sperm count<20×10^6/mL, normal sperm <65% and average motility <60%	History of anatomical disorders, endocrinopathy, previous hormonal therapy, use of androgens, antiandrogens, anticoagulants, cytotoxic drugs, or immunosuppressants Alcohol and drug abuse BMI≥30 kg/m^2	1	No	Improved volume, TSC, concentration, total motility, TAC ($p<0.05$)	N/A	2	2
21	Micic et al (2019) [94]	Double-blind RCT	Treatment (n=125) *vs.* placebo (n=50)	Total sperm number ≤15×10^6/mL; progressive motility <32%; normal viscosity and normal leucocytes number (<1×10^6/mL); sperm vitality ≤58%; normal sperm morphology <4%	Motility<5% Sperm concentration <1×10^6/mL History of therapy for infertility within the last 2 months Alcohol consumption Undescended testes, post-pubertal mumps, endocrine and autoimmune diseases, cystic fibrosis, or testicular cancer Hypersensitivity to ingredients in Proxeed Plus Presence of endocrine disorders, anti-sperm antibodies, leukocytospermia Use of antioxidant agents or vitamins Involvement in other clinical trials	0	Yes	Improved ejaculated volume ($p=0.001$), progressive motility ($p<0.001$), vitality ($p=0.002$) after treatment Reduced SDF by Halosperm test Increased seminal carnitine and α-glucosidase activity, positively correlated with improved progressive motility	N/A	4	3

Data are summarized and ranked based on the study design, the population investigated, the inclusion/exclusion criteria, the analysis of the female partner, the main outcomes reported, and the power of the statistical analysis.

SN: serial number, RCT: randomized controlled trial, TMSC: total motile sperm count, N/A: not available, IVF: *in vitro* fertilization, SDF: sperm DNA fragmentation, MD: median, CI: confidence interval, β-HCG: beta-human chorionic gonadotropin, LBR: live birth rate, ns: non-significant, DFI: DNA fragmentation index, SCSA: sperm chromatin structure assay, AOX: antioxidant, IMI: idiopathic male infertility, ORP: oxidation reduction potential, UMI: unexplained male infertility, BMI: body mass index, 8-OHdG: 8-hydroxy-2' -deoxyguanosine, MDA: malondialdehyde, PR: pregnancy rate, CoQ: coenzyme Q, TAC: total antioxidant capacity, GPx: glutathione peroxidase, ROS: reactive oxygen species, SCD: sperm chromatin dispersion, OAT: oligoasthenoteratozoospermia, TUNEL: terminal deoxynucleotidyl transferase dUTP nick end labeling, CMA3: chromomycin A3, TSC: total sperm count.

more, all the high-quality studies reported a significant improvement in the semen and sperm function parameters after antioxidant treatment in men with UMI.

5. Analysis of the most recent publications

A total of 21 articles published between January 2019 and July 2020 investigated the effects of antioxidant treatment on semen quality (Table 4) [25,42-46,56,57,92-101,104,115,121]. Based on our analysis, 13 and 8 studies were ranked as low and high-quality, respectively. Of these, 19 out of 21 (90.5%) showed improvement in semen parameters, while 4 out of 6 (66.7%) reported a significant improvement in sperm function. The number of studies investigating reproductive outcomes after antioxidant treatment was very limited, with only 3 out of 5 (60.0%) reporting an improvement in pregnancy rate, while birth rate showed no variation in the two studies reporting its evaluation.

DISCUSSION

Male infertility is a relatively common concern, contributing significantly to poor reproductive outcomes in couples. Oxidative stress has been increasingly identified as a common mechanism that mediates not only the pathophysiology, but also the many etiologies and risk factors associated with male infertility [1,2,5,6]. Within this context, there is increased use of antioxidants as a therapeutic option in male infertility, however, there remains no consensus on the efficacy, indications, dosage or length of treatment [8-11]. Therefore, the objective of this study was to systematically review the literature of trials investigating antioxidant use in male infertility, and to propose some broad guidelines for the practicing clinicians based on the currently available evidence. The results (Table 2) were stratified based on the currently available evidence on the clinical conditions investigated and the data were further analysed. Most studies reported men with abnormal semen quality (n=45) and infertile men (n=20) as well as male infertility conditions such as varicocele (n=11), IMI (n=10), UMI (n=5), and urogenital inflammation (n=3). Although there is no doubt that assessing sperm quality is just a first approach to a diagnosis and that evaluation of it as a predictor of fecundity or a couple's fertility success may lead to imprudent conclusions, there is no consensus whether the intake of exogenous antioxidants should be routinely done in clinical practice.

Majzoub and Agarwal (2018) [10] performed a systematic review and identified 26 studies showing positive effects of exogenous antioxidant intake on sperm quality and relevant outcomes of assisted reproduction such as live birth rates. The authors critically discussed the studies and highlighted that the treatment was given only for a short period to a small number of men. In addition, the lack of a standardized test to estimate oxidative stress levels in sperm and seminal fluid was another flaw in these studies, while the heterogeneity of the study designs made it particularly challenging to compare the effects and reach a robust conclusion. This has also been observed in our study, where 60 studies (61.9%) analyzed a variety of seminal oxidative stress markers, including the levels of seminal ROS and/or several endogenous antioxidants (*i.e.*, total antioxidant capacity assay, superoxide dismutase, catalase, glutathione), markers of lipid peroxidation (*i.e.*, malondialdehyde), oxidative DNA damage (8-hydroxy-2' -deoxyguanosine), and oxidation-reduction potential (ORP) (Table 2). The lack of standardization in the evaluation of oxidative stress in seminal fluid before and after therapy hinders a definitive conclusion regarding the implementation of oral antioxidant supplementation for infertile men in the clinical practice. Moreover, the lack of detailed methodological descriptions in most articles testing oral antioxidants supplementation is a major shortcoming that makes comparisons between different studies difficult. The evaluation of the length of treatment in the analyzed studies also does not help in this regard, as it is variable, with 15 (15.5%) studies reporting the treatment for an unclear amount of time or less than 3 months (Table 2). This might result in difficulties to observe any significant influence on human spermatogenesis.

More recently, a systematic review and meta-analysis with data from seven RCTs using L-carnitine (LC) and L-acetyl carnitine (LAC) as treatment (LC 2 g/day+LAC 1 g/day in six studies and LC 150 mg/day+LAC 50 mg/day in one study during 12 or 24 weeks) enrolling a total of 693 patients, concluded that a combined therapy of LC and LAC is effective in men with idiopathic oligoasthenoteratozoospermia [122]. This conclusion was supported by a significant increase in forward sperm motility and total motile sperm count. All the other sperm characteristics analyzed including semen volume, sperm concentration and per-

centage of abnormal spermatozoa showed no change. Since most of the selected studies lacked consistent and detailed information, pregnancy of the female partner as an endpoint was not considered in the analysis. Nevertheless, the authors found that the combined therapy of LC+LAC could lead to higher pregnancy rates. Although this meta-analysis provides evidence for a positive effect of dietary supplementation with LC+LAC for 3–6 months in men with idiopathic oligoasthenoteratozoospermia seeking fertility treatment, there are several limitations that hamper a robust conclusion. First, the clinical diagnosis of idiopathic oligoasthenoteratozoospermia is open to interpretation. Thus, each study may have considered and recruited distinct types of patients. In addition, since the studies had a significant variance regarding the number of selected patients (21 patients in two studies and up to 175 patients in one study), this impedes the robustness of the conclusions. The most striking limitation, common to most studies, is the fact that the bioavailability of the compounds is unknown. Moreover, the mechanisms by which they target testicular function and exert their action is not well established and these studies do not provide evidence for a synergistic action *versus* a single compound action, nor do they clearly show how the compounds act.

CRITICAL EVALUATION OF THE NECESSITY OF ADDITIONAL DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED TRIALS

An important issue that must be pointed out in this context is that out of 90 studies that reported the effects of antioxidant treatment on semen parameters, 70 were low-quality studies, whereas only 20 were ranked as high-quality. The overall statistical analysis of both low- and high-quality studies indicates that the antioxidant treatment has a significant positive effect on semen parameters. A similar result could still be obtained for the effect of the antioxidant treatment on seminal oxidative stress and SDF. Only 60 of these studies evaluated oxidative stress markers. For the reproductive outcome, however, only a total of 19 studies, 5 of which were of high-quality, have reported this outcome parameter. Out of these 5 studies, 3 reported a positive effect, while in 2 studies either no effect or a negative effect was observed. These data clearly indicates that the number of high-quality studies is too low to obtain significant results. Some of the reasons are that fertilization is a multifactorial process. In this case the man is treated for oxidative stress, which reflects in improvements of semen parameters and seminal oxidative stress, even in high-quality studies. However, when looking at fertilization, pregnancy and live birth rates oocyte quality has to be considered as a confounding variable. These factors are also the reason why the number of studies necessary (n=202) to obtain a significant result for the high-quality studies is so high and is therefore unrealistic. Secondly, the general cost for high-quality double-blind, randomized, and placebo-controlled studies with a sufficiently high number of participants is also very high.

Smits et al (2019) [11] performed an extensive meta-analysis to evaluate if dietary supplementation with oral antioxidants was effective and safe. The authors analysed 61 studies involving 6,264 subfertile men seeking fertility treatment. The authors found that 18 different oral antioxidants were used in these studies. The most relevant conclusion was that oral antioxidant supplementation can improve the reproductive capacity of subfertile men and even enhance live birth rates. However, the evidence collected was considered of low or very low-quality due to serious study limitations, making the comparison and/or aggregation to perform robust statistical analysis difficult. The studies also showed a significant variation in the antioxidant supplementation regimens (type, dose, or even combined intake). Some studies used a placebo group to compare with, while others chose to compare with no treatment or treatment with another antioxidant.

The endpoint for couples attending a fertility treatment has to be clinical pregnancy or live birth, but most studies fail to present these data. Only 12 of the 44 studies that were included in the previously cited meta-analysis reported on clinical pregnancy and live birth, which is considered a major limitation [11]. The latter evidence has also been highlighted in our systematic analysis of the literature, where only 22 (22.7%) and 4 (4.1%) out of 97 studies reported pregnancy and live birth rates as a clinical outcome, respectively. Pregnancy and live birth are highly influenced by a wide variety of embryological and female factors. This highlights one of the major problems in literature as the studies on the use and effectiveness of antioxidants are mostly focused on the males and not on the couple.

Studies need standardization not only regarding the selection of the males, but also the female partners to evaluate clinical pregnancy, achievement of live birth, and the influence of the treatment. Many confounding factors may interfere with such outcomes including female age, ovarian reserve, anatomic, inflammatory and endocrinal disorders, and as such, the impact of any fertility treatment can only be assessed after adjusting for such confounding factors. All these factors determine the type of ART treatment that is employed, namely *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI), and eventually also have a significant impact on the reproductive outcome. Hence, the outcomes, positive or negative, can be skewed due to numerous confounding factors. Consequently, the focus of the effectiveness of an antioxidant treatment of men should be whether or not the treatment improves seminal parameters and sperm function, rather than reproductive outcomes which are influenced by numerous other variables that are, i) not/insufficiently considered in recent studies, and ii) can have a significant negative effect on blastulation, embryo development, onset/continuation of pregnancy, and live birth. Furthermore, in order to be able to judge the effectiveness of treatment, it is necessary to specify the andrological condition for which patients are being treated. Without this, one might include patients with a condition in a study which are not or only poorly responding to the treatment. In turn, this would negatively affect the outcome of the study.

We have identified 21 studies investigating the effects of antioxidant treatment on semen quality that were published from January 2019 to July 2020. The majority of these studies showed an improvement in semen parameters and sperm function. With a more detailed assessment of quality reported, 13 of the 21 were ranked as low-quality. Steiner et al [99] and Micic et al [94] strictly excluded couples with female factors, while most of the studies inadequately documented such criteria in their methodologies. From the molecular point of view, this integrative focus on the couple is essential. The sperm-oocyte interaction is sensitive to the redox balance, and excessive ROS in spermatozoa can lead to impaired oocyte function [123]. Hence, the effects of sperm oxidative damage go far beyond fertilization since they can have a negative impact on embryo development or even pregnancy loss [124,125]. On the other hand, the oocyte has the capability to repair damages in spermatozoa [126], and this is another unknown variable that needs to be considered when thinking of reproductive outcomes as a therapeutic effect of treating men with antioxidants. A number of critical points can further be pointed out from individual studies. Schisterman et al [46] lost 31% of their subjects during follow-up and a substantial number of couples received non-specified treatment off-site, which could alter the study results. The Steiner et al's [99] study was terminated early as they failed to show a >10% difference in pregnancy/live birth rates. Moreover, the authors failed to confirm adherence with antioxidant treatment, and ovarian stimulation was used in couples who did not conceive after 3 months of therapy, which could affect the outcomes. Terai et al [98] included men between 20 and 60 years of age, which is atypical of the reproductive age group. Abstinence was defined as 4 or more days without an upper limit [98]. Advanced male age and delayed abstinence are associated with increased oxidative stress measures and with alteration in semen parameters [127,128]. Furthermore, Terai et al [98] did not include a placebo group, with an experimental group investigating a Chinese herbal formula. Hadi et al [45], Alahmar et al [97], Hamidian et al [121], Salehi et al [42], Hasoon [43], Gambera et al [93], Arafa et al [25], Nazari et al [101], and Jannatifar et al [92] all reported improved semen parameters, but these studies are uncontrolled open label trials and are relatively underpowered. Furthermore, Alkumait et al [100] and Terai et al [98] were unblinded trials with relatively small sample sizes (n=51 and 31, respectively). On the other hand, the reviewed studies also have several positive virtues that can be pointed out. For example, the study by Agarwal et al [129] investigated changes in protein expression following antioxidant therapy, thereby enhancing our understanding of the physiologic alterations at the molecular level. Several studies assessed the effect of antioxidant therapy on SDF levels (Table 2). SDF is increasingly being utilized in the evaluation of male factor infertility and is believed to be an important determinant of fertility potential [130]. Furthermore, SDF is considered as an indirect measure of oxidative stress and can validate the benefits of antioxidant therapy in restoring the body's redox potential.

Therefore, for the above discussed reasons, having more double-blind RCT studies with a large enough sample size are neither feasible nor could they provide

the expected clear result in terms of improved live birth rates after the antioxidant treatment.

STRENGTH WEAKNESS OPPORTUNITY THREAT (SWOT) ANALYSIS

1. Strengths

Antioxidant supplementation for the treatment of male infertility has been increasingly investigated in the past decade. Reports suggest that different antioxidant formulations were used to improve sperm quality and function in infertile men with various clinical circumstances (Table 2). These improvements were reflected on reproductive outcomes such as pregnancy rate (Table 2). An increasing number of studies have investigated the effect of antioxidant therapy on measures of oxidative stress, perhaps indicating it is a feasible treatment approach/option for patients with alteration in seminal redox potential.

2. Weaknesses

The contradictory results in reproductive outcomes seen in a number of studies can be considered as the main factor limiting the routine use of antioxidants for the treatment of male infertility, while female and embryological confounding factors were not taken into account (Table 2). Yet, in these studies, the male was treated with the false expectation that this treatment would automatically increase the success of the reproductive outcome. In addition, the low level of evidence was extrapolated from the clinical trials reporting benefit due to non-homogenous study designs, or inconsistencies in the treatment regimens (individual or combined) used (Table 2). Furthermore, the majority of these studies failed to adjust for confounding factors (*e.g.*, female factors) that are essential for conception or establishment of pregnancy (Table 2).

3. Opportunities

Selection of suitable candidates for antioxidant supplementation by oxidative stress measurement seems a logical approach [25]. Indeed, the concept of MOSI has been recently proposed. The classification may guide the identification of a specific group of idiopathic infertile men who will be more likely to benefit from the treatment [2]. Secondly, evaluation of the sperm proteome of idiopathic infertile men before and after oral oxidant supplementation offers a window to better understanding the molecular mechanisms associated with sperm function (Fig. 3) [129]. Oral antioxidants may be an alternative cost-effective treatment option for infertile couples who desire to avoid assisted reproduction.

4. Threats

Despite the conclusion from Cochrane collaborations

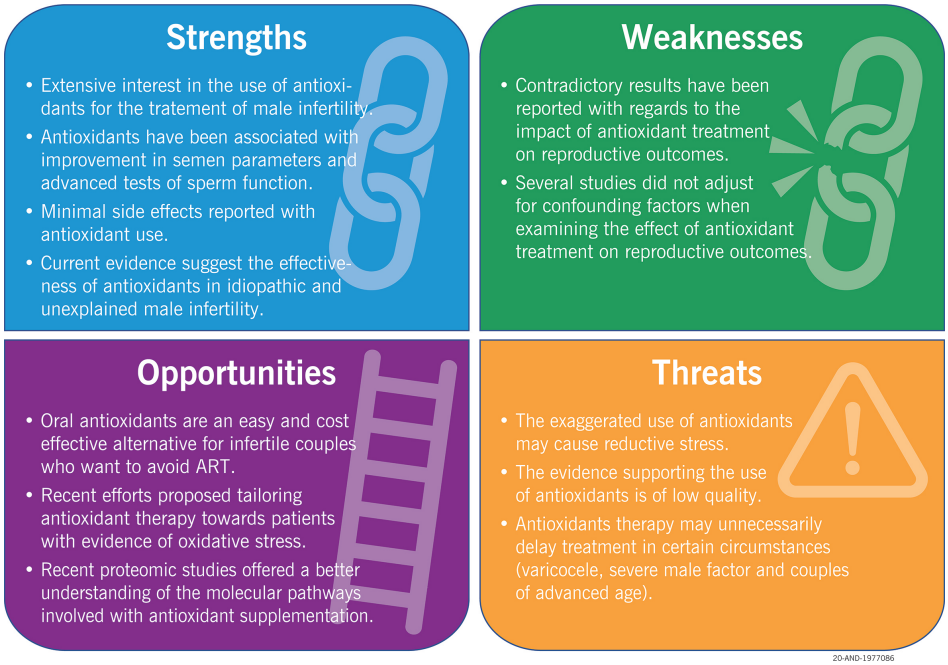

Fig. 3. Strength Weakness Opportunity Threat (SWOT) analysis. SWOT has been conducted to describe the impact of antioxidant supplementation in the treatment of male infertility. ART: assisted reproductive technology.

that oral antioxidant therapy may improve semen parameters and the likelihood of pregnancy, the lack of sufficient high-quality evidence still hinders a consensus among clinicians [11]. In addition, the wide variation in the treatment regimen raises concerns about overzealous use of antioxidants. The detrimental effects of reductive stress may be as pathological as that of oxidative stress [23]. Moreover, the often unpredictable outcome after antioxidant supplementation in the context of multiple confounding factors in reproduction may delay the definitive treatment, particularly for couples of advanced age.

CLINICAL GUIDELINES

While antioxidant supplementation is frequently utilized for the treatment of male factor infertility, no clear recommendations exist endorsing their use for specific clinical indications. Therefore, we aimed to develop clinical practice guidelines based on the available evidence to help in identifying the clinical circumstances in which antioxidant supplementation appears to be most beneficial. This systematic review included 97 articles, which investigated antioxidant treatment for various etiologies of male infertility. Very few studies explored the effect of antioxidants on semen quality of men with genitourinary inflammation (n=3), a hyperinsulinemic state (n=1) and recurrent pregnancy loss in female partners (n=1) (Table 2), and are hence insufficient for evidence-based recommendations for their use. On the other hand, there are some perceived high-quality studies available but, as highlighted above, due to the difficulties recruiting a sufficient number of patients and the small number of these studies, a statistical analysis will be underpowered and therefore does not provide the required answer. As we have shown in our analysis, the number of such studies conducted under the given circumstances and with a pre-selected set of criteria would have to be unreasonably high. Yet, looking at the diverse groups of conditions where oxidative stress is significantly involved and an antioxidant treatment would make sense, it is clear that in absence of prior testing for oxidative stress and without proper identification of suitable patient groups, the treatment will fail. Hence, appropriate patient identification is essential for the success of the treatment. We were able to formulate recommendations for antioxidant treatment for men with abnormal semen quality, IMI, UMI, and clinical varicocele based on studies reviewed (Table 2). However, it should be noted that our recommendations are based on the previously published studies, which, in our analysis, cannot be considered as high-quality because a number of variables (such as female factor, inclusion criteria, sample size esteem, *etc.*) were not properly reported. Therefore, our recommendation is that antioxidant treatment is possible and can result in improved male seminal parameters if the condition is caused by oxidative stress. It is self-evident that the therapy has to be monitored to not only avoid over-dosage of antioxidants, but also to see if it is successful or if alternative treatments are to be considered.

1. Abnormal semen quality

Antioxidants have long been investigated as a therapeutic option to counteract the harmful effects of ROS toxicity on various body systems. The reproductive system is one good example, as seminal oxidative stress is believed to be a common pathophysiology and various oxidative stress-associated aetiologies can alter sperm quality and function. Results of our review demonstrated that the majority of low-quality studies reported a significant improvement in conventional semen parameters and measures of sperm function. However, this result was not observed by high-quality studies. The recent Cochrane review revealed a somewhat similar result [11]. While an improvement in conventional semen parameters was noted over time, the findings were not reliable as a great deal of heterogeneity was observed across the included studies. Moreover, antioxidants were found to lower SDF compared to placebo.

Recommendation: antioxidants can improve conventional semen parameters and measures of sperm function (grade C recommendation).

2. Varicocele

Varicocele is the most common correctable cause of male infertility, prevalent in about 40% of men with primary infertility and up to 80% of men with secondary infertility [131]. Several studies have confirmed the presence of higher levels of oxidative stress in infertile men with varicocele in comparison to fertile men with or without varicocele and infertile men with idiopathic infertility [132-136]. This finding may justify the utility of antioxidants as a medical treatment strategy for varicocele. Nonetheless, in most patients, varicocelec-

tomy remains the gold standard modality that results in sustained improvement in semen parameters and natural conception [137].

Most of the studies exploring the effect of antioxidant supplementation on semen parameters and sperm function were of low-quality. While the majority of these studies reported an improvement in outcome, the evidence supporting antioxidant use as a sole treatment for varicocele is not sufficient. Our sample size calculation confirms the need for further research in this regard in order to obtain a statistically significant effect. However, the reported improvement may be clinically relevant supporting antioxidant use as an adjunct therapy to varicocele ligation. A recent systematic review and meta-analysis explored antioxidant efficacy on improving semen quality after varicocelectomy [138]. The authors included 6 RCT with 576 patients receiving various antioxidant regimens or placebo following varicocelectomy. Significant improvements in sperm concentration ($p<0.001$), total motility ($p=0.03$), progressive motility ($p<0.001$) and normal morphology ($p<0.001$) were reported for the treatment group. However, pregnancy rate did not improve ($p=0.36$). Nonetheless, this finding confirms the presence of an additive effect of the antioxidant therapy in patients undergoing varicocelectomy.

Recommendation: antioxidants in addition to varicocele ligation result in further improvement in semen parameters (grade C recommendation).

3. Unexplained male infertility and idiopathic male infertility

Antioxidants are also commonly utilized for the treatment of patients with UMI or IMI. The former is defined by failure of conception despite having normal semen parameters, while the latter is characterized by the presence of semen abnormalities due to unknown etiology. The prevalence of UMI and IMI ranges between 6%–27% and 30%–58%, respectively [3,139]. Oxidative stress is believed to play a significant role in the pathophysiology of infertility of unknown origin and has been identified in 30%–40% of patients with UMI and up to 80% of patients with IMI [140-142].

Our analysis revealed that antioxidant use in men with IMI and UMI resulted in significant improvement in semen parameters and sperm function, as reported by high-quality studies. While the majority of low-quality studies echoed similar improvements, a larger number of studies are required to reach statistical significance.

A systematic review of 32 studies which assessed the impact of antioxidant therapy in IMI patients revealed an improvement of semen parameters, with the biggest benefit observed in sperm motility [143]. Fewer studies have assessed antioxidant treatment in patients with UMI. A recent study included 29 UMI patients who were treated with a combination of antioxidants for 3 months. The authors reported a significant increase in progressive motility ($p=0.002$), and a decrease in SDF ($p=0.03$) and ORP levels ($p=0.02$) following treatment. Greco et al [117] randomized 64 patients with UMI and elevated SDF to either treatment with vitamins C and E for 2 months or placebo. While there was no significant difference in semen parameter results, significant reduction in SDF was noted in the treatment group ($p<0.001$).

Recommendation: antioxidants significantly increase sperm quality in men with IMI and UMI (grade B recommendation).

CURRENT STATE AND FUTURE RECOMMENDATIONS FOR ANTIOXIDANT RESEARCH

The bivalent action of ROS as essential signaling molecules in physiological functions for sperm to fertilize an oocyte [144-146], as well as in mediating a detrimental effect on sperm functionality [147-149], suggests that appropriate clinical indications for prescribing antioxidants and monitoring the treatment are critical clinical considerations. Therefore, it is crucial to continue to study the physiological and pathophysiological mechanisms of ROS in the male reproductive tract and its relevance for sperm production and conception. In this regard, the physiological needs of functional spermatozoa, *e.g.*, for the induction of capacitation and acrosome reaction, have to be considered. It appears that three aspects of sperm physiology have to be addressed, namely i) preservation and support of metabolism, ii) improvement of sperm maturation and function, and iii) protection against ROS-related trauma. Therefore, the correct ratio and concentration of antioxidants is essential for this therapeutic effect to occur and any new studies must take this into account.

Standardization of oxidative stress measurement must also be implemented, not only because a repro-

ducible baseline is needed, but also because many of the current studies are either lacking or using different oxidative stress measurement and are therefore difficult to compare. The assessment of oxidative stress may also facilitate the identification of the best candidates and responders to antioxidant therapy (Fig. 4) [2].

Recently, the MiOXSYS system has be proposed as a standardized assessment of seminal oxidative stress due to its ease of use, cost effectiveness, and reproducibility, as well as proposed evidence based clinical guidelines [150,151]. The MiOXSYS system measures the overall redox balance in seminal fluid to directly evaluate oxidative or reductive stress. Monitoring the treatment with antioxidants is another important aspect as overtreatment could lead to reductive stress-related infertility because the little amount of ROS, which is essential to trigger physiological functions of spermatozoa for it to fertilize oocytes, would be scavenged if the antioxidants are overdosed.

For a safe implementation of oral antioxidants use in a clinical setting, it is important not only to discover the molecular mechanisms of action of the bioactive compounds, but also the secondary effects that may arise. Personalized or adjusted prescription of oral antioxidants can enhance efficacy, without promoting over-dosage and deleterious health effects. Methodology and couple selection must be well reported. Doses and duration of the treatment should be adjusted according to several factors including the detected levels of ROS and antioxidant enzymes in seminal fluid and spermatozoa. Bioavailability of the compounds and their mechanism of action should also be thoroughly studied. More time-points to detect the oxidative balance of spermatozoa and seminal fluid should also be considered. Finally, one should not only look at the oral antioxidant therapy as a clinical treatment of the male and then expect that this treatment will result in the live birth of a healthy baby, but rather consider reproduction as a joint responsibility of both partners where a male and a female equally contribute to the reproductive outcome. Currently, the female partner is not examined for possible oxidative stress, but there is also a lack of knowledge about the impact of ROS and the redox level (oxidative and reductive stress) in the female reproductive system on oocyte development and maturation as well as on embryo development. A study by Ufer et al (2010) [152] indicated that proper embryo development depends on finely tuned redox control. On the other hand, elevated levels of antioxidants may result in teratogenic developments [153].

Currently, there is significant heterogeneity in trials reporting antioxidants on male infertility. This includes the condition investigated, type of antioxidant used, duration of the study, and the outcomes measured (Table 2). Furthermore, it is essential to select previously studied antioxidant candidates as well as new potential compounds, and also determine if they act better alone or in synergy with other antioxidants

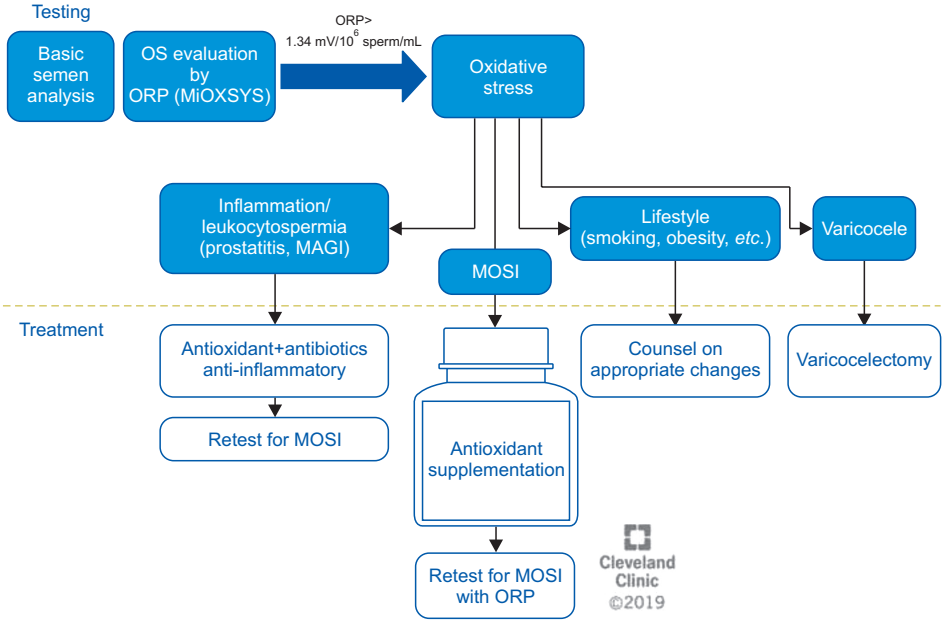

Fig. 4. Treatment options for male oxidative stress infertility (MOSI). OS: oxidative stress, ORP: oxidation-reduction potential, MAGI: male accessory gland infection.

as has been shown in some studies [154]. Due to several factors, including the high costs and the high number of participants needed to reach a statistically robust study, the individuals recruited in any new study must be well selected based on strict inclusion/exclusion criteria. This means the patients should have the same infertility diagnosis and similar semen analysis: for example, mixing patients with azoospermia, mild oligozoospermia or asthenozoospermia should be avoided. Meeting these criteria will likely be difficult at a single institution and does not even include recruiting patients for an adequate control group. Use of placebo in the control group is also mandatory to avoid bias. Notably, confounding factors, including dietary habits, are often overlooked in most RCTs, but when patients are being enrolled in a study with antioxidant supplementation, baseline antioxidant intake and diet should be evaluated in both partners, male and female. Furthermore, an appropriate primary outcome, such as decrease in oxidative stress or SDF, should also be evaluated. Finally, it is critical to set a correct time period for the study. A short to medium time period of 3 to 6 months is considered ideal to study the antioxidant effects on semen parameters.

UNRESOLVED QUESTIONS

What makes the situation even more complicated is that there are no dose-response studies in humans to pinpoint the optimum antioxidant dosages needed to produce improvement in semen quality. We also still do not know the normal physiological range of the redox levels in both males and females. For spermatozoa, a recent study by Panner Selvam et al (2020) [24] indicated normal redox values between -9.76 and 1.48 mV/10^6 sperm/mL. Although these values already indicate oxidative or reductive stress conditions, the normal physiological range will be much narrowed in between these values. In the female, one might have to consider variations depending on the menstrual cycle and/or the onset of pregnancy. Besides, these values might change with age and/or health status. Furthermore, the effective bioavailability of antioxidants in the testes, the epididymis and the semen is still unknown. Since many antioxidants (*e.g.*, co-enzyme Q10, vitamin E, and carnitines) can easily cross the blood-testis barrier, a proper balance for the redox level has to be achieved because an over-dosage might lead to reductive stress, which has been shown to be as harmful as oxidative stress [22].

CONCLUSIONS

This systematic review identified a significant number of well-designed studies that unequivocally show the beneficial effects of oral antioxidants in improving semen parameters and pregnancy outcome. However, despite the safety and efficacy of the antioxidant therapy, five main factors have hindered its wide acceptance and implementation in the treatment of male infertility: i) lack of randomized placebo-controlled studies that show the safety and efficacy of antioxidants in improving pregnancy rates in infertile couples; ii) type of antioxidant to be used; iii) dose; iv) duration of treatment; and v) costs.

Although randomized placebo-controlled studies are regarded as the gold standard in the validation of the safety and efficacy of therapies, given the fact that the occurrence of a pregnancy is a multifactorial process mainly determined by the genomic quality of the egg, to show the impact of antioxidant therapy on pregnancy outcome in randomized placebo-controlled studies would be unrealistic and extremely difficult to perform. Given the difficulty in carrying out these randomized placebo-controlled studies and the existence of significant clinical evidence supporting the safety and efficacy of antioxidants in improving pregnancy outcome in infertile couples, the use of antioxidant therapy should be recommended.

To answer the question of what type of antioxidant should be used, we believe that antioxidants that readily cross the blood-testis and blood-epididymis barriers should be recommended. The formulation should be well-balanced as lipid-soluble and water-soluble antioxidants together with other factors are closely interacting, thereby regenerating lipid-located antioxidants. If this balance is not given, it may not only result in suboptimal antioxidant effects, but also in paradoxical prooxidant effects due to interference in redox reactions.

Concerning the dose to be used, the dose should be high enough to restore the normal physiological cellular functions by reducing oxidative stress without compromising the physiological role of ROS in sperm maturation and fertilization reactions. An overdosage which may lead to reductive stress should be avoided.

The duration of an antioxidant therapy would have to be adjusted according to the place where the damage

occurs. If it is in the epididymis, a treatment course of at least two weeks should be sufficient to counteract ROS-induced damage. In addition, since oxidative stress in the epididymis is a constitutive process and antioxidants have no side-effects, antioxidant therapy should be recommended until pregnancy is achieved. This would apply to couples undergoing timed intercourse as well as to couples undergoing *in vitro* fertilization. On the other hand, if the oxidative damage is occurring in the testes such as in the case of clinical varicocele, the duration of antioxidant treatment should be of at least three months.

Finally, the cost argument has to be seen from the perspective of the sponsors of high quality randomized, double-blind placebo-controlled clinical trials. Natural antioxidant formulations have very low cost, and their use would be amply justified based on efficacy and safety as well as for cost-saving aspects for patients and health systems. The problem for the possible funding by the pharmaceutical industry of the studies is to recover the high costs of a trial on a cheap antioxidant, which can be available over the counter, and for which not even intellectual property rights are available.

In conclusion, the use of antioxidants that readily cross the blood-testis and blood-epididymis barriers should be recommended. Their efficacy, lack of side-effects and low costs should encourage their wider acceptance and implementation among infertility specialists for their use in the treatment of male infertility and infertile couples. Despite these favorable aspects of antioxidant treatments for idiopathic and UMI, more high-quality research is needed to understand the effects of ROS and antioxidants on the human fertilizing potential in both male and female. However, the practicality of conducting such studies remains questionable.

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Conflict of Interest

The authors have nothing to disclose.

Author contribution

Conceptualization: AA. Writing – original draft: all the authors. Writing – review & editing: all the authors.

Supplementary Materials

Supplementary materials can be found *via* https://doi.org/10.5534/wjmh.200196

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