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Impact of Antioxidant Therapy on Natural Pregnancy Outcomes and Semen Parameters in Infertile Men: A Systematic Review and Meta-Analysis of Randomized Controlled Trials

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Purpose: Seminal oxidative stress (OS) is a recognized factor potentially associated with male infertility, but the efficacy of antioxidant (AOX) therapy is controversial and there is no consensus on its utility. Primary outcomes of this study were to investigate the effect of AOX on spontaneous clinical pregnancy, live birth and miscarriage rates in male infertile patients. Secondary outcomes were conventional semen parameters, sperm DNA fragmentation (SDF) and seminal OS.

Materials and Methods: Literature search was performed using Scopus, PubMed, Ovid, Embase, and Cochrane databases. Only randomized controlled trials (RCTs) were included and the meta-analysis was conducted according to PRISMA guidelines.

Results: We assessed for eligibility 1,307 abstracts, and 45 RCTs were finally included, for a total of 4,332 infertile patients. We found a significantly higher pregnancy rate in patients treated with AOX compared to placebo-treated or untreated controls, without significant inter-study heterogeneity. No effects on live-birth or miscarriage rates were observed in four studies. A significantly higher sperm concentration, sperm progressive motility, sperm total motility, and normal sperm morphology was found in patients compared to controls. We found no effect on SDF in analysis of three eligible studies. Seminal levels of total antioxidant capacity were significantly higher, while seminal malondialdehyde acid was significantly lower in patients than controls. These results did not change after exclusion of studies performed following varicocele repair.

Conclusions: The present analysis upgrades the level of evidence favoring a recommendation for using AOX in male infertility to improve the spontaneous pregnancy rate and the conventional sperm parameters. The failure to demonstrate an increase in live-birth rate, despite an increase in pregnancy rates, is due to the very few RCTs specifically assessing the impact of AOX on live-birth rate. Therefore, further RCTs assessing the impact of AOX on live-birth rate and miscarriage rate, and SDF will be helpful.

Keywords: Antioxidants; Male infertility; Meta-analysis; Pregnancy; Semen parameters; Sperm DNA fragmentation

INTRODUCTION

Infertility affects about one in six couples worldwide, and approximately 50% of infertility is due to male factor [1,2]. Many causes have been attributed to male factor infertility, such as varicocele, endocrine disturbances, genetic abnormalities, immunological factors, urogenital abnormalities or infections, lifestyle, malignancy, systemic disease, gonadotoxins, or obstruction of the reproductive tract [1]. About 30% to 40% of cases are labeled as idiopathic male infertility (IMI) which is diagnosed when there are normal findings on physical examination, and on genetic and hormonal evaluation, but semen analysis reveals abnormal parameters with failure to achieve fatherhood despite unprotected sexual intercourse [3].

Seminal oxidative stress (OS) is now recognized as a potential contributing factor to the various causes of infertility, including IMI. Seminal reactive oxygen species (ROS) are produced by immature sperm, leukocytes, and as by-products of metabolic pathways, and are needed for the normal function of spermatozoa [4]. Excess production of ROS occurs under several circumstances, including smoking, alcohol, other lifestyle factors, varicocele, radiation exposure, and several other conditions [5]. OS occurs when there is a high concentration of ROS leading to an imbalance between ROS and antioxidants (AOXs) [5]. Several studies have suggested that OS plays a significant role in male infertility [6-8]. OS can interfere with capacitation, sperm DNA integrity, and cause sperm membrane damage and this can impact the fertilization process [9,10]. Spermatozoa are particularly susceptible to the action of high concentrations of ROS due to the presence of a large amount of polyunsaturated fatty acids in their plasma membrane as well as a low concentration of enzymes in the cytoplasm that can neutralize ROS [11]. Recently, the term Male Oxidative Stress Infertility (MOSI) was proposed by Agarwal et al [12] to encompass men with abnormal semen analysis and high OS, who were previously classified as having IMI.

The rational for AOX therapy is based on the understanding that AOXs may neutralize these potentially harmful oxidants. An AOX is a substance that neutral-

izes or protects the cells against the detrimental effects of oxidation and free radicals. The AOX system has enzymatic or non-enzymatic factors. Enzymatic AOXs include superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase. Non-enzymatic AOXs include glutathione, cysteine, N-acetylcysteine (NAC), carotenoids, vitamin C, vitamin E, carnitine, ferritin, L-arginine, transferrin, coenzyme Q10 (CoQ10), myo-inositol, lycopene, selenium, zinc, and folate [13,14]. The mechanism of action of these AOXs includes free radical scavenging and neutralization as well as preserving sperm DNA integrity and mitochondrial transport [15].

In 2021, a systematic review by Agarwal et al [16] identified 97 clinical trials (52 uncontrolled, 12 unblinded and 33 blinded randomized controlled trials [RCTs]) evaluating the efficacy of a single or combined AOXs for treatment of male infertility. By conducting a qualitative analysis of the evidence, they suggested that a review of the guidelines is needed, as the role of AOXs should be supported in case of (1) abnormal semen quality (grade C recommendation), (2) varicocele (grade C recommendation), and (3) idiopathic and unexplained male infertility (grade B recommendation) [16]. Studies on single supplements or on specific clinical contexts have demonstrated the positive role of AOXs on semen quality and reproductive outcomes. Thus, two recent meta-analyses of RCTs showed a significant positive effect of NAC and L-carnitine/L-acetyl-carnitine (LC/LAC) on semen parameters [17,18]. In addition, a meta-analysis of six studies with a total of 576 patients, found that the administration of AOXs after varicocelectomy resulted in a greater improvement in sperm concentration ($p<0.0001$), total sperm motility ($p=0.03$), progressive sperm motility ($p<0.00001$), and sperm morphology ($p<0.00001$) compared to placebo [19]. Furthermore, AOXs improved progressive sperm motility and sperm vitality, and significantly reduced sperm DNA fragmentation (SDF) when used in patients undergoing sperm freezing and thawing [20].

The 2019, Cochrane review assessed the benefit of different AOXs on pregnancy, live-birth, and miscarriage rates [14]. Despite the low quality of the evidence, this latest Cochrane review validated the efficacy of AOXs in improving the pregnancy rate (odds ratio [OR] 2.97, 95% confidence interval [CI] 1.91–4.63). However, the review failed to provide evidence of improvement of live-birth rate because of the low quality of the studies and limited amount of data available.

Despite these potential benefits, the role of AOX treatment in male infertility is still controversial. Major scientific societies, including the European Association of Urology (EAU) [21] and the American Urological Association/American Society for Reproductive Medicine (AUA/ASRM) [22], are not supporting the routine use of AOXs in infertile men, mainly due to the heterogeneity of data. Hence, there is a need for further studies.

The aim of the present systematic review and meta-analysis is to provide an updated, analysis on the impact of AOX therapy for male infertility as compared to placebo or no treatment. Spontaneous clinical pregnancy rate, live-birth, and miscarriage rates were selected as primary outcomes, while conventional sperm parameters, SDF, and seminal OS indices were considered as secondary outcomes. To the best of our knowledge, this is the first study conducting a meta-analysis of seminal OS indices in infertile men after AOX therapy.

MATERIALS AND METHODS

1. Protocol and outcome measures

This meta-analysis was conducted on previously published articles investigating the role of AOX therapy in the management of male infertility and followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) protocols [23]. The PRISMA checklist has been included as Supplement Table 1. The primary outcome was defined as the impact of AOXs on spontaneous clinical pregnancy rate, live-birth rate, and miscarriage rate. Secondary outcomes were the impact on basic semen parameters (sperm concentration, progressive sperm motility, total sperm motility, sperm morphology using the percentage of normal sperm forms), SDF, and indices of seminal OS such as seminal total AOX capacity (TAC) and malondialdehyde acid (MDA). This protocol has been registered in the PROSPERO database (PROSPERO registration number: CRD42022304600).

2. Eligibility criteria

This systematic review and meta-analysis included all English-language RCTs on male factor subfertility/infertility published until July 2021 that reported spontaneous pregnancy outcomes, and conventional

semen parameters, SDF, or seminal OS in AOX-treated patients *vs.* placebo-treated or untreated controls. AOX is defined as a supplement (containing a single or a combination of compounds) that can be obtained without a prescription, is not regulated as a pharmaceutical drug, and has an AOX effect. Combined AOXs are those that are composed of two or more AOXs. All RCT studies were included regardless of the type or dose of the oral AOX. Studies that include AOXs plus a plant extract were included if the AOX was the main compound used in the intervention group. All different study designs other than RCTs, animal studies, *in vitro* studies, case reports, case series, studies of plant extracts or herbal substances, and studies enrolling men taking hormonal or any other fertility drugs were excluded.

3. Search strategy

A systematic search was conducted using Scopus, PubMed, Ovid, Embase, and Cochrane databases. The initial query string on Scopus search was: (((TITLE-ABS-KEY (antioxidant) AND TITLE-ABS-KEY (infertil*) AND TITLE-ABS-KEY ((male) OR (man) OR (men))) AND NOT (TITLE-ABS-KEY ((mouse) OR (mice) OR (rat) OR (animal)))) AND NOT (TITLE-ABS-KEY ((meta-analysis) OR (metaanalysis)))) AND (TITLE-ABS-KEY ((year*) OR (month*) OR (day*) OR (year*) OR (time) OR (duration))) AND (LIMIT-TO (DOCTYPE,"ar")) AND (EXCLUDE (SUBJAREA,"AGRI") OR EXCLUDE (SUBJAREA,"ENVI")) AND (EXCLUDE (SUBJAREA,"CENG")) AND (EXCLUDE (SUBJAREA,"ENGI")) AND (EXCLUDE (SUBJAREA,"COMP")) AND (EXCLUDE (SUBJAREA,"IMMU")) AND (EXCLUDE (SUBJAREA,"HEAL")) AND (EXCLUDE (SUBJAREA,"SOCI")) AND (EXCLUDE (SUBJAREA,"MATE")) AND (EXCLUDE (SUBJAREA,"MULT") OR EXCLUDE (SUBJAREA,"VETE")) AND (EXCLUDE (LANGUAGE,"Portuguese")) AND (EXCLUDE (LANGUAGE,"Turkish") OR EXCLUDE (LANGUAGE,"Chinese") OR EXCLUDE (LANGUAGE,"Dutch") OR EXCLUDE (LANGUAGE,"German")) AND (EXCLUDE (LANGUAGE,"Hungarian")) AND (EXCLUDE (LANGUAGE,"French")) AND (EXCLUDE (LANGUAGE,"Persian")). PubMed, Ovid, Embase, and Cochrane's search string used was: ((antioxidant) AND (infertil*) AND ((male) OR (man) OR (men))) AND (((year*) OR (month*) OR (day*) OR (year*) OR (time) OR (duration))).

All the eligible studies were selected following the PICO (Population, Intervention, Comparison/Comparator, Outcomes) model (Supplement Table 2). Abstracts of the retrieved articles were independently screened and assessed to confirm their eligibility by four researchers (GS, AF, SK, AR). They worked in pairs, and disagreements were resolved by the fifth researcher (RC).

4. Assessment of quality of included studies

To evaluate the quality of evidence (QoE) of the included studies three tools were used: the Cochrane Risk of Bias of RCTs [24], the JADAD score [25], and CONSORT guidelines [26]. The QoE was assessed by four researchers (GS, AF, SK, AR) who worked in pairs. Disagreements were resolved by a fifth researcher (RC).

5. Data extraction

Data extraction was conducted for all eligible articles with full-text availability. Extracted information included the following: first author, year of publication, country, study design, the total number of patients, types of AOX, duration of treatment in months, semen parameters (volume, total motility, progressive motility, concentration, and morphology), OS indices (TAC and MDA), SDF, and pregnancy-related outcomes (pregnancy rate, live-birth rate, and miscarriage rate).

6. Accuracy of data collection

To ensure accuracy of search results and to reduce potential errors due to manual collection, screening for eligibility, data extraction, and quality assessment were done in duplicates and cross-matched in each subgroup. In case of discrepancy between screener and verifier, the results in dispute were verified by a third senior author to make a final decision.

7. Statistical analysis

The meta-analysis was performed using the random effect model. Measures of heterogeneity included Cochrane-Q test and I^2 statistics. The weight of each individual study was determined using the inverse variance method and the Mantel–Haenszel methods for continuous and binary data, respectively. The pub-

lication bias was assessed with the color-contoured funnel plot and the asymmetry of the plot was tested by the Egger and Harbord tests for mean difference (MD) and log-OR respectively. For the SDF, we used the standardized mean difference (SMD), as this outcome was evaluated using different protocols. A subgroup analysis was performed as a sensitivity analysis and to determine the importance of subgroups on the pooled effect size. All p-values lower than 0.05 were considered statistically significant. The analysis was performed using RevMan software v. 5.4 (Cochrane Collaboration, Oxford, UK) and the IBM v 25 statistical software (IBM Corp., Armonk, NY, USA) and R-programing language v 4.1.0.

RESULTS

Using the above-mentioned search strategy, we extracted 1,307 abstracts. After the exclusion of 328 duplicates, the remaining 979 abstracts were assessed for inclusion in the meta-analysis. Of these, 868 were judged not eligible based on their title and the abstract, or because they were narrative reviews, comments, systematic reviews and meta-analyses, or letters to the editor. Among the remaining 111 articles that were initially deemed eligible, 66 were excluded due to unavailable full-text, absence of untreated or placebo-treated control group, non-RCT study design, not extractable data or presence of fertile men in the control group. Finally, 45 RCTs met the study inclusion criteria and were included in the meta-analysis, for a total of 4,332 infertile patients (Fig. 1).

Six of the included studies were performed in an infertile population receiving AOX or no treatment following varicocele repair [27-32]. Therefore, for each outcome which included a varicocele repair group we performed a sub-analysis after exclusion of these studies. The main characteristics of the included studies are shown in Table 1. The duration of AOX therapy of the analyzed studies ranged from 1 to 12 months. The quality of the included studies is shown in Table 2. The inclusion and exclusion criteria for each included study are detailed in Supplement Table 3.

1. Spontaneous pregnancy rate

Sixteen studies [28,29,32-45] assessed spontaneous pregnancy rate in association with AOX, including 1,355 infertile patients (761 in the AOX-treated group and 594 in the untreated or placebo-treated control group) and 190 pregnancies were recorded. We found a positive effect of AOX treatment on spontaneous pregnancy rate (OR 1.97 [95% CI: 1.28, 3.04]; p<0.01), with absence of significant inter-study heterogeneity (I^2=20%; χ^2 p=0.20) (Fig. 2). There was no significant publication bias (Fig. 3).

The sub-analysis performed after the exclusion of

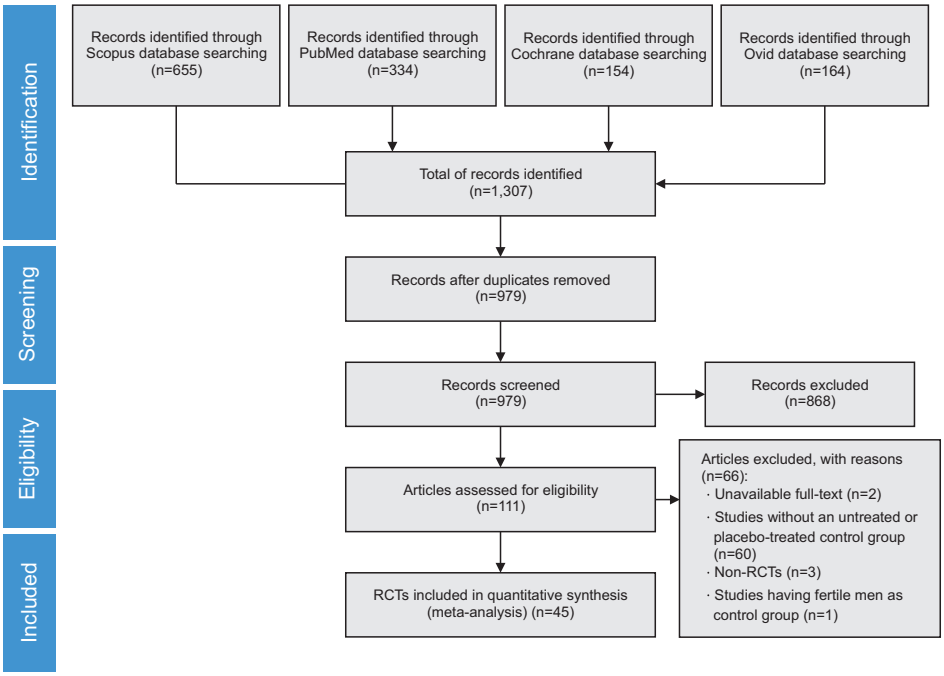

Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow chart of the included studies. RCT: randomized controlled trial.

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Table 1. Main characteristics of the 45 randomized controlled trials included in this meta-analysis

Reference	Year	Population	Intervention	Control	Duration (mo)	Outcome
Eslamian et al [51]	2020	Idiopathic asthenozoospermic men	465 mg DHA plus 600 IU vitamin E (n=41), or 465 mg DHA plus vitamin E–resembling placebo (n=41), or 600 IU vitamin E plus DHA-resembling placebo (n=45)	Placebo (n=45)	3	Basic semen parameters, OS
Kopets et al [38]	2020	Patients with IMI	LC/LAC 1,990 mg, coQ10 40 mg, L-arginine 250 mg, glutathione 100 mg, Zinc 7.5 mg, vitamin B9 234 μg, vitamin B12 2 μg, Selenium 50 μg (n=42)	Placebo (n=41)	6	Basic semen parameters, natural pregnancy
Steiner et al [42]	2020	Patients with OAT or high SDF	500 mg of vitamin C, 400 mg of vitamin E, 0.20 mg of selenium, 1,000 mg of LC, 20 mg of zinc, 1,000 mcg of folic acid, 10 mg of lycopene daily (n=85)	Placebo (n=86)	6	Semen parameters, SDF, live-birth rate
Ardestani Zadeh et al [27]	2019	Infertile patients with varicocele undergone to varicocele repair	Folic acid 5 mg, Selenium 200 μg, vitamin E 400 IU (n=27)	No treatment (n=30)	6	Semen parameters
Kızılay and Altay [28]	2019	Infertile patients with OAT and varicocele, following varicocele repair	LC fumarate 2 g, LAC 1 g, fructose 2 g, citric acid 100 mg, vitamin C 180 mg, Zinc 20 mg, Folic acid 400 mcg, Selenium 100 mcg, CoQ10 40 mg, vitamin B12 3 mcg (n=62)	No treatment (n=28)	6	Basic semen parameters, natural pregnancy/live-birth
Nouri et al [60]	2019	Idiopathic asthenozoospermic men	Lycopene 25 mg daily (n=17)	Placebo (n=19)	3	Basic semen parameter, OS
Blomberg Jensen et al [69]	2018	Men part of an infertile couple with impaired semen quality	Vitamin D 1,400 IU+calcium 500 mg plus vitamin D 300,000 IU oil once orally (n=136)	Placebo (n=133)	5	Semen parameters, live-birth rate (ART)[b]
Busetto et al [36]	2018	Infertile men with oligo- and/or astheno- and/or teratozoospermia, divided into patients with varicocele grade I–III, and patients without varicocele	LC 1,000 mg, fumarate 725 mg, LAC 500 mg, fructose 1,000 mg, CoQ10 20 mg, vitamin C 90 mg, zinc 10 mg, folic acid 200 μg and vitamin B12 1.5 μg/daily (n=49)	Placebo (n=45)	6	Basic semen parameters, natural pregnancy/live-birth, miscarriage
Lu et al [55]	2018	Infertile men with left-sided clinical varicocele	400 mg melatonin daily (n=27)	Placebo (n=27)	3	Basic semen parameters, OS
Stenqvist et al [43]	2018	Subfertile men	Vitamin C 3 mg, vitamin E 5 mg, vitamin B12 0.5 μg, LC 750 mg, CoQ10 10 mg, folic acid 100 μg, zinc 5 mg, selenium 25 μg (n=37)	Placebo (n=40)	6	Basic semen parameters, SDF, pregnancy rate
Busetto et al [35]	2017	Infertile patients with OAT	2 sachets of Proxeed Plus daily (1,000 mg l-carnitine, 725 mg fumarate, 500 mg LAC, 1,000 mg fructose, 20 mg CoQ10, 90 mg vitamin C, 10 mg zinc, 200 μg folic acid and1.5 μg vitamin B12) (n=52)	Placebo (n=52)	6	Basic semen parameters, natural pregnancy/live-birth
Barekat et al [29]	2016	Subfertile men with varicocele grade 2–3 following varicocele repair	NAC 200 mg (n=20)	No treatment (n=20)	3	Basic semen parameters, OS, SDF, natural pregnancy/live-birth

Table 1. Continued 1

Reference	Year	Population	Intervention	Control	Duration (mo)	Outcome
Ener et al [30]	2016	Infertile men with a left-sided clinical varicocele following to varicocele repair	Vitamin E 600 mg (n=22)	No treatment (n=23)	12	Basic semen parameters, natural pregnancy/live-birth
Boonyarangkul et al [46]	2015	Men with abnormal semen analysis	Tamoxifen citrate 20 mg (n=15)[a] or Folate 5 mg (n=15) or Tamoxifen citrate 20 mg plus Folate 5 mg (n=15)[a]	Placebo (n=15)	3	Basic semen parameters
Cyrus et al [31]	2015	Infertile men with palpable varicocele grade 2–3 underwent to varicocele repair	Vitamin C 500 mg (n=46)	Placebo (n=69)	3	Basic semen parameters
Haghighian et al [53]	2015	Infertile men with idiopathic astheno-zoospermia	ALA 600 mg (n=23)	Placebo (n=21)	3	Basic semen parameters, OS
Moslemi Mehni et al [57]	2014	Infertile men with idiopathic OAT	Pentoxifylline 800 mg+L-carnitine 1,000 mg (n=58) or Pentoxifylline 800 mg+Placebo (n=59) or LC 1,000 mg+Placebo (n=59)	Placebo (n=59)	3	Basic semen parameters
Azizollahi et al [32]	2013	Infertile men with varicocele grade III following varicocele repair	Zinc 66 mg (n=32) or Folic acid 5 mg (n=26) or Zinc 66 mg+Folic acid 5 mg (n=29)	Placebo (n=25)	6	Sperm concentration, morphology, motility, forward progressive motility
da Silva et al [49]	2013	Subfertile men	Folic acid 5 mg daily (n=23)	Placebo (n=26)	3	Basic semen parameters
Gopinath et al [37]	2013	Idiopathic OA	2 tablets of CoQ10 50 mg+L-carnitine 500 mg+lycopene 2.5 mg+zinc 12.5 mg (n=46) or 1 tablet (n=43)	Placebo (n=36)	6	Basic semen parameters, spontaneous pregnancy rate
Safarinejad et al [64]	2012	Infertile men with primary infertility for at least 2 years	CoQ10 (Ubiquinol) 200 mg (n=114)	Placebo (n=114)	6.5	Basic semen parameters, seminal plasma antioxidant status
Nadjarzadeh et al [59]	2011	Infertile men with OAT who have been trying for pregnancy for >1 year of unprotected intercourse	CoQ10 200 mg (n=23)	Placebo (n=24)	3	Basic semen parameters, OS
Safarinejad [65]	2011	Infertile men with idiopathic OAT	Omega-3 fatty acids: EPA and DHA, 1.84 g per day (n=119)	Placebo (n=119)	8	Basic semen parameters, OS
Dimitriadis et al [50]	2010	Infertile men with OA	Vardenafil 10 mg (n=23)[a] or Sildenafil 50 mg (n=25)[a] or LC 1000 mg (n=26)	No treatment (n=22)	5.5	Basic semen parameters
Martinez-Soto et al [56]	2010	Infertile men	DHA 1,000 mg+EPA 135 mg (n=35)	Placebo (n=29)	2.5	SDF, basic semen parameters, antioxidant capacity
Morgante et al [58]	2010	Infertile men with asthenospermia	L-arginine 1,660 mg+carnitine 150 mg+LAC 50 mg+ginseng 200 mg in one vial (n=90)	No treatment (n=90)	3	Basic semen parameters
Peivandi et al [62]	2010	Infertile men	LC 2 g (n=15)	Placebo (n=15)	2	Basic semen parameters
Balercia et al [34]	2009	Infertile men with idiopathic astheno-zoospermia	CoQ10 200 mg (n=30)	Placebo (n=30)	6	Basic semen parameters, spontaneous pregnancy rate

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Table 1. Continued 2

Reference	Year	Population	Intervention	Control	Duration (mo)	Outcome
Ciftci et al [47]	2009	IMI	NAC (600 mg daily) (n=60)	Placebo (n=60)	3	Basic semen parameters, OS
Safarinejad and Safarinejad [66]	2009	Men with idiopathic OAT, asthenospermia or teratospermia of 2 years duration	Selenium 200 μg (n=116) or NAC 600 mg (n=118) or Selenium 200 μg+NAC 600 mg (n=116)	Placebo (n=118)	6.5	Basic semen parameters
Safarinejad [67]	2009	Subfertile men with idiopathic OAT	CoQ10 300 mg (n=106)	Placebo (n=106)	6.5	Basic semen parameters
Stanislavov et al [68]	2009	Subfertile patients	80 mg Pycnogenol and 3 g L-arginine aspartate (n=25)	Placebo (n=25)	1	Basic semen parameters
Omu et al [61]	2008	Men with asthenozoospermia attending infertility and andrology clinic	Zinc 400 mg (n=11) or Zinc 400 mg+vitamin E 20 mg (n=12) or Zinc 400 mg+vitamin E 20 mg+vitamin C 10 mg (n=14)	No treatment (n=8)	3	Basic semen parameters, spontaneous pregnancy rate, live-birth rate, miscarriage
Paradiso Galatioto et al [40]	2008	Men with persistent oligospermia (5–20 million/mL) 6 months after retrograde embolization	NAC 600 mg, vitamin C 180 mg, vitamin E 12 mg, vitamin A 3.6 IU, thiamine 24 mg, riboXavin 6 mg, piridoxin 12 mg, nicotinamide 60 mg, pantothenate 12 mg, biotin 2.4 mg, cyanocobalamin 6 mg, ergocalciferol 480 IU, calcium 60 mg, magnesium 21.5 mg, phosphate 27 mg, iron 12 mg manganese 0.6 mg, copper 1.2 mg, zinc 0.6 mg (n=20)	No treatment (n=22)	3	Basic semen parameters, natural pregnancy
Sigman et al [71]	2006	Subfertile men aged 18 to 65 years	LC 2,000 mg+LAC 1,000 mg (n=12)	Placebo (n=9)	4	Basic semen parameters, pregnancy rate (ART)[b]
Balercia et al [33]	2005	Subfertile men with idiopathic asthenozoospermia	LC 3 g (n=15) *vs*. LAC 3 g (n=15) *vs*. LC 2 g+LAC 1 g (n=14)	Placebo (n=15)	6	Basic semen parameters, spontaneous pregnancy rate
Greco et al [52]	2005	Subfertile men	Vitamin C 1 g and Vitamin E 1 g (n=32)	Placebo (n=32)	2	Basic semen parameters, SDF
Lenzi et al [54]	2003	Subfertile men with OAT	LC 2 g+LAC 1,000 mg (n=43)	Placebo (n=43)	2	Basic semen parameters, spontaneous pregnancy rate
Závaczki et al [45]	2003	Subfertile men	Magnesium 3,000 mg (n=10)	Placebo (n=10)	3	Basic semen parameters, spontaneous pregnancy rate
Conquer et al [48]	2000	Healthy asthenozoospermic men who were patients of an infertility clinic	DHA 400 mg (n=9) or DHA 800 mg (n=10)	Placebo (n=9)	3	Basic semen parameters
Rolf et al [63]	1999	Men with infertility for over one year	Vitamin C 1,000 mg+vitamin E 800 mg (n=15)	Placebo (n=16)	2	Basic semen parameters and pregnancy rate
Omu et al [39]	1998	Men with asthenozoospermia attending infertility and andrology clinic	Zinc 500 mg (n=49)	No therapy (n=48)	3	Basic semen parameters, natural pregnancy/live-birth

Table 1. Continued 3

Reference	Year	Population	Intervention	Control	Duration (mo)	Outcome
Scott et al [41]	1998	Men attending subfertility clinic with low sperm motility	Selenium 100 mg daily alone (n=16) or selenium 100 mg plus selenium combined with vitamins A (1 mg), C (10 mg), and E (15 mg) supplements daily (n=30)	Placebo (n=18)	3	Basic semen parameters, natural pregnancy/ live-birth
Suleiman et al [44]	1996	Asthenozospermic men attending a fertility center	Vitamin E 100 mg (n=52)	Placebo (n=35)	6	Sperm motility, spontaneous live-birth, pregnancy, miscarriage and MDA levels
Dawson et al [70]	1990	Men with sperm agglutination	Ascorbic acid 1,000 mg (n=10) or Ascorbic acid 200 mg (n=10)	Placebo (n=10)	0.75	Basic semen parameters

ALA: alpha-lipoic acid, ART: assisted reproductive technique, CoQ10: coenzyme Q10, DHA: docosahexaenoic acid, EPA: eicosapentaenoic, IMI: idiopathic male infertility, LAC: L-acetyl-carnitine, LC: L-carnitine, MDA: malondialdehyde acid, NAC: N-acetylcysteine, OA: oligo-asthenoteratozoospermia, OAT: oligo-asthenoteratozoospermia, OS: oxidative stress, SDF: sperm DNA fragmentation.
[a]This group was excluded from the analysis.
[b]Outcome excluded from the present meta-analysis, since it does not assess spontaneous pregnancy rate.

Table 2. Quality of evidence assessment results of the Cochrane Risk of Bias for Randomized Controlled Trials [21], CONSORT guidelines [23], and JADAD score [22]

Reference	Year	Cochrane Risk of Bias for Randomized Controlled Trials								CONSORT guideline (1–25)	JADAD (1–5)	Total quality score (9–51)
		Selection bias Random sequence generation[a]	Selection bias Allocation concealment[a]	Reporting bias Selective reporting[a]	Other bias Other sources of bias[a]	Performance bias Blinding (participants and personnel)[a]	Detection bias Blinding (outcome assessment)[a]	Attrition bias Incomplete outcome data[a]	Total score (7–21)			
Eslamian et al [51]	2020	3	3	2	2	2	2	3	17	22	5	44
Kopets et al [38]	2020	3	3	3	2	3	2	2	18	18	5	41
Steiner et al [42]	2020	3	3	2	2	3	2	2	17	15	3	35
Ardestani Zadeh et al [27]	2019	3	3	2	2	2	2	2	16	15	3	34
Kızılay and Altay [28]	2019	3	2	2	2	1	1	3	14	12	3	29
Nouri et al [60]	2019	3	2	3	3	3	3	3	20	17	5	42
Blomberg Jensen et al [69]	2018	3	3	3	2	3	3	3	20	24	4	48
Busetto et al [36]	2018	3	3	3	2	3	3	3	20	20	4	44
Lu et al [55]	2018	3	3	3	2	3	3	3	20	14	3	37
Stenqvist et al [43]	2018	2	3	3	3	3	3	3	20	21	5	46
Busetto et al [35]	2017	3	2	2	2	2	2	3	16	17	4	37
Barekat et al [29]	2016	2	2	3	2	1	1	3	14	13	2	29

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Table 2. Comtinued 1

Reference	Year	Cochrane Risk of Bias for Randomized Controlled Trials								CONSORT guideline (1–25)	JADAD (1–5)	Total quality score (9–51)
		Selection bias Random sequence generation[a]	Selection bias Allocation concealment[a]	Reporting bias Selective reporting[a]	Other bias Other sources of bias[a]	Performance bias Blinding (participants and personnel)[a]	Detection bias Blinding (outcome assessment)[a]	Attrition bias Incomplete outcome data[a]	Total score (7–21)			
Ener et al [30]	2016	2	2	1	2	1	1	3	12	13	2	27
Boonyarangkul et al [46]	2015	2	2	2	3	2	2	3	16	13	3	32
Cyrus et al [31]	2015	3	3	2	3	3	2	3	19	21	5	45
Haghighian et al [53]	2015	3	3	3	2	3	3	3	20	22	5	47
Moslemi Mehni et al [57]	2014	2	1	2	2	1	1	2	11	13	2	26
Azizollahi et al [32]	2013	2	2	2	1	2	2	1	12	10	3	25
da Silva et al [49]	2013	3	3	2	2	3	2	3	18	17	5	40
Gopinath et al [37]	2013	2	2	2	2	2	2	2	14	17	3	34
Safarinejad et al [64]	2012	3	3	3	3	3	3	3	21	20	5	46
Nadjarzadeh et al [59]	2011	2	2	3	3	3	3	3	19	19	4	42
Safarinejad [65]	2011	3	3	3	3	3	2	3	20	19	5	44
Dimitriadis et al [50]	2010	2	1	2	2	1	1	3	12	14	2	15
Martinez-Soto et al [56]	2010	3	3	3	2	3	3	2	19	15	4	38
Morgante et al [58]	2010	2	2	2	1	1	1	3	12	9	2	23
Peivandi et al [62]	2010	2	2	2	3	3	3	3	18	8	3	29
Balercia et al [34]	2009	2	2	2	3	3	3	2	17	12	2	31
Ciftci et al [47]	2009	3	3	3	1	2	2	3	17	14	4	35
Safarinejad and Safarinejad [66]	2009	3	3	3	2	3	3	3	20	20	4	44
Safarinejad [67]	2009	3	3	3	2	3	3	3	20	20	4	44
Stanislavov et al [68]	2009	1	3	2	2	3	3	3	17	14	3	34
Omu et al [61]	2008	1	1	2	1	1	1	2	9	9	2	20
Paradiso Galatioto et al [40]	2008	3	3	2	3	1	1	3	16	18	3	37
Sigman et al [71]	2006	2	2	2	2	2	1	3	14	15	3	32
Balercia et al [33]	2005	2	2	2	2	2	2	2	14	12	3	29
Greco et al [52]	2005	2	2	2	2	2	2	3	15	13	3	31
Lenzi et al [54]	2003	2	2	3	2	2	2	3	16	15	3	34
Závaczki et al [45]	2003	2	2	2	3	1	2	1	13	8	2	23
Conquer et al [48]	2000	2	2	3	2	2	2	3	16	16	3	35

Table 2. Continued 2

Reference	Year	Cochrane Risk of Bias for Randomized Controlled Trials								CONSORT guideline (1–25)	JADAD (1–5)	Total quality score (9–51)
		Selection bias Random sequence generation[a]	Selection bias Allocation concealment[a]	Reporting bias Selective reporting[a]	Other bias Other sources of bias[a]	Performance bias Blinding (participants and personnel)[a]	Detection bias Blinding (outcome assessment)[a]	Attrition bias Incomplete outcome data[a]	Total score (7–21)			
Rolf et al [63]	1999	2	3	2	3	2	2	3	17	16	3	36
Omu et al [39]	1998	2	2	2	2	1	1	1	11	13	1	25
Scott et al [41]	1998	3	3	2	2	2	2	3	17	14	4	35
Suleiman et al [44]	1996	2	2	2	1	2	2	1	12	6	3	21
Dawson et al [70]	1990	2	2	2	1	3	2	3	15	8	2	28

[a]High risk of bias=1; Unclear risk of bias=2; Low risk of bias=3.

the studies administering AOX or no treatment following varicocele repair confirmed the benefit of AOX administration on spontaneous pregnancy rate (OR 2.12 [95% CI: 1.23, 3.65]; n=157 events; p<0.01] (Fig. 4), in the absence of significant inter-study heterogeneity (I^2=36%; χ^2 p=0.08), and no significant publication bias as confirmed by the funnel plot (Fig. 5).

2. Live-birth rate

Only four studies could be included in the analysis of live-birth rate [33,34,39,42], overall including 388 infertile patients (209 in the AOX-treated group and 179 in the control one) and with 65 events (live-birth) recorded.

The analysis revealed no effect of AOX treatment on live-birth rate (OR 1.21 [95% CI: 0.53, 2.76]; p=0.64) (Fig. 6).

3. Miscarriage rate

Four studies reported data on the miscarriage rate and could be included in the analysis [36,39,42,44]. We found no effect of AOX treatment on miscarriage rate (OR 1.01 [95% CI: 0.34, 3.00]; n=13 events; p=0.98), in a total population of 459 infertile patients (Fig. 7).

4. Sperm concentration

A total of thirty-six studies were included [27-37,41,45-68], allowing to analyze this outcome in 4,310 infertile patients (2,407 AOX-treated patients and 1,903 placebo-treated or untreated controls).

We found a significantly positive effect of AOX treatment on sperm concentration (weighted MD 5.93 mil/mL [95% CI: 4.43, 7.43]; p<0.01), with the presence of significant inter-study heterogeneity (I^2=94%; χ^2 p<0.01). There was no significant publication bias (p=0.5) (Fig. 8, 9).

The results of sub-group analysis performed after exclusion of 7 studies on patients with varicocele that underwent varicocele repair followed by AOX or placebo/no treatment [27,32,55] on a total of 3,653 infertile men, indicated a significant persistent positive effect of AOX supplementation on sperm concentration (weighted MD 5.55 mil/mL [95% CI: 3.87, 7.22]; 2,023 patients *vs.* 1,630 controls; p<0.01). A significant inter-study heterogeneity was also found in this subgroup analysis (I^2=95%; χ^2 p<0.01), with no significant publication bias (p=0.8) (Fig. 10, 11).

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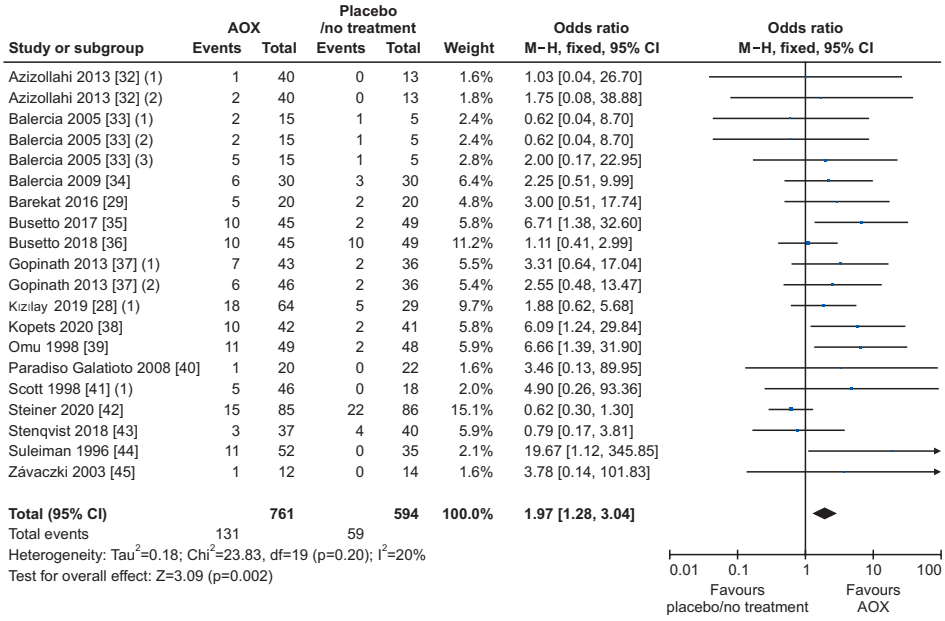

Fig. 2. Forest plot of the spontaneous pregnancy rate in infertile patients treated with antioxidants (AOXs) compared to placebo or untreated infertile controls.

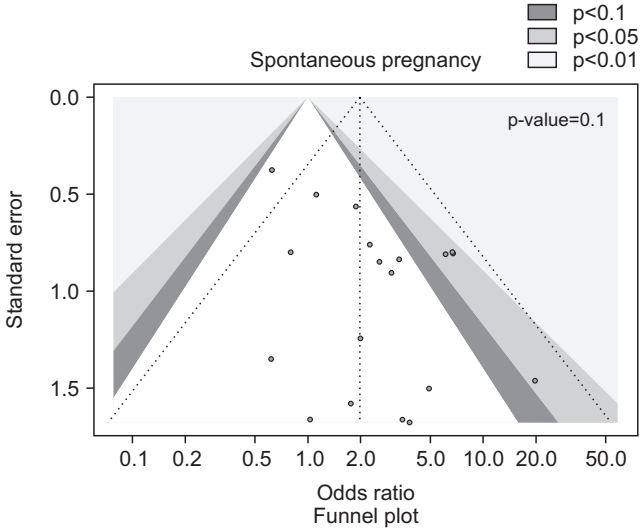

Fig. 3. Funnel plot of the spontaneous pregnancy rate in infertile patients treated with antioxidants compared to placebo or untreated infertile controls.

5. Progressive sperm motility

Twenty studies have been included in the analysis of the effect of AOX on progressive sperm motility [28,31-35,37,46,49,51,53,56-60,62,63,69,70], overall including 2,345 infertile patients (1,297 AOX-treated patients and 1,048 placebo-treated or untreated controls). The analysis showed a significant positive effect of AOX on progressive sperm motility (weighted MD 7.21% [95% CI: 3.66, 10.76]; p<0.01). Significant inter-study heterogeneity was observed (I^2=99%; χ^2 p<0.01). The Funnel plot was significantly asymmetrical denoting the presence of publication bias (p=0.02) (Fig. 12, 13).

The benefit of AOX supplementation on progressive sperm motility was also confirmed by the analysis performed after the exclusion of studies carried out in patients with varicocele or treated with varicocele repair and AOX or placebo/no treatment [28,31,32], including a total of 1,923 infertile men (weighted MD 7.78% [95% CI: 3.86, 11.70]; 1,020 patients *vs.* 903 controls; p<0.01). We found significant inter-study heterogeneity (I^2=99%; χ^2 p<0.01) Also, significant publication bias persisted even after the exclusion of the studies with varicocele repair (Fig. 14, 15).

6. Total sperm motility

Thirty-six studies with a total of 4,452 infertile patients (2,516 AOX-treated patients and 1,936 placebo-treated or untreated controls) were included in the analysis of total sperm motility [27-37,39,41,44-48,50-56,58-62,64,66,67,69-71]. We found a significant positive effect of AOX on total sperm motility (weighted MD 7.52% [95% CI: 3.11, 11.94]; p<0.01).

Significant inter-study heterogeneity was observed (I^2=100%; χ^2 p<0.01). The asymmetry of the Funnel plot denoted significant publication bias (p<0.001) (Fig. 16, 17).

We also found a positive effect of AOX supplementation on total sperm motility after the exclusion of studies carried out in patients with varicocele or treated with varicocele repair and AOX or placebo/no treatment [27-32,50,55], including a total of 4,291 infertile men (MD 7.29% [95% CI: 2.75, 11.83]; 2,435 patients *vs.*

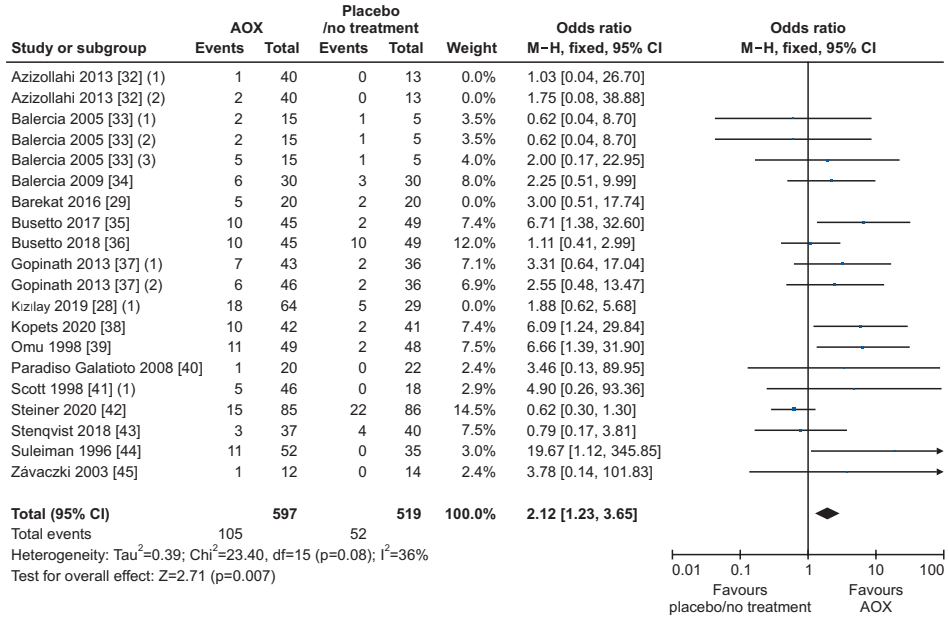

Fig. 4. Forest plot of the spontaneous pregnancy rate in infertile patients treated with antioxidants (AOXs) compared to placebo or untreated infertile controls, after the removal of studies including patients with varicocele undergoing varicocele repair.

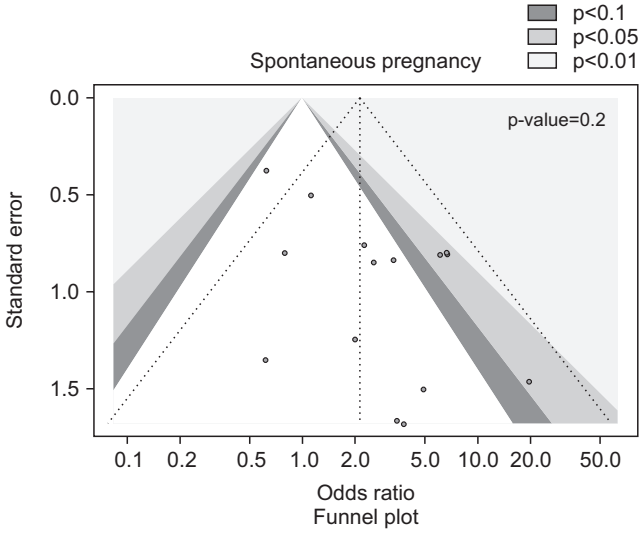

Fig. 5. Funnel plot of the spontaneous pregnancy rate in infertile patients treated with antioxidants compared to placebo or untreated infertile controls, after the removal of studies including patients with varicocele undergoing varicocele repair.

1,856 controls; p<0.01).

The analysis resulted in a significant inter-study heterogeneity (I^2=100%; χ^2 p<0.01) and the publication bias persisted even after the exclusion of the studies with varicocele repair (p<0.001) (Fig. 18, 19).

7. Sperm morphology

Eighteen studies, with a total of 1828 infertile men (975 in the AOX-treated group and 853 in the placebo-treated or untreated one), were included in the analysis of sperm morphology [29,31,32,35,45,49-53,55,59-61,63,64,66,67]. We found a significant positive effect of AOX on sperm morphology (weighted MD 3.28% [95% CI: 2.40, 4.17]; p<0.01). Significant inter-study heterogeneity was observed (I^2=96%; χ^2 p<0.01). In addition, a significant funnel plot asymmetry was found (p=0.04), thus consistent with the presence of publication bias (Fig. 20, 21).

We also found a positive effect of AOX supplementation on sperm morphology after the exclusion of studies carried out in patients with varicocele or treated with varicocele repair and AOX or placebo/no treatment [29,31,32,50,55], including a total of 1,413 infertile men (weighted MD 1.34% [95% CI: 0.13, 2.56]; 718 patients *vs.* 695 controls; p=0.03).

The analysis showed significant inter-study heterogeneity (I^2=88%; χ^2 p<0.01). The publication bias disappeared after the exclusion of varicocele repair studies (p=0.6) (Fig. 22, 23).

8. SDF

Only three studies, with a total of 68 AOX-treated patients and 67 placebo-treated or untreated controls, were included in the analysis of SDF [29,52,56]. All three studies used the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) test. No effect of AOX on SDF was found compared to placebo or no treatment (SMD -0.63 [95% CI: -2.29, 1.02]; p=0.45) (Fig. 24).

We found a significant lower SDF in patients on AOX compared to controls when the analysis was

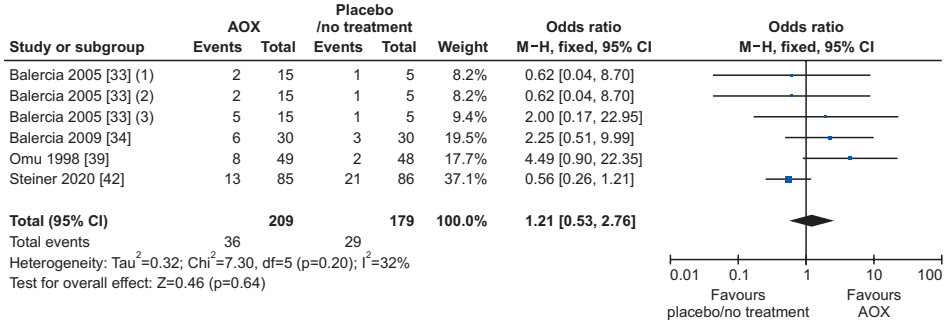

Fig. 6. Forest plot of the live-birth rate in infertile patients treated with antioxidants (AOXs) compared to placebo or untreated infertile controls.

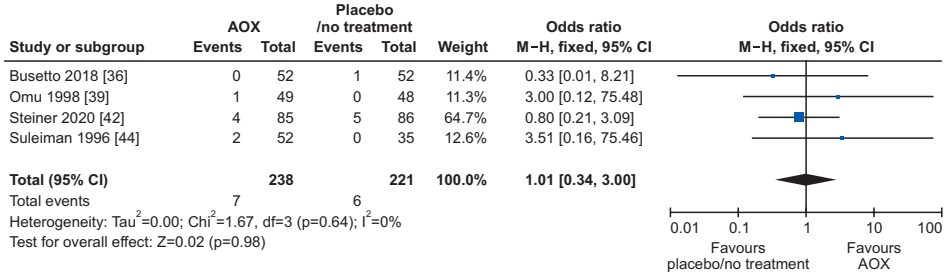

Fig. 7. Forest plot of the miscarriage rate in infertile patients treated with antioxidants (AOXs) compared to placebo or untreated infertile controls.

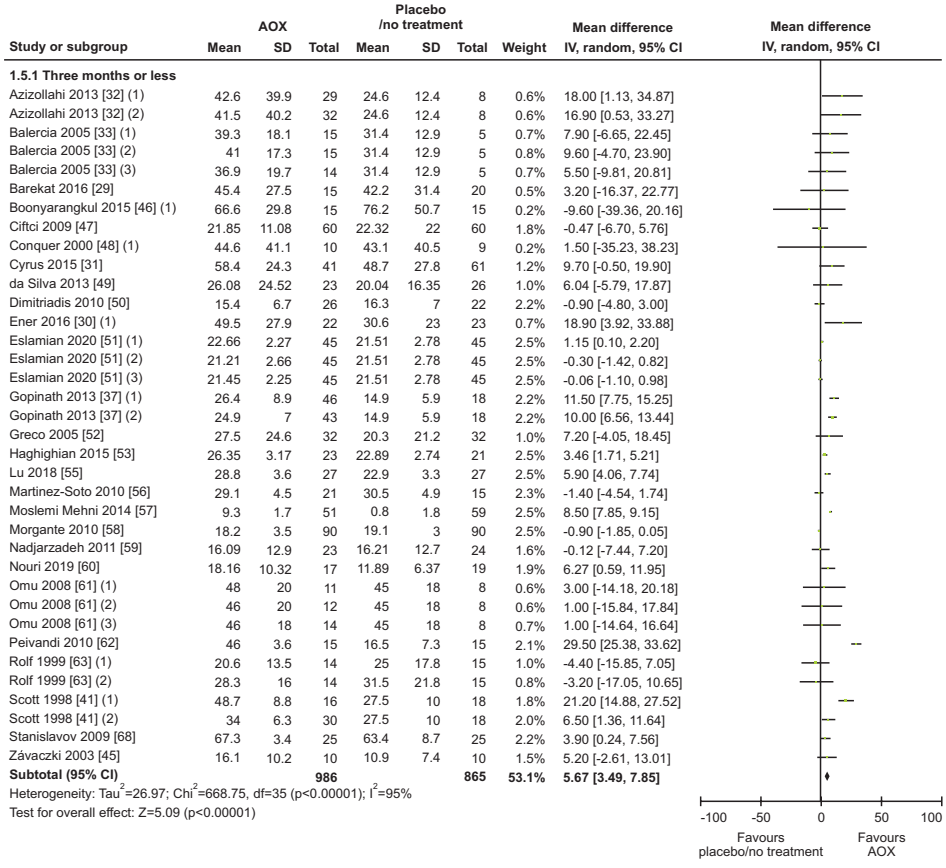

Fig. 8. Forest plot of the sperm concentration in infertile patients treated with antioxidants (AOXs) compared to placebo or untreated infertile controls.

repeated after the exclusion of one study carried out in patients treated with varicocele repair and AOX or placebo/no treatment [29] (SMD -1.47 [95% CI: -2.18, -0.77]; 53 patients *vs.* 47 controls; p<0.01), although this analysis was performed in only two studies.

Furthermore, the analysis showed a significant

Study or subgroup	AOX Mean	AOX SD	AOX Total	Placebo /no treatment Mean	Placebo /no treatment SD	Placebo /no treatment Total	Weight	Mean difference IV, random, 95% CI
1.5.2 Six months								
Ardestani Zadeh 2019 [27]	41.26	24.52	30	35.83	23.21	30	1.0%	5.43 [-6.65, 17.51]
Azizollahi 2013 [32] (4)	47.6	40.4	29	29.9	18.7	8	0.5%	17.70 [-1.90, 37.30]
Azizollahi 2013 [32] (5)	39.6	30.5	32	29.9	18.7	8	0.6%	9.70 [-7.02, 26.42]
Azizollahi 2013 [32] (6)	49.1	16.8	26	29.9	18.7	8	0.8%	19.20 [4.72, 33.68]
Balercia 2005 [33] (4)	45.53	21.42	15	33.73	14.36	15	0.9%	11.80 [-1.25, 24.85]
Balercia 2005 [33] (5)	39.57	19.99	15	33.73	14.36	15	0.9%	5.84 [-6.62, 18.30]
Balercia 2005 [33] (6)	37.4	16.42	14	33.73	14.36	15	1.0%	3.67 [-7.59, 14.93]
Balercia 2009 [34]	44.9	19.3	30	46.4	19.8	30	1.2%	-1.50 [-11.39, 8.39]
Boonyarangkul 2015 [46] (2)	53.3	22.8	15	76.1	70.8	15	0.1%	-22.80 [-60.44, 14.84]
Busetto 2017 [35]	51.4	13.9	45	43.7	13.6	49	1.9%	7.70 [2.13, 13.27]
Busetto 2018 [36]	40.8	18.2	52	41.4	17.9	52	1.6%	-0.60 [-7.54, 6.34]
Ener 2016 [30] (2)	53.9	22	22	48	34.2	23	0.6%	5.90 [-10.83, 22.63]
Gopinath 2013 [37] (1)	33.2	12.4	46	15.9	7.7	18	2.0%	17.30 [12.25, 22.35]
Gopinath 2013 [37] (2)	31.7	9.7	43	15.9	7.7	18	2.1%	15.80 [11.21, 20.39]
Kızılay 2019 [28] (1)	13.12	1.89	18	11.19	1.95	9	2.5%	1.93 [0.39, 3.47]
Kızılay 2019 [28] (2)	18.19	3.3	39	15.22	3.11	17	2.5%	2.97 [1.16, 4.78]
Lenzi 2003 [54]	22.1	9.1	30	22.2	17	26	1.6%	-0.10 [-7.40, 7.20]
Safarinejad 2009 [66] (1)	26.8	5.3	105	23.5	5.8	35	2.4%	3.30 [1.13, 5.47]
Safarinejad 2009 [66] (2)	32.1	6.8	104	23.5	5.8	35	2.4%	8.60 [6.28, 10.92]
Safarinejad 2009 [66] (3)	27.6	6.4	105	23.5	5.8	35	2.4%	4.10 [1.82, 6.38]
Safarinejad 2009 [67]	26.4	4.4	98	20.8	4.3	96	2.5%	5.60 [4.38, 6.82]
Safarinejad 2012 [64]	28.7	4.6	101	16.8	4.4	102	2.5%	11.90 [10.66, 13.14]
Subtotal (95% CI)			**1,014**			**659**	33.9%	**6.66 [4.29, 9.02]**
Heterogeneity: Tau^2=18.30; Chi^2=189.91, df=21 (p<0.00001); I^2=89%								
Test for overall effect: Z=5.52 (p<0.00001)								
1.5.3 Nine months or more								
Balercia 2005 [33] (7)	39.4	13.9	15	30.1	9.3	5	1.1%	9.30 [-1.47, 20.07]
Balercia 2005 [33] (8)	31.2	8.6	15	30.1	9.3	5	1.3%	1.10 [-8.14, 10.34]
Balercia 2005 [33] (9)	33.3	13.6	14	30.1	9.3	5	1.1%	3.20 [-7.63, 14.03]
Balercia 2009 [34]	44.2	20.4	30	49.6	20.5	30	1.1%	-5.40 [-15.75, 4.95]
Ener 2016 [30] (3)	58.6	20.2	22	47.2	27.2	23	0.8%	11.40 [-2.56, 25.36]
Safarinejad 2009 [67]	22.8	3.8	98	21.2	3.8	96	2.5%	1.60 [0.53, 2.67]
Safarinejad 2011 [65]	28.7	4.4	101	16.8	4.4	102	2.5%	11.90 [10.69, 13.11]
Safarinejad 2012 [64]	22.4	4.2	112	16.2	3.7	113	2.5%	6.20 [5.17, 7.23]
Subtotal (95% CI)			**407**			**379**	13.0%	**5.19 [0.96, 9.41]**
Heterogeneity: Tau^2=23.87; Chi^2=163.26, df=7 (p<0.00001); I^2=96%								
Test for overall effect: Z=2.41 (p=0.02)								
Total (95% CI)			**2,407**			**1,903**	**100.0%**	**5.93 [4.43, 7.43]**
Heterogeneity: Tau^2=22.90; Chi^2=1,099.49, df=65 (p<0.00001); I^2=94%								
Test for overall effect: Z=7.76 (p<0.00001)								
Test for subgroup differences: Chi^2=0.53, df=2 (p=0.77); I^2=0%								

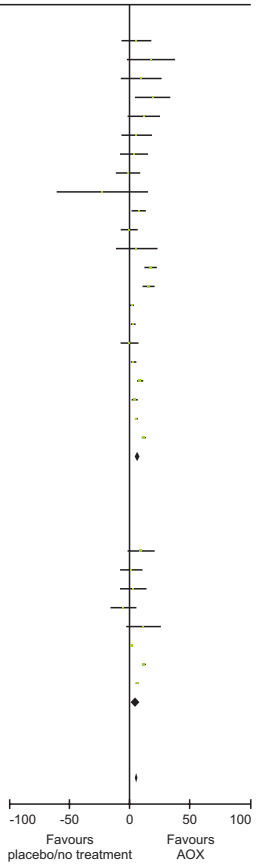

Fig. 8. Continued

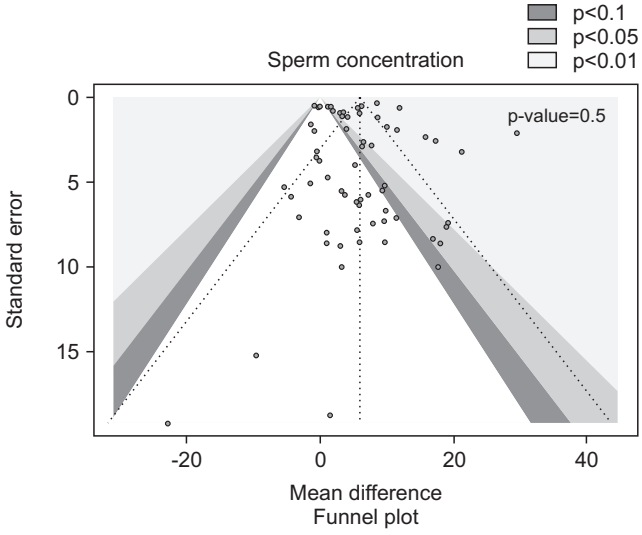

Fig. 9. Funnel plot of the sperm concentration in infertile patients treated with antioxidants compared to placebo or untreated infertile controls.

inter-study heterogeneity both overall (I^2=95%; χ^2 p<0.01), and in subgroup analysis (I^2=95%; χ^2 p<0.01) (Fig. 25).

9. Seminal TAC

Six studies, with a total of 172 AOX-treated patients and 144 placebo-treated or untreated controls, were included in the analysis of seminal TAC [51,53,55,59-61]. Seminal levels of TAC were significantly higher in patients compared to controls (weighted MD 1.87 mmol Trolox/L [95% CI: 1.26, 2.48; p<0.01]). The analysis demonstrated in a significant inter-study heterogeneity (I^2=97%; χ^2 p<0.01) (Fig. 26).

10. Seminal MDA

Seven studies, with a total of 224 patients and 179 controls, analyzed levels of seminal MDA [44,51,53,55,59-61]. Seminal levels of MDA were significantly lower in patients treated with AOX compared to placebo-treated or untreated controls (weighted MD -0.39 nmol/mL [95% CI: -0.65, -0.14; p<0.01]). The analysis showed a significant inter-study heterogeneity (I^2=96.2%; χ^2 p<0.01) (Fig. 27).

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Study or subgroup	AOX Mean	AOX SD	AOX Total	Placebo /no treatment Mean	Placebo /no treatment SD	Placebo /no treatment Total	Weight	Mean difference IV, random, 95% CI
1.5.1 Three months or less								
Azizollahi 2013 [32] (1)	42.6	39.9	29	24.6	12.4	8	0.0%	18.00 [1.13, 34.87]
Azizollahi 2013 [32] (2)	41.5	40.2	32	24.6	12.4	8	0.0%	16.90 [0.53, 33.27]
Balercia 2005 [33] (1)	39.3	18.1	15	31.4	12.9	5	0.9%	7.90 [-6.65, 22.45]
Balercia 2005 [33] (2)	41	17.3	15	31.4	12.9	5	0.9%	9.60 [-4.70, 23.90]
Balercia 2005 [33] (3)	36.9	19.7	14	31.4	12.9	5	0.9%	5.50 [-9.81, 20.81]
Barekat 2016 [29]	45.4	27.5	15	42.2	31.4	20	0.0%	3.20 [-16.37, 22.77]
Boonyarangkul 2015 [46] (1)	66.6	29.8	15	76.2	50.7	15	0.3%	-9.60 [-39.36, 20.16]
Ciftci 2009 [47]	21.85	11.08	60	22.32	22	60	2.1%	-0.47 [-6.70, 5.76]
Conquer 2000 [48] (1)	44.6	41.1	10	43.1	40.5	9	0.2%	1.50 [-35.23, 38.23]
Cyrus 2015 [31]	58.4	24.3	41	48.7	27.8	61	0.0%	9.70 [-0.50, 19.90]
da Silva 2013 [49]	26.08	24.52	23	20.04	16.35	26	1.2%	6.04 [-5.79, 17.87]
Dimitriadis 2010 [50]	15.4	6.7	26	16.3	7	22	2.5%	-0.90 [-4.80, 3.00]
Ener 2016 [30] (1)	49.5	27.9	22	30.6	23	23	0.0%	18.90 [3.92, 33.88]
Eslamian 2020 [51] (1)	22.66	2.27	45	21.51	2.78	45	2.9%	1.15 [0.10, 2.20]
Eslamian 2020 [51] (2)	21.21	2.66	45	21.51	2.78	45	2.9%	-0.30 [-1.42, 0.82]
Eslamian 2020 [51] (3)	21.45	2.25	45	21.51	2.78	45	2.9%	-0.06 [-1.10, 0.98]
Gopinath 2013 [37] (1)	26.4	8.9	46	14.9	5.9	18	2.6%	11.50 [7.75, 15.25]
Gopinath 2013 [37] (2)	24.9	7	43	14.9	5.9	18	2.6%	10.00 [6.56, 13.44]
Greco 2005 [52]	27.5	24.6	32	20.3	21.2	32	1.3%	7.20 [-4.05, 18.45]
Haghighian 2015 [53]	26.35	3.17	23	22.89	2.74	21	2.9%	3.46 [1.71, 5.21]
Lu 2018 [55]	28.8	3.6	27	22.9	3.3	27	0.0%	5.90 [4.06, 7.74]
Martinez-Soto 2010 [56]	29.1	4.5	21	30.5	4.9	15	2.7%	-1.40 [-4.54, 1.74]
Moslemi Mehni 2014 [57]	9.3	1.7	51	0.8	1.8	59	2.9%	8.50 [7.85, 9.15]
Morgante 2010 [58]	18.2	3.5	90	19.1	3	90	2.9%	-0.90 [-1.85, 0.05]
Nadjarzadeh 2011 [59]	16.09	12.9	23	16.21	12.7	24	1.9%	-0.12 [-7.44, 7.20]
Nouri 2019 [60]	18.16	10.32	17	11.89	6.37	19	2.2%	6.27 [0.59, 11.95]
Omu 2008 [61] (1)	48	20	11	45	18	8	0.7%	3.00 [-14.18, 20.18]
Omu 2008 [61] (2)	46	20	12	45	18	8	0.7%	1.00 [-15.84, 17.84]
Omu 2008 [61] (3)	46	18	14	45	18	8	0.8%	1.00 [-14.64, 16.64]
Peivandi 2010 [62]	46	3.6	15	16.5	7.3	15	2.5%	29.50 [25.38, 33.62]
Rolf 1999 [63] (1)	20.6	13.5	14	25	17.8	15	1.2%	-4.40 [-15.85, 7.05]
Rolf 1999 [63] (2)	28.3	16	14	31.5	21.8	15	1.0%	-3.20 [-17.05, 10.65]
Scott 1998 [41] (1)	48.7	8.8	16	27.5	10	18	2.1%	21.20 [14.88, 27.52]
Scott 1998 [41] (2)	34	6.3	30	27.5	10	18	2.3%	6.50 [1.36, 11.64]
Stanislavov 2009 [68]	67.3	3.4	25	63.4	8.7	25	2.6%	3.90 [0.24, 7.56]
Závaczki 2003 [45]	16.1	10.2	10	10.9	7.4	10	1.8%	5.20 [-2.61, 13.01]
Subtotal (95% CI)			**820**			**718**	**55.5%**	**5.02 [2.66, 7.39]**
Heterogeneity: Tau^2=28.21; Chi^2=652.53, df=29 (p<0.00001); I^2=96%								
Test for overall effect: Z=4.16 (p<0.0001)								
1.5.2 Six months								
Ardestani Zadeh 2019 [27]	41.26	24.52	30	35.83	23.21	30	0.0%	5.43 [-6.65, 17.51]
Azizollahi 2013 [32] (4)	47.6	40.4	29	29.9	18.7	8	0.0%	17.70 [-1.90, 37.30]
Azizollahi 2013 [32] (5)	39.6	30.5	32	29.9	18.7	8	0.0%	9.70 [-7.02, 2.6.42]
Azizollahi 2013 [32] (6)	49.1	16.8	26	29.9	18.7	8	0.0%	19.20 [4.72, 33.68]
Balercia 2005 [33] (4)	45.53	21.42	15	33.73	14.36	15	1.1%	11.80 [-1.25, 24.85]
Balercia 2005 [33] (5)	39.57	19.99	15	33.73	14.36	15	1.1%	5.84 [-6.62, 18.30]
Balercia 2005 [33] (6)	37.4	16.42	14	33.73	14.36	15	1.3%	3.67 [-7.59, 14.93]
Balercia 2009 [34]	44.9	19.3	30	46.4	19.8	30	1.5%	-1.50 [-11.39, 8.39]
Boonyarangkul 2015 [46] (2)	53.3	22.8	15	76.1	70.8	15	0.2%	-22.80 [-60.44, 14.84]
Busetto 2017 [35]	51.4	13.9	45	43.7	13.6	49	2.2%	7.70 [2.13, 13.27]
Busetto 2018 [36]	40.8	18.2	52	41.4	17.9	52	2.0%	-0.60 [-7.54, 6.34]
Ener 2016 [30] (2)	53.9	22	22	48	34.2	23	0.0%	5.90 [-10.83, 22.63]
Gopinath 2013 [37] (1)	33.2	12.4	46	15.9	7.7	18	2.3%	17.30 [12.25, 22.35]
Gopinath 2013 [37] (2)	31.7	9.7	43	15.9	7.7	18	2.4%	15.80 [11.21, 20.39]
Kızılay 2019 [28] (1)	13.12	1.89	18	11.19	1.95	9	0.0%	1.93 [0.39, 3.47]
Kızılay 2019 [28] (2)	18.19	3.3	39	15.22	3.11	17	0.0%	2.97 [1.16, 4.78]
Lenzi 2003 [54]	22.1	9.1	30	22.2	17	26	1.9%	-0.10 [-7.40, 7.20]
Safarinejad 2009 [66] (1)	26.8	5.3	105	23.5	5.8	35	2.8%	3.30 [1.13, 5.47]
Safarinejad 2009 [66] (2)	32.1	6.8	104	23.5	5.8	35	2.8%	8.60 [6.28, 10.92]
Safarinejad 2009 [66] (3)	27.6	6.4	105	23.5	5.8	35	2.8%	4.10 [1.82, 6.38]
Safarinejad 2009 [67]	26.4	4.4	98	20.8	4.3	96	2.9%	5.60 [4.38, 6.82]
Safarinejad 2012 [64]	28.7	4.6	101	16.8	4.4	102	2.9%	11.90 [10.66, 13.14]
Subtotal (95% CI)			**818**			**556**	**30.1%**	**7.00 [4.33, 9.68]**
Heterogeneity: Tau^2=17.60; Chi^2=123.88, df=14 (p<0.00001); I^2=89%								
Test for overall effect: Z=5.13 (p<0.00001)								
1.5.3 Nine months or more								
Balercia 2005 [33] (7)	39.4	13.9	15	30.1	9.3	5	1.3%	9.30 [-1.47, 20.07]
Balercia 2005 [33] (8)	31.2	8.6	15	30.1	9.3	5	1.6%	1.10 [-8.14, 10.34]
Balercia 2005 [33] (9)	33.3	13.6	14	30.1	9.3	5	1.3%	3.20 [-7.63, 14.03]
Balercia 2009 [34]	44.2	20.4	30	49.6	20.5	30	1.4%	-5.40 [-15.75, 4.95]
Ener 2016 [30] (3)	58.6	20.2	22	47.2	27.2	23	0.0%	11.40 [-2.56, 25.36]
Safarinejad 2009 [67]	22.8	3.8	98	21.2	3.8	96	2.9%	1.60 [0.53, 2.67]
Safarinejad 2011 [65]	28.7	4.4	101	16.8	4.4	102	2.9%	11.90 [10.69, 13.11]
Safarinejad 2012 [64]	22.4	4.2	112	16.2	3.7	113	2.9%	6.20 [5.17, 7.23]
Subtotal (95% CI)			**385**			**356**	**14.3%**	**4.77 [0.40, 9.14]**
Heterogeneity: Tau^2=24.03; Chi^2=162.70, df=6 (p<0.00001); I^2=96%								
Test for overall effect: Z=2.14 (p=0.03)								
Total (95% CI)			**2,023**			**1,630**	**100.0%**	**5.55 [3.87, 7.22]**
Heterogeneity: Tau^2=24.84; Chi^2=1,065.04, df=51 (p<0.00001); I^2=95%								
Test for overall effect: Z=6.48 (p<0.00001)								
Test for subgroup differences: Chi^2=1.40, df=2 (p=0.50); I^2=0%								

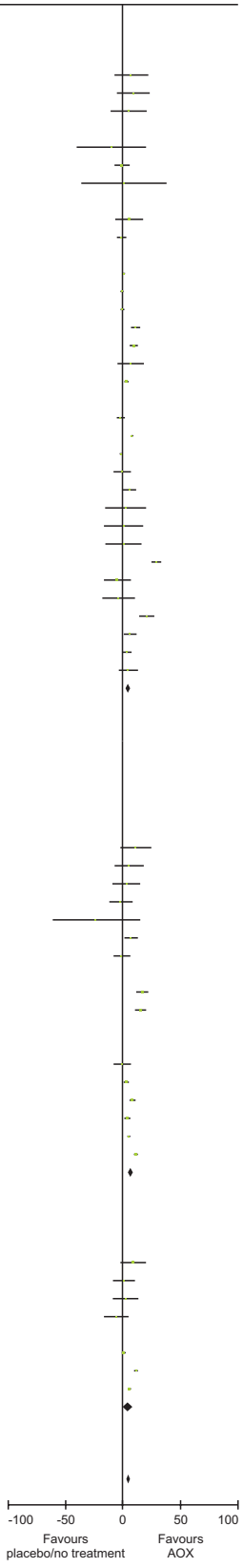

Fig. 10. Forest plot of the sperm concentration in infertile patients treated with antioxidants (AOXs) compared to placebo or untreated infertile controls, after the removal of studies including patients with varicocele undergoing varicocele repair.

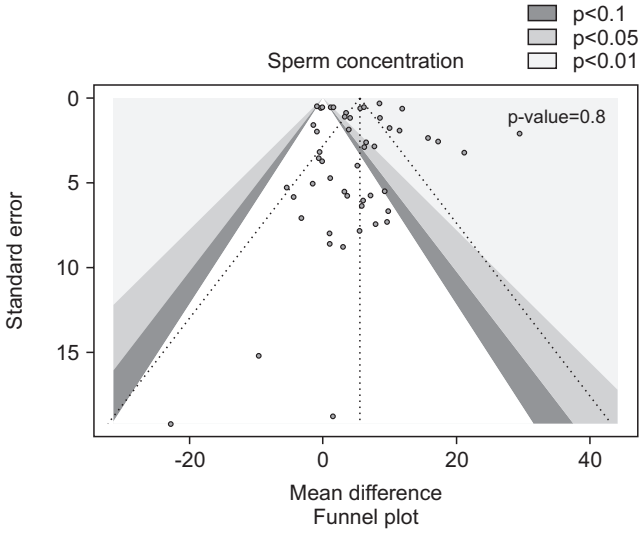

Fig. 11. Funnel plot of the sperm concentration in infertile patients treated with antioxidants compared to placebo or untreated infertile controls, after the removal of studies including patients with varicocele undergoing varicocele repair.

DISCUSSION

1. Impact of AOX therapy on spontaneous pregnancy outcomes

When treating infertile men with AOXs, the main desired outcomes include an improvement in clinical pregnancy and live-birth rates, and a reduction in miscarriage rates. According to the results of the current meta-analysis, the odds for a spontaneous clinical pregnancy are almost double (OR 1.97 [95% CI: 1.28, 3.04]; p<0.01) in infertile men after treatment with AOX compared to controls who have received placebo or no treatment. These results are in line with the latest 2019 Cochrane review and meta-analysis by Smits et al [14], that included 11 studies and reported an increased clinical pregnancy rate with various AOX treatments (OR 2.97, p<0.0001). The latter study only analyzed 105 events from the 11 RCTs as compared to 190 events from 16 RCTs of 1,355 patients in our study.

Another meta-analysis specifically evaluating combined LC/LAC supplementation in infertile men with idiopathic oligo-asthenoteratozoospermia found significantly higher clinical pregnancy rates in the treatment group compared to the control group (OR 3.76, p=0.002) [18]. A beneficial effect of AOX therapy on clinical pregnancy after spontaneous or assisted reproduction has also been concluded by several reviews [72-74]. Conversely, the recent MOXI trial did not report a favorable effect of AOX treatment in infertile men in terms of improving clinical pregnancy, showing similar clinical pregnancy rates in both the treatment and placebo groups (9% in both groups, p=0.98) [42]. This is likely due to evaluation of outcome after only 3 months of treatment and a small sample size. Furthermore, a meta-analysis on the effect of CoQ10 in infertile men did not report higher pregnancy rates in the treatment group despite improvement in semen parameters [75]. The recent Cochrane review also included 3 studies that found no difference in miscarriage rates between AOX and placebo or untreated groups [14]. The same meta-analysis reported significantly higher live-birth rates among the treatment group (OR 1.79, p=0.005) [14]. The MOXI trial, however, did not demonstrate any benefit of AOX in improving live-birth rates [42]. In our meta-analysis, no impact on miscarriage and live-birth rates following AOX therapy in infertile men was observed, despite higher pregnancy rates. This is likely due to the small number of studies and events.

In light of these mixed findings, there is a need for further RCTs specifically assessing these outcomes with adequate follow up as these events may not occur soon after the start of therapy. In addition, as suggested by Steiner et al [42], the lack of effect on these parameters could result from the lack of selection of patients who should receive AOXs as well as confounding factors affecting pregnancy rates. In fact, it may be hypothesized that the main beneficiaries of AOX therapy are those with high seminal OS. Future RCTs should be designed to include mainly patients with elevated OS markers.

2. Impact of AOX therapy on basic semen parameters

Semen analysis is the cornerstone of the male infertility work up and often the first laboratory test ordered. The ultimate measure of success for treatment of infertility is a clinical pregnancy or live-birth but these outcomes may need up to 10 months to manifest. In the meantime, semen parameters can be monitored to determine if the prescribed treatment is having a positive effect in improving the couple's chance to conceive. In our meta-analysis, treatment with AOX (either single or combined) significantly improved sperm concentration, progressive and total motility, and sperm morphology. Similarly, a previously published systematic review reported that vitamin E, vitamin C, NAC, carnitines, CoQ10, lycopene, selenium, and zinc

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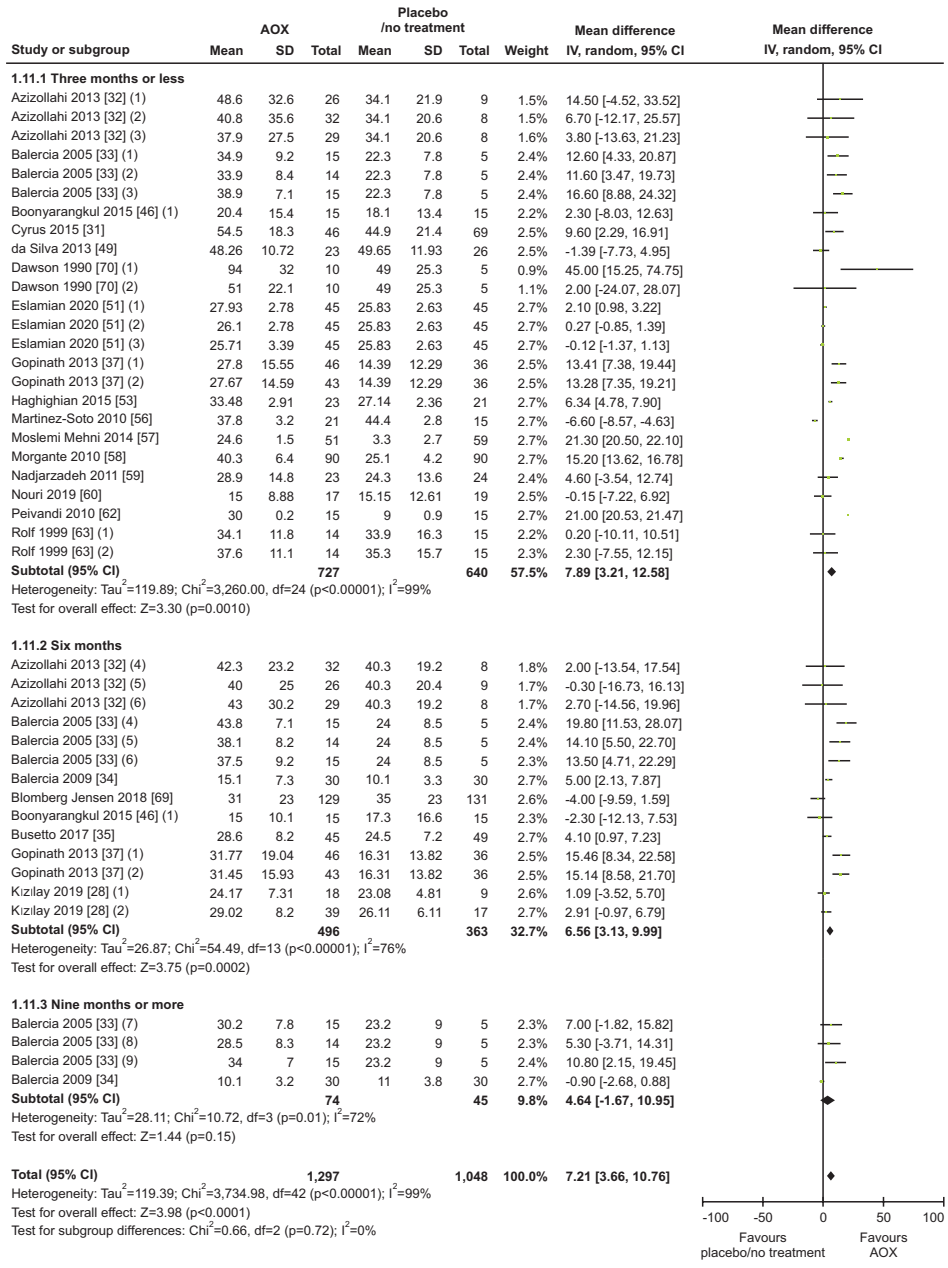

Fig. 12. Forest plot of the sperm progressive motility in infertile patients treated with antioxidants (AOXs) compared to placebo or untreated infertile controls.

were associated with improved sperm concentration, motility, and morphology [73]. The latest Cochrane database systematic review stated that there was high heterogeneity in published studies and reliable conclusions could not be drawn regarding the effect of AOX on sperm concentration, total motility, and progressive motility [14]. However, the authors suggested that carnitines and combined AOX led to improvement in sperm motility and polyunsaturated fats while zinc improved sperm concentration when compared to placebo or no treatment [14]. When taking the data from previous studies and from our new analysis together, there appears to be benefit of AOX on semen analysis parameters, regardless of the supplement used. In contrast to previous studies, our meta-analysis focused on the use of AOX in general, and not on specific molecules or combinations, for which the studies are very heterogenous.

3. Impact of AOX therapy on SDF

Given the well-established role of OS in the pathogenesis of SDF [76], we aimed to identify articles that have investigated the effect of AOX therapy on SDF. The very limited number of controlled studies on

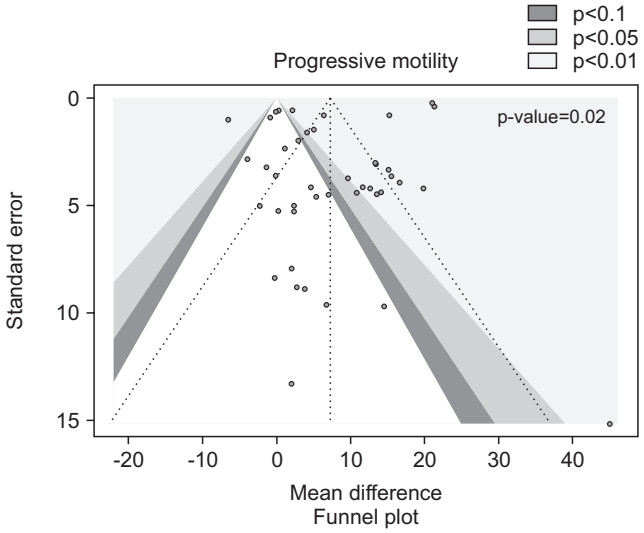

Fig. 13. Funnel plot of the sperm progressive motility in infertile patients treated with antioxidants compared to placebo or untreated infertile controls.

AOXs and SDF, likely precluded our ability to further evaluate the effect of AOX on SDF. However, several prospective studies reported a significant reduction in SDF from baseline after AOX therapy in infertile men, regardless of the assay used or the type of AOX. For example, supplementation of NAC for three months resulted in significant reductions in DNA fragmentation as measured by TUNEL assay (from 19.3% to 15.1%, p=0.01) [77]. Supplying a combination AOX of vitamin C, vitamin E, and CoQ10 resulted in significant improvement in DNA fragmentation index (DFI) as measured by Sperm Chromatin Structure Assay (SCSA) [78]. Significant reductions in SDF percentage as measured by Sperm Chromatin Dispersion (SCD) were also reported after three months of CoQ10 treatment [79]. Two trials investigated AOX therapy after varicocelectomy in men with clinical varicocele; both reported no additional benefit of AOX in reduction of SDF after varicocelectomy in these men compared to varicocelectomy

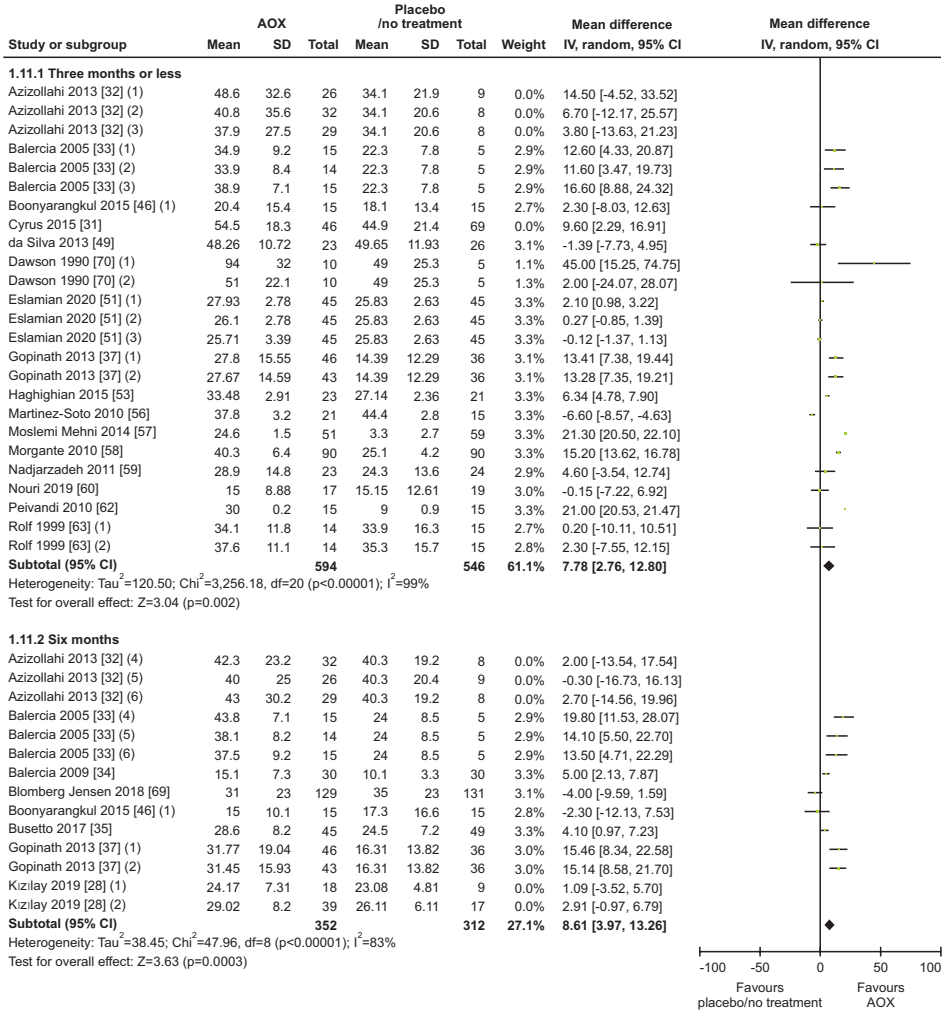

Fig. 14. Forest plot of the progressive sperm motility in infertile patients treated with antioxidants (AOXs) compared to placebo or untreated infertile controls, after the removal of studies including patients with varicocele undergoing varicocele repair.

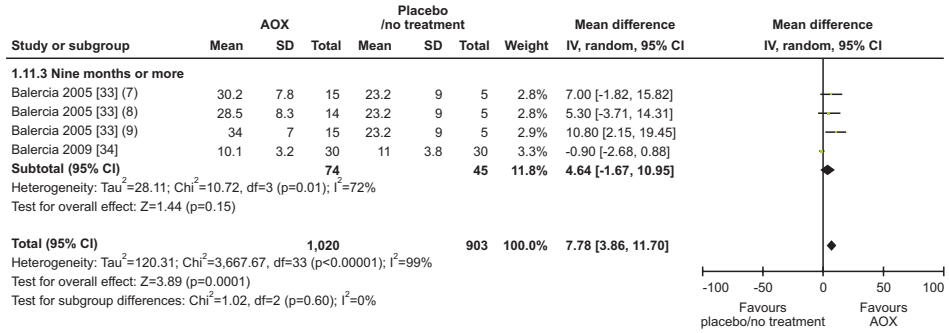

Fig. 14. Continued.

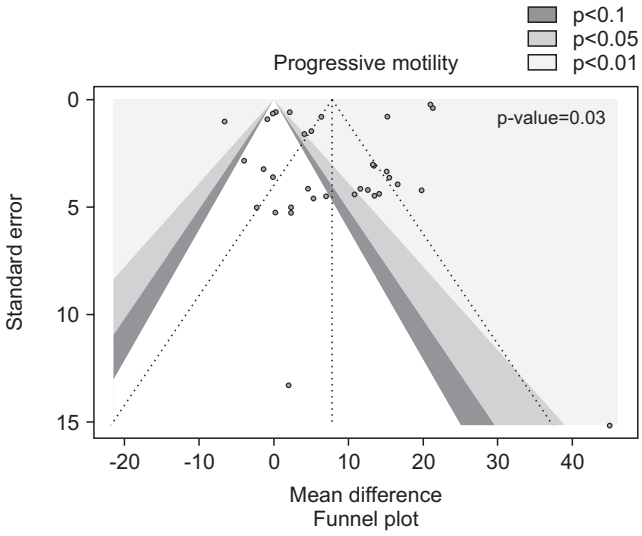

Fig. 15. Funnel plot of the progressive sperm motility in infertile patients treated with antioxidants compared to placebo or untreated infertile controls, after the removal of studies including patients with varicocele undergoing varicocele repair.

alone, signifying the importance of addressing the underlying condition when possible, rather than empiric AOX supplementation [29,80]. One trial on astaxanthin supplementation reported no reduction in SDF compared to placebo [81], while another on folic acid reported SDF improvement only in carriers of *MTHFR* gene 677 thymidine/thymidine polymorphism [82]. Conversely, a recent multicenter RCT administering folic acid and zinc or placebo to 2,370 infertile men for six months reported a significantly lower SDF in patients compared to controls [83]. Therefore, it is difficult to reach a firm conclusion as to the impact of AOX on levels of SDF due to many factors including the small number of available studies, the variable AOXs regimens, the different assays used for SDF, and the different conditions associated with SDF. Additional well-designed studies are warranted.

4. Impact of AOX therapy on seminal OS indices

The most pragmatic use of AOX in male infertility is in cases of elevated seminal OS for men classified as having MOSI. Normally there is a homeostasis between ROS and AOX. If the scale tips towards ROS, then dietary supplementation with AOX may help restore this balance and improve seminal quality. TAC is a measure of total AOX present in the seminal plasma and provides a measure of reductive potential [84]. Studies have demonstrated that infertile men have lower TAC when compared to fertile men, and semen parameters such as concentration, motility, and morphology have been positively correlated with TAC [85]. Our analysis identified that seminal TAC improved after treatment with AOX. There was also a significant decrease in seminal MDA, an indicator of lipid peroxidation, after treatment with AOX. However, significant inter-study heterogeneity was found. Several earlier studies have demonstrated improvement in OS and a decrease in MDA after treatment with vitamin C, vitamin E, beta-carotene, zinc, selenium, and NAC used either alone or in combination [44,47,61,86,87]. Our meta-analysis is consistent with the results of these previous studies and, as far as we know, this is the first meta-analysis assessing the impact of AOX on TAC and MDA. However, while AOXs seem to improve seminal OS indices, clinicians need also to be aware that over treatment with AOXs can lead to toxicity and reductive stress [88]. Thus, identification and selection of patients with high risk for MOSI could be useful to maximize the benefits of AOXs on sperm quality and prevent reductive stress toxicity. When the assessment of seminal OS becomes more standardized, this test could be important to identify those who could benefit from AOX.

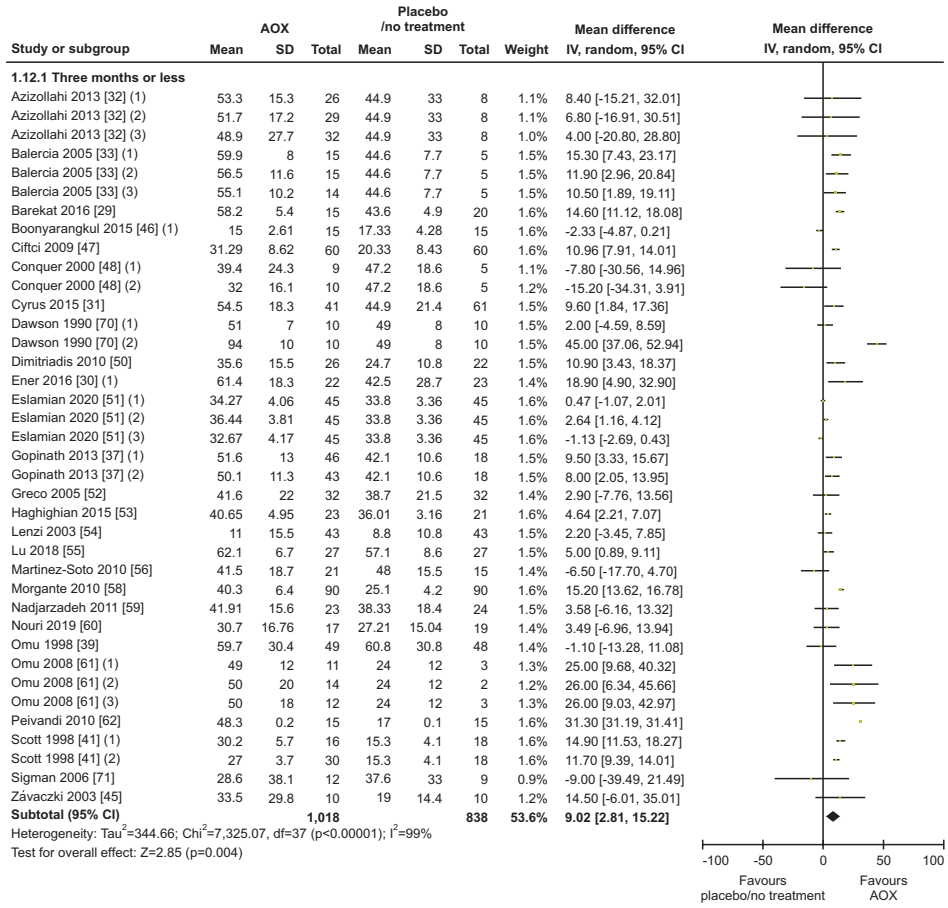

Fig. 16. Forest plot of the total sperm motility in infertile patients treated with antioxidants (AOXs) compared to placebo or untreated infertile controls.

5. What could this study change?

Currently, AOX therapy is being widely used in male infertility management, even though there is only limited evidence and no practical guidelines on the duration or even the type of AOX to be used [73]. Our meta-analysis has provided encouraging evidence, demonstrating the positive impact of AOX therapy on clinical pregnancy rate, seminal parameters, and OS levels in men for whom a careful diagnostic evaluation has excluded major comorbidities, genetic, anatomical, inflammatory, traumatic or testicular cause of male infertility, or associated female infertility (Supplement Table 3). Some of the included studies were performed in a population of men with varicocele who underwent varicocele repair and were subsequently given AOX or placebo or untreated. The exclusion of these varicocele studies did not change the results, suggesting that the effect of AOX on the analyzed outcomes is independent of varicocele repair.

Compared to the last Cochrane review [14], the present study increased the number of RCTs, allowing the study of the largest population analyzed so far (Table 3). This allowed us to confirm the Cochrane's study findings and upgrade the level of evidence of many of the investigated outcomes [14,75,89-91] (Table 3). Moreover, to the best of our knowledge, this is the first study to offer a meta-analytic investigation of seminal levels of TAC and MDA in patients with male infertility after AOX administration and to confirm a positive effect on both of these outcomes.

6. Comparison with other studies

The MOXI trial has investigated the impact of AOX therapy on male infertility [42]. The authors conclude that AOX therapy neither improves semen parameters and DNA integrity, nor improves *in vivo* pregnancy or live-birth rates among men with male factor infertility. However, its study design had some limitations.

First, only 144 of the required 790 couples were recruited to achieve 80% power for the primary outcome (live-birth rates). Secondly, at baseline, the placebo group had a higher proportion of men with secondary infertility and the AOX group had a lower percentage of morphologically normal sperm. Third, the partici-

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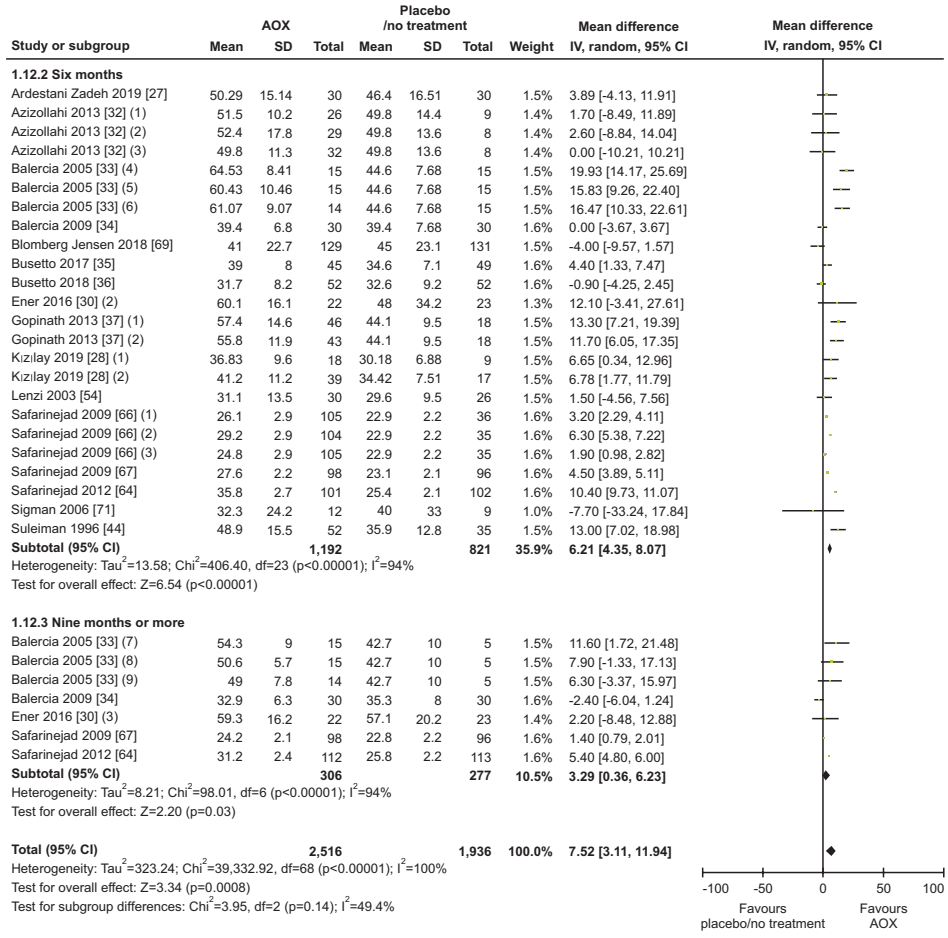

Fig. 16. Continued.

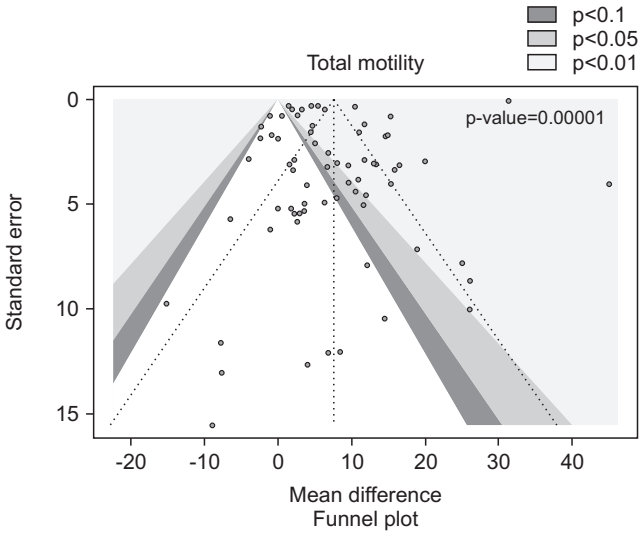

Fig. 17. Funnel plot of the sperm total motility in infertile patients treated with antioxidants compared to placebo or untreated infertile controls.

pants were assigned to either AOX or placebo based on semen parameters and female partner's age, while other important issues related to the underlying etiological factors for diagnoses of male infertility were ignored. This may be an additional source of selection bias since some genital abnormalities such as varicocele may impact the outcome of AOX therapy of infertile men [36,92].

Fourth, the MOXI trial used a combined AOX formula containing vitamin C (500 mg), vitamin E (400 mg), selenium (0.20 mg), LC (1,000 mg), zinc (20 mg), folic acid (1,000 mg), lycopene (10 mg), and vitamin D (2,000 IU). The authors state that they selected this formulation based on the finding of a previous Cochrane systematic review reporting that each individual component has a positive impact on sperm structure or function and/or pregnancy rates after assisted reproductive technique (ART). However, it remains unclear whether the formulation chosen in the MOXI trial is appropriate or not.

Fifth, the authors of the MOXI trial state that AOX therapy was given for at least 3 months and up to 6 months. The US Food and Drug Administration (FDA) recommends a minimum duration of 26 weeks for as-

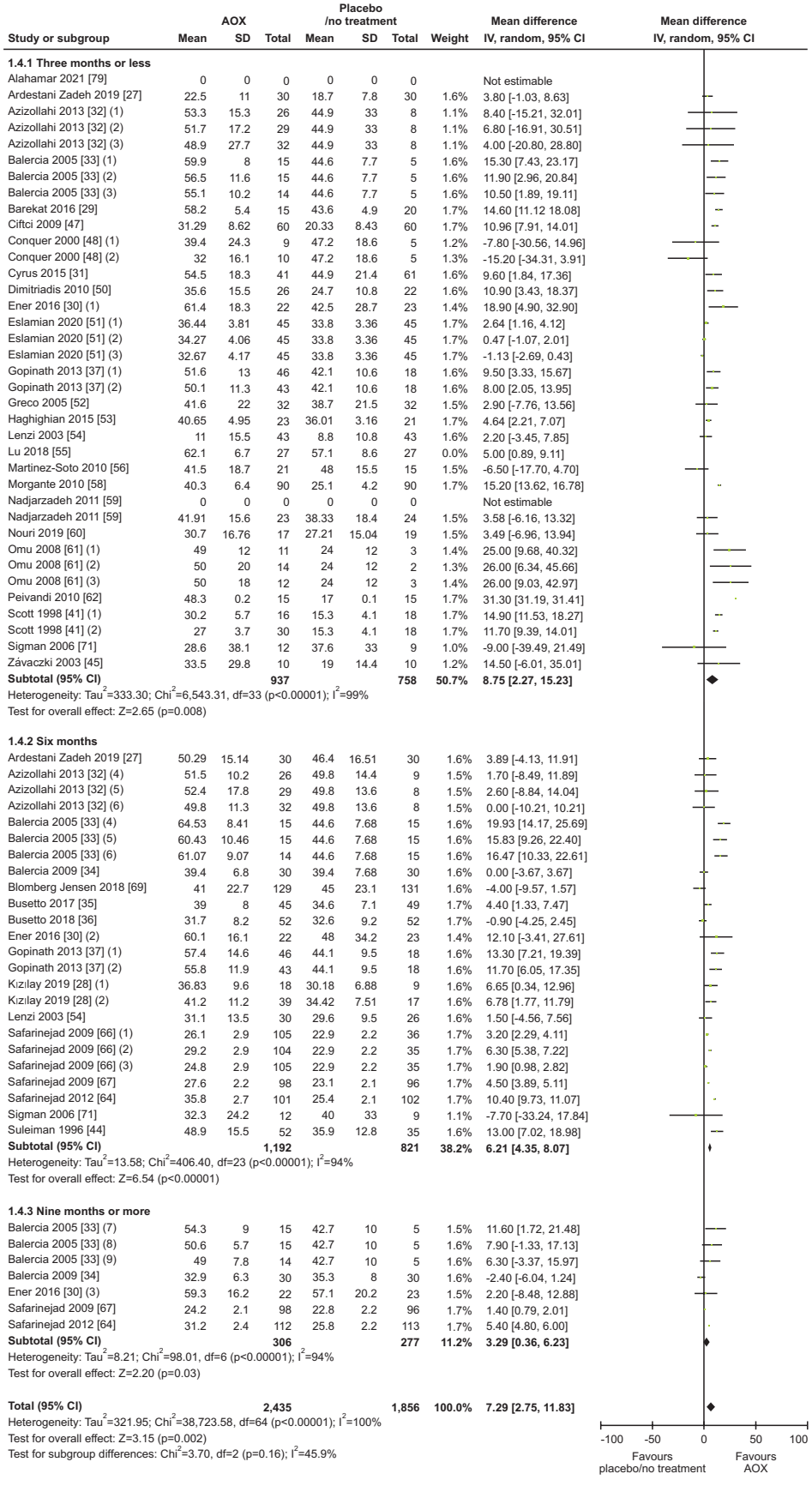

Fig. 18. Forest plot of the sperm total motility in infertile patients treated with antioxidants (AOXs) compared to placebo or untreated infertile controls, after the removal of studies including patients with varicocele undergoing varicocele repair.

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sessing the impact of a therapeutic agent used for the treatment of male factor infertility. Using different durations of AOX therapy may add an important confounder that may impact the interpretation of the results. Lastly, ovarian stimulation with clomiphene

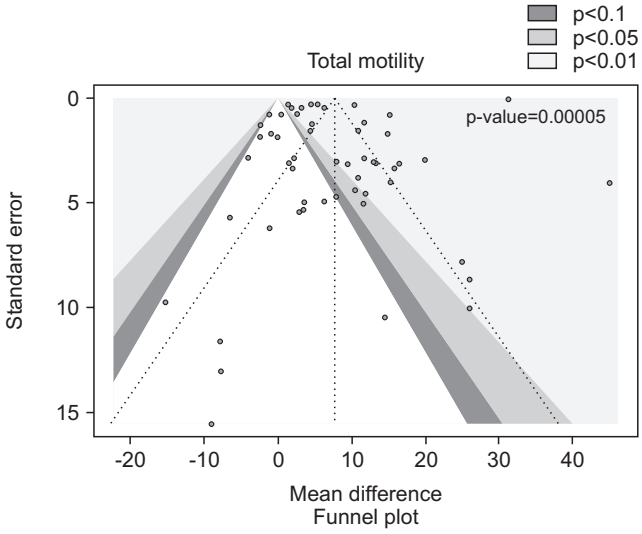

Fig. 19. Funnel plot of the total sperm motility in infertile patients treated with antioxidants compared to placebo or untreated infertile controls, after the removal of studies including patients with varicocele undergoing varicocele repair.

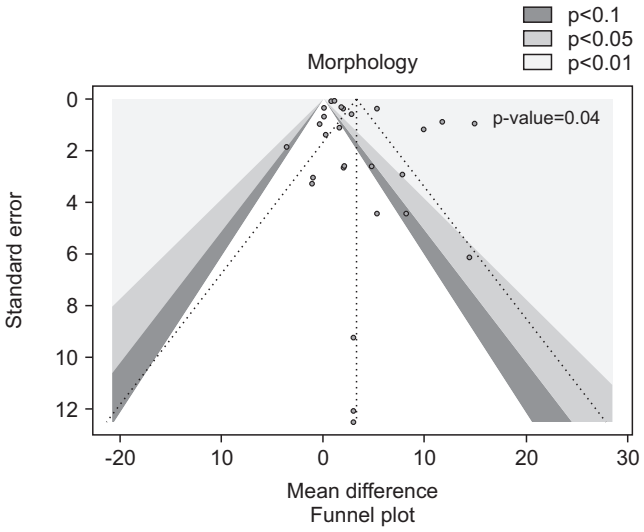

Fig. 21. Funnel plot of the sperm morphology in infertile patients treated with antioxidants compared to placebo or untreated infertile controls.

Study or subgroup	AOX Mean	AOX SD	AOX Total	Placebo /no treatment Mean	Placebo /no treatment SD	Placebo /no treatment Total	Weight	Mean difference IV, random, 95% CI
1.13.1 Three months or less								
Azizollahi 2013 [32] (1)	49.9	2.2	29	38.1	2.5	10	5.5%	11.80 [10.06, 13.54]
Azizollahi 2013 [32] (2)	53	2.8	26	38.1	2.5	10	5.3%	14.90 [13.01, 16.79]
Azizollahi 2013 [32] (3)	48	2.3	32	38.1	2.5	5	4.7%	9.90 [7.57, 12.23]
Barekat 2016 [29]	2.71	0.3	15	1.9	0.2	20	7.0%	0.81 [0.63, 0.99]
Cyrus 2015 [31]	75.3	13.1	41	67.5	16.4	61	1.8%	7.80 [2.05, 13.55]
da Silva 2013 [49]	23.91	3.68	23	24.23	3.06	26	5.3%	-0.32 [-2.23, 1.59]
Dimitriadis 2010 [50]	25.8	9.8	26	23.7	8.1	22	2.1%	2.10 [-2.96, 7.16]
Eslamian 2020 [51]	14.44	3.16	45	14.36	3.4	45	6.0%	0.08 [-1.28, 1.44]
Greco 2005 [52]	8	7.1	32	11.6	7.8	32	3.2%	-3.60 [-7.25, 0.05]
Haghighian 2015 [53]	15.39	3.6	23	13.8	3.73	21	4.9%	1.59 [-0.58, 3.76]
Lu 2018 [55]	5.3	0.3	27	4.2	0.3	27	7.0%	1.10 [0.94, 1.26]
Nadjarzadeh 2011 [59]	6.52	5.1	23	6.29	4.3	24	4.2%	0.23 [-2.47, 2.93]
Nouri 2019 [60]	1.88	0.99	17	1.78	1.08	19	6.7%	0.10 [-0.58, 0.78]
Omu 2008 [61] (1)	72	14	11	69	16	2	0.1%	3.00 [-20.67, 26.67]
Omu 2008 [61] (2)	72	16	12	69	16	4	0.2%	3.00 [-15.11, 21.11]
Omu 2008 [61] (3)	72	20	14	69	16	2	0.1%	3.00 [-21.52, 27.52]
Rolf 1999 [63] (1)	12.2	5.4	14	13.3	11.4	15	1.5%	-1.10 [-7.53, 5.33]
Rolf 1999 [63] (2)	13.4	7.8	14	14.4	8.6	15	1.7%	-1.00 [-6.97, 4.97]
Závaczki 2003 [45]	57.2	12.5	10	42.8	14.8	10	0.5%	14.40 [2.39, 26.41]
Subtotal (95% CI)			**434**			**370**	**67.7%**	**3.30 [2.26, 4.34]**
Heterogeneity: Tau^2=2.66; Chi^2=445.41, df=18 (p<0.00001); I^2=96%								
Test for overall effect: Z=6.23 (p<0.00001)								
1.13.2 Six months								
Azizollahi 2013 [32] (4)	56.6	2.2	29	48.4	9.9	5	0.9%	8.20 [-0.51, 16.91]
Azizollahi 2013 [32] (5)	53.7	1.7	26	48.4	9.9	5	0.9%	5.30 [-3.40, 14.00]
Azizollahi 2013 [32] (6)	53.2	2.7	32	48.4	9.9	15	2.1%	4.80 [-0.30, 9.90]
Busetto 2017 [35]	17.7	15.2	44	15.7	9.4	49	2.0%	2.00 [-3.21, 7.21]
Safarinejad 2009 [66] (1)	9.2	2.9	105	7.2	2.6	106	6.7%	2.00 [1.26, 2.74]
Safarinejad 2009 [67]	9.6	2.4	98	7.8	2.1	96	6.8%	1.80 [1.17, 2.43]
Safarinejad 2012 [64]	17.6	4.4	101	14.8	4.1	102	6.2%	2.80 [1.63, 3.97]
Subtotal (95% CI)			**435**			**378**	**25.6%**	**2.06 [1.62, 2.50]**
Heterogeneity: Tau^2=0.00; Chi^2=5.76, df=6 (p=0.45); I^2=0%								
Test for overall effect: Z=9.15 (p<0.00001)								
1.13.3 Nine months or more								
Safarinejad 2011 [65]	12.8	2.6	106	7.5	2.7	105	6.7%	5.30 [4.58, 6.02]
Subtotal (95% CI)			**106**			**105**	**6.7%**	**5.30 [4.58, 6.02]**
Heterogeneity: Not applicable								
Test for overall effect: Z=14.52 (p<0.00001)								
Total (95% CI)			**975**			**853**	**100.0%**	**3.28 [2.40, 4.17]**
Heterogeneity: Tau^2=2.88; Chi^2=598.39, df=26 (p<0.00001); I^2=96%								
Test for overall effect: Z=7.31 (p<0.00001)								
Test for subgroup differences: Chi^2=57.34, df=2 (p<0.00001); I^2=96.5%								

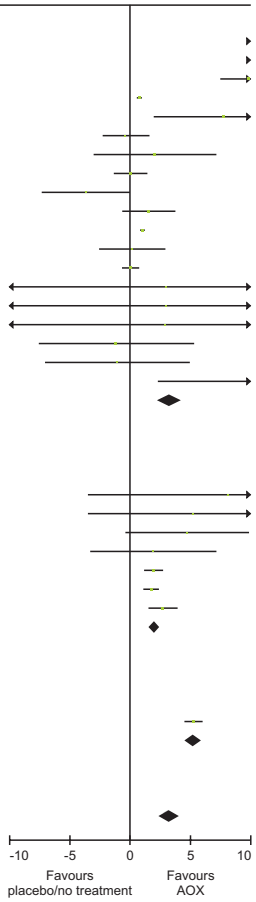

Fig. 20. Forest plot of the sperm morphology in infertile patients treated with antioxidants (AOXs) compared to placebo or untreated infertile controls.

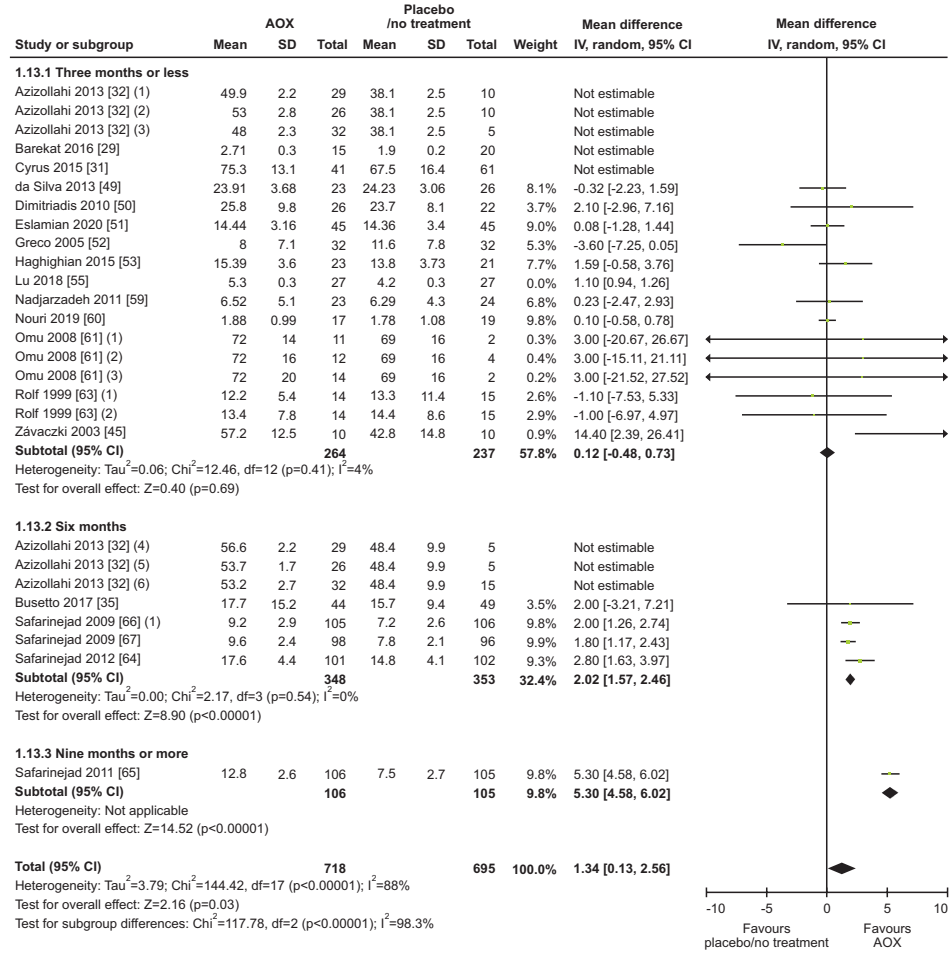

Fig. 22. Forest plot of the sperm morphology in infertile patients treated with antioxidants (AOXs) compared to placebo or untreated infertile controls, after the removal of studies including patients with varicocele undergoing varicocele repair.

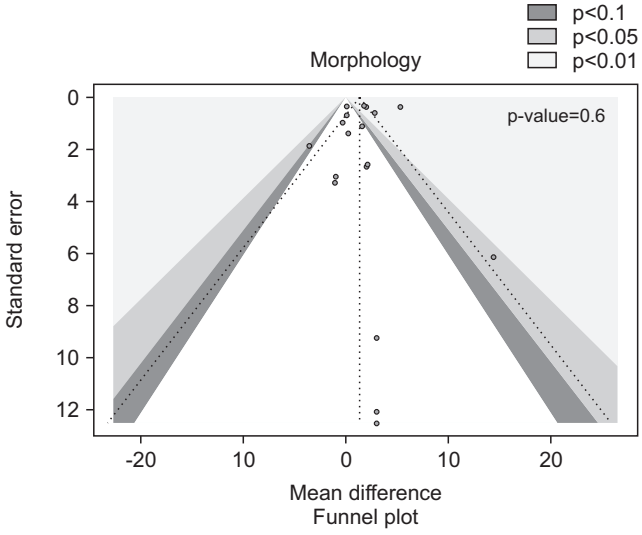

Fig. 23. Funnel plot of the sperm morphology in infertile patients treated with antioxidants compared to placebo or untreated infertile controls, after the removal of studies including patients with varicocele undergoing varicocele repair.

citrate followed by intrauterine insemination (IUI) was used for couples who had not conceived after a trial with AOX *vs.* placebo. Thus, there is a concern of having heterogeneous responses due to combining AOX supplementation with IUI.

Several meta-analyses have evaluated the potential benefit of AOX for treatment of male infertility. Despite being considered the gold standard, the last Cochrane meta-analysis [14] has some objective limitations, that the present study has overcome (Table 3). Compared to the last Cochrane review, our study increased the number of RCTs, and investigated the largest population analyzed so far on this topic. This allowed us to score the evidence on clinical pregnancy as moderate-quality and, accordingly, the analysis of this outcome resulted in minimal inter-study heterogeneity (Table 3). Regarding live-birth rate, the population analyzed in the present study is smaller than that assessed in the Cochrane meta-analysis, since we focused only on spontaneous pregnancies. Also, some of the outcomes of the present study, such as sperm morphology, seminal levels of TAC and of MDA, were not analyzed in the study by Smits et al [14]. In addition, concerning

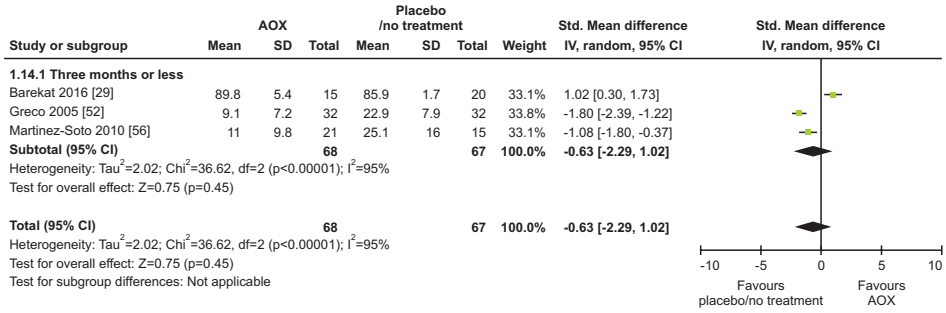

Fig. 24. Forest plot of the sperm DNA fragmentation in infertile patients treated with antioxidants (AOXs) compared to placebo or untreated infertile controls.

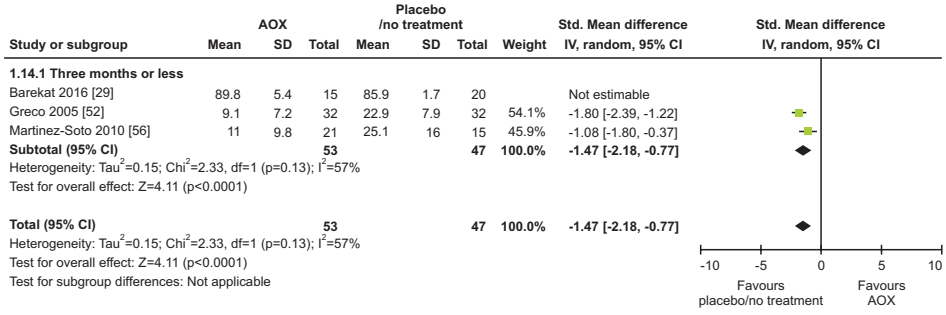

Fig. 25. Forest plot of the sperm DNA fragmentation in infertile patients treated with antioxidants (AOXs) compared to placebo or untreated infertile controls, after the removal of studies including patients with varicocele undergoing varicocele repair.

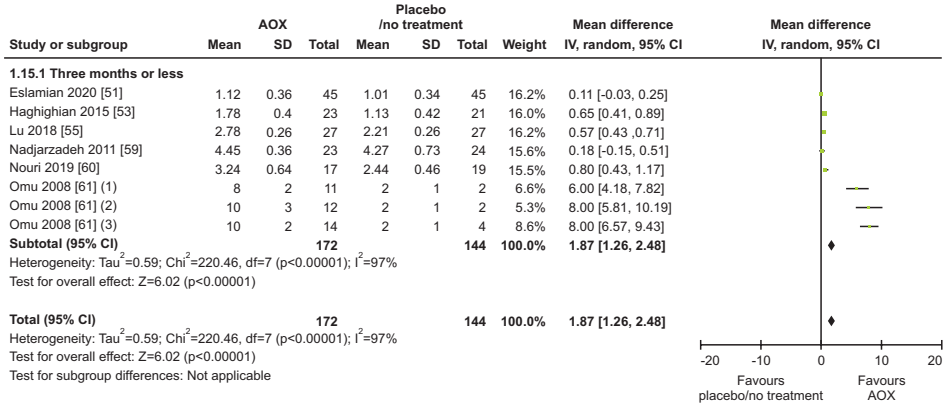

Fig. 26. Forest plot of the total antioxidant (AOX) capacity in infertile patients treated with antioxidants compared to placebo or untreated infertile controls.

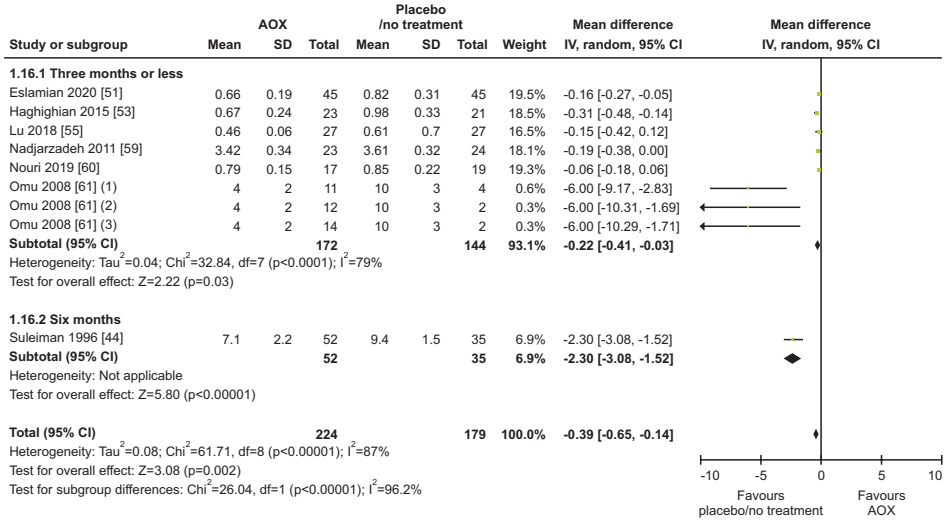

Fig. 27. Forest plot of the malondialdehyde in infertile patients treated with antioxidants (AOXs) compared to placebo or untreated infertile controls.

Table 3. Comparison of the findings of the present study with the last Cochrane meta-analysis [14]

Outcome	Present meta-analysis		Cochrane meta-analysis [14]	
	Summary of the results	Number of participants (n studies)	Summary of the results	Number of participants (n studies)
Spontaneous pregnancy rate[a]	OR 1.97 (95% CI: 1.28, 3.04); n=190 events; p<0.01; I^2=20%; χ^2 p=0.20 (Fig. 2)	1,355 (16 RCTs)	OR 2.97 (95% CI: 1.20, 2.67); n=105 events; p<0.05; I^2=0%	786 (11 RCTs)
Live-birth rate[a]	OR 1.21 (95% CI: 0.53, 2.76); n=65 events; p=0.64 (Fig. 6)	388 (4 RCTs)	OR 1.79 (95% CI: 1.20, 2.67); n=124 events; p<0.05; I^2=40%	750 (7 RCTs)
Miscarriage rate[a]	OR 1.01 (95% CI: 0.34, 3.00); n=13 events; p=0.98 (Fig. 7)	459 (4 RCTs)	OR 1.74 (95% 0.40, 7.60); n=8 events; p=0.46	247 (3 RCTs)
Sperm concentration	MD 5.93 mil/mL (95% CI: 4.43, 7.43); p<0.01; I^2=94%; χ^2 p<0.01 (Fig. 8)	4,310 (36 RCTs)	Overall effect not assessed[b]	3,456 (26 RCTs)
Progressive sperm motility	MD 7.21% (95% CI: 3.66, 10.76); p<0.01; I^2=99%; χ^2 p<0.01 (Fig. 12)	2,345 (20 RCTs)	Overall effect not assessed[b]	1,523 (15 RCTs)
Total sperm motility	MD 7.52% (95% CI: 3.11, 11.94); p<0.01; I^2=100%; χ^2 p<0.01 (Fig. 16)	4,452 (36 RCTs)	Overall effect not assessed[b]	3,456 (25 RCTs)
Sperm morphology	MD 3.28% (95% CI: 2.40, 4.17); p<0.01; I^2=96%; χ^2 p<0.01 (Fig. 20)	1,828 (18 RCTs)	Not assessed	Not assessed
Sperm DNA fragmentation	SMD -0.63 (95% CI: -2.29, 1.02); p=0.45 (Fig. 24)	135 (3 RCTs)	MD -5.0 (95% CI: -12.61, 2.61); p=0.20	254 (6 RCTs)
Seminal TAC levels	MD 1.87 (95% CI: 1.26, 2.48; p<0.01; I^2=97%; χ^2 p<0.01 (Fig. 26)	316 (6 RCTs)	Not assessed	Not assessed
Seminal MDA levels	MD -0.39 (95% CI: -0.65, -0.14; p<0.01; I^2=87%; χ^2 p<0.01 (Fig. 27)	403 (7 RCTs)	Not assessed	Not assessed

CI: confidence interval, MD: mean difference, MDA: malondialdehyde acid, OR: odds ratio, RCT: randomized controlled trial, SMD: standardized mean difference, TAC: total antioxidant capacity.
[a]Only spontaneous events were considered in the present meta-analysis.
[b]Subgroup analysis based on treatment duration was performed.

the conventional sperm parameters (sperm concentration, progressive and total motility), the Cochrane review provided the results of the sub-group analysis only (*e.g.*, effect of a single AOX, or of a specific time of assessment), without analyzing the overall effect (hence, independently from the time and the specific AOX used). The detailed analysis of the differences in the sample size, in the number of RCTs included for each outcome, and in the results between the present study and the Cochrane meta-analysis is detailed in Table 3.

Pitfalls of the Cochrane and several other meta-analyses are highlighted in the strengths and weakness analysis provided in Table 4. If we consider the previous meta-analyses, the number of analyzed studies has generally been limited most of the time [75,90], thus providing very-low or low-quality evidence. Furthermore, some meta-analyses evaluated the impact of only one or two AOX, failing to provide a comprehensive picture on their use in male infertility [75,90] (Table 4). Comprehensively, the present study analyzed the largest cohort so far, which allowed to upgrade the level of the evidence.

7. Limitations of the study

The present meta-analysis demonstrates the positive impact of AOX on spontaneous pregnancy, seminal OS indices and basic sperm parameters. However, the limited number of controlled studies investigating the effect of AOXs on live-birth rate and SDF prevented us from reaching a firm conclusion about these outcomes. This study is also not evaluating the effects of AOXs based on subgroup analysis. The heterogeneity in the

Table 4. Summary of the strengths and weakness of the meta-analyses on AOX published in the last ten years

Society	Year	Strengths	Weakness
Present study	2022	Evidence based on the highest number of RCTs and on the largest population analyzed so far Highest level of evidence provided so far Absence of inter-study heterogeneity for clinical pregnancy Evidence on seminal indices of OS (TAC and MDA) Sub-analysis performed after the exclusion of patients with varicocele Evidence on RCTs only Score of the QoE using the GRADE system	Limited number of controlled trials on live-birth rate, miscarriage and SDF Inter-study heterogeneity for secondary outcomes No subgroup analysis according to the type of AOX, and heterogeneity in the formulation of AOX analyzed
[14]	2019	Subgroup analysis according to the type of AOX Evidence on RCTs only Score of the QoE using the GRADE system High number of studies Absence of inter-study heterogeneity for clinical pregnancy	Limited number of controlled trials on live-birth rate, miscarriage, and SDF Inter-study heterogeneity for secondary outcomes Only the Cochrane Risk of Bias of RCTs is used for QoE No details on the training of researchers are provided Patients with varicocele are included in the analysis Evidence on seminal indices of OS (*e.g.*, TAC and MDA) is not provided Low, very-low QoE
[89]	2021	Evidence on RCTs only High selected cohort (idiopathic asthenozoospermia) Absence of heterogeneity in the formulation of AOX analysed	The idiopathic etiology is not clearly mentioned in the inclusion criteria of the included studies Only two AOXs are analyzed (N-acetyl-cysteine, L-carnitine/L-acetyl-carnitine) Limited number of studies (n=7) Only the Cochrane Risk of Bias of RCTs is used for QoE No details on the training of researchers are provided
[90]	2021	Evidence on RCTs only High selected cohort (idiopathic infertility) Absence of heterogeneity in the formulation of AOX analyzed	Limited number of studies (n=3) Only the Cochrane Risk of Bias of RCTs is used for QoE No details on the training of researchers are provided Inter-study heterogeneity Only one antioxidant is analyzed (N-Acetyl-cysteine)
[91]	2014	Evidence on RCTs only Score of the QoE using the GRADE system	Low sample size for each assessed outcome Only the Cochrane Risk of Bias of RCTs is used for QoE No details on the training of researchers are provided Inter-study heterogeneity Low, very-low QoE
[75]	2013	Evidence on RCTs only Absence of heterogeneity in the formulation of AOX analysed	Limited number of studies (n=3) Only the Cochrane Risk of Bias of RCTs is used for QoE No details on the training of researchers are provided Only one AOX is analyzed (Co-enzyme Q10) Inter-study heterogeneity

AOX: antioxidant, GRADE: Grading of Recommendations Assessment, Development and Evaluation, MDA: malondialdehyde acid, OS: oxidative stress, RCT: randomized controlled trial, QoE: quality of evidence, TAC: total antioxidant capacity.

formulations studied in the literature also resulted in the presence of outliers encountered during our analysis, but we were able to adjust for this in our statistical approach. This foreseeable and expected outcome represents the limitation of the present study, based on the scarcity of well-designed studies to be included in such a meta-analysis. Beside these limitations, it is important to underline that until now there are no robust studies that have investigated the relationship between micronutrient patterns and infertility and have highlighted the role of AOXs in those patients with specific nutrient deficiency.

Despite this, our study was still capable of generating statistical results that are consistent with the scientific narrative. However, we need more well-designed large RCTs to reach more definitive conclusions. These points are highlighted in the Strengths, Weaknesses, Opportunities, and Threats (SWOT) analysis, which is shown in Fig. 28.

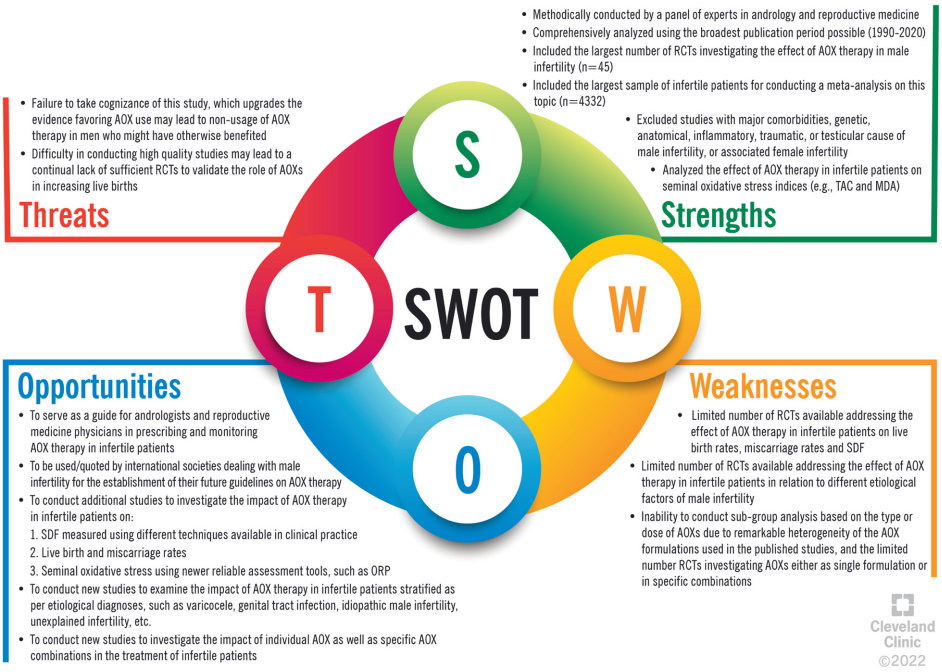

Fig. 28. Strengths, Weaknesses, Opportunities, and Threats (SWOT) analysis of the present study. AOX: antioxidant, MDA: malondialdehyde acid, ORP: oxidation-reduction potential, RCT: randomized controlled trial, SDF: sperm DNA fragmentation, TAC: total antioxidant capacity.

CONCLUSIONS

Our study included the largest population analyzed for the impact of AOX therapy on male infertility. The current meta-analysis demonstrates low to moderate evidence on the positive impact of AOX therapy on spontaneous pregnancy rate, and conventional sperm parameters in infertile men. Additionally, our results provide a very low to low evidence on the potential positive effect of AOX therapy on levels of seminal TAC and MDA in infertile men. The exclusion of studies that examined patients treated with AOX or placebo or untreated after varicocele repair did not change the results. This suggests that the effect of AOX on the analyzed outcomes is independent of varicocele repair. Compared to the last Cochrane review in 2019 [14], our study increased the number of RCTs included in the meta-analysis, creating the largest population analyzed with regards to the benefits of AOXs on male fertility. Also, some of the outcomes of the present study, such as sperm morphology, seminal levels of TAC and of MDA, had not been analyzed in the 2019 Cochrane review [14].

Finally, the present analysis provides additional evidence in favor of recommending the use of AOX therapy in male infertility (Table 5). The results of the current meta-analysis may help update those guidelines of the scientific societies that do not express a clear position on the use of AOX for the treatment of the infertile male due to the lack of evidence [22,93-95] (Table 6). For the clinical use of AOX to be standardized, further studies are needed, as at present there is not a specific AOX or AOX combination with a particular dosing that can be recommended for infertile men due to the heterogeneity of the available data.

Accordingly, based on our results, the following suggestions can be made on the use of AOX for the treatment of patients with male infertility:

1. We suggest the use of AOX to improve spontaneous pregnancy rates in patients diagnosed with IMI after a careful diagnostic work-up and the exclusion of major causes of infertility, and in those with varicocele following varicocele repair (2 ØØØO).
2. We suggest the use of AOX to improve conventional sperm parameters in couples diagnosed with IMI after a careful diagnostic work-up and the exclusion of other causes of infertility, and in those with varicocele following varicocele repair (2 ØØØO).
3. We suggest the use of AOX to improve the seminal indices of OS (TAC and MDA) in patients diagnosed with IMI and OS (2 ØØOO).

The present study identifies the need for further RCTs assessing the impact of AOX on live-birth rate, miscarriage rate, and SDF, as only few studies have analyzed these outcomes in infertile patients treated

Table 5. Summary of the findings of the present study

Outcome	Results	Number of participants (n studies)	Level of the evidence (scored using the GRADE[a] system)
Spontaneous pregnancy rate	OR 1.97 (95% CI: 1.28, 3.04); n=190 events; p<0.01; I^2=20%; χ^2 p=0.20 (Fig. 2)	1,355 (16 RCTs)	⊕⊕⊕⊖ (Moderate)[b]
Live-birth rate	OR 1.21 (95% CI: 0.53, 2.76); n=65 events; p=0.64 (Fig. 6)	388 (4 RCTs)	⊕⊖⊖⊖ (Very low)[b,c,d]
Miscarriage rate	OR 1.01 (95% CI: 0.34, 3.00); n=13 events; p=0.98 (Fig. 7)	459 (4 RCTs)	⊕⊖⊖⊖ (Very low)[b,c,d]
Sperm concentration	MD 5.93 mil/mL (95% CI: 4.43, 7.43); p<0.01; I^2=94%; χ^2 p<0.01 (Fig. 8)	4,310 (35 RCTs)	⊕⊕⊕⊖ (Moderate)[b]
Progressive sperm motility	MD 7.21% (95% CI: 3.66, 10.76); p<0.01; I^2=99%; χ^2 p<0.01 (Fig. 12)	2,345 (20 RCTs)	⊕⊕⊕⊖ (Moderate)[b]
Total sperm motility	MD 7.52% (95% CI: 3.11, 11.94); p<0.01; I^2=100%; χ^2 p<0.01 (Fig. 16)	4,452 (36 RCTs)	⊕⊕⊕⊖ (Moderate)[b]
Sperm morphology	MD 3.28% (95% CI: 2.40, 4.17); p<0.01; I^2=96%; χ^2 p<0.01 (Fig. 20)	1,828 (18 RCTs)	⊕⊕⊖⊖ (Low)[b,c]
Sperm DNA fragmentation	SMD -0.63 (95% CI: -2.29, 1.02); p=0.45 (Fig. 24)	135 (3 RCTs)	⊕⊖⊖⊖ (Very low)[b,c,d]
Seminal TAC levels	MD 1.87 (95% CI: 1.26, 2.48; p<0.01; I^2=97%; χ^2 p<0.01 (Fig. 26)	316 (6 RCTs)	⊕⊕⊖⊖ (Low)[b,c]
Seminal MDA levels	MD -0.39 (95% CI: -0.65, -0.14; p<0.01; I^2=87%; χ^2 p<0.01 (Fig. 27)	403 (7 RCTs)	⊕⊕⊖⊖ (Low)[b,c]

CI: confidence interval, GRADE: Grading of Recommendations Assessment, Development and Evaluation, MD: mean difference, MDA: malondialdehyde acid, OR: odds ratio, RCT: randomized controlled trial, SMD: standardized mean difference, TAC: total antioxidant capacity.
[a]High level: We are very confident that the true effect lies close to that of the estimate of the effect. Moderate level: We are moderately confident in the effect estimate. The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different. Low level: Our confidence in the effect estimate is limited. The true effect may be substantially different from the estimate of the effect. Very low level: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect.
[b]Downgraded one level for risk of bias: lack of allocation concealment, lack of blinding and incomplete accounting of patients and outcome events.
[c]Downgraded one level for serious imprecision: small sample size.
[d]Downgraded one level for serious imprecision: crossing the line of no effect.

Table 6. Summary of the Societies' recommendations on the use of AOX for the treatment of male infertility

Society	Year	Recommendation
European Academy of Andrology (EAA) [92]	2018	Recommendation 9. According to the current evidence, we cannot recommend either for or against antioxidants, and for antiestrogens (tamoxifen or clomiphene) or aromatase inhibitors (2ØOOO)
American Urological Association (AUA)/American Society for Reproductive Medicine (ASRM) [22]	2021	Recommendation 43. Clinicians should counsel patients that the benefits of supplements (*e.g.*, antioxidants, vitamins) are of questionable clinical utility in treating male infertility. Existing data are inadequate to provide recommendation for specific agents to use for this purpose (Conditional Recommendation; Evidence Level: Grade B) Current data suggest that they are likely not harmful, but it is questionable whether they will provide tangible improvements in fertility outcomes
Italian Society of Andrology and Sexual Medicine (SIAMS) [93]	2021	We recommend against treatment with nutraceuticals/antioxidants in unselected infertile men to increase sperm parameters (Expert Opinion) We suggest considering the use of nutraceuticals/antioxidants in selected patients with idiopathic oligozoospermia and/or asthenozoospermia and/or clear signs of high OS, since in some cases they might improve sperm parameters (2, ØOOO) We cannot recommend either for or against the use of nutraceuticals/antioxidants to increase pregnancy rate (Expert Opinion)
European Association of Urology – Sexual and Reproductive Health Guidelines [94]	2022	No clear recommendation can be made for treatment of patients with idiopathic infertility using antioxidants, although antioxidant use may improve semen parameters (weak)

AOX: antioxidant, OS: oxidative stress.

with AOX compared to placebo-treated or untreated controls. Finally, the ideal treatment duration, as well as best therapeutic regimen (single *vs.* combined AOX) remains unknown and further studies will be needed to address these issues.

Conflict of Interest

The authors have nothing to disclose.

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Author Contribution

Conceptualization: AA, RS. Data curation: R.Sa, RC, AH. Formal analysis: RC, AH. Funding acquisition: None. Investigation: GS, SK, AF. Methodology: R.Sa, RC, AH. Project administration: AA, RS. Supervision: FB, TAAMH. Validation: AR, AZ, GC, MG, PK, EK, GC, TT, GP, HJP, RAG, SM, GMB, MEB, AK, EC, GIR, AEC, RFA, CNJ. Writing – original draft: RC, GS, R.Sa, FB, AF. Writing – review & editing & final approval: AA, RS.

Supplementary Materials

Supplementary materials can be found *via* https://doi.org/10.5534/wjmh.220067.

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Supplement Table 1. PRISMA checklist.

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	4-5
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	6-7
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	8
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	//
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Table 1, Suppl. Table 2, page 8-10
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	8-10
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	8-10
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	8-10
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	8-10
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Suppl. Table 2
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	10
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	10
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	10

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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	10
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	10
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	11
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Table 2 and Funnel plots
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	11-39
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	11-39
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	11-39
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	11-39
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	40-43
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	49-50
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	43
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	//

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Supplement Table 2. Eligibility criteria based on the PICOS (Population, Intervention, Comparison/Comparator, Outcomes, Study design) model

	Inclusion	Exclusion
Population	Infertile men	Adolescent, patients taking any other fertility drugs, fertile men, animal models
Intervention	AOX therapy (single or combined), AOX plus plant extract wherein AOX is the main supplementation Duration: ≤3 months, 6 months or ≥9 months	Use of plant extracts or herbal substances as the main supplementation
Comparison	Placebo or no treatment	
Outcome	Primary outcome i) Natural pregnancy (per couple) ii) Live birth (per couple) iii) Miscarriage rate (per pregnancy) Secondary outcome i) Sperm parameters ii) Sperm DNA fragmentation Oxidative Stress	

AOX: antioxidant.

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Supplement Table 3. Inclusion and exclusion criteria of the patients enrolled in the studies included in this meta-analysis

Reference	Year	Study design	Inclusion criteria	Exclusion criteria
Eslamian et al [51]	2020	RCT	Healthy, voluntary, idiopathic asthenozoospermic men, aged 20–45 years, unwanted childlessness for ≥1 years with the same female partner, normal endocrine function, and with the total number (or concentration) of spermatozoa, and percentage of morphologically normal spermatozoa, equal to or above the lower WHO reference limits.	1) To have abnormal testis, cryptorchidism, varicocele, had genital surgery, abnormal karyotypes, or endocrine hypogonadism detected via physical examination and paraclinical testing; 2) A history of the use of antioxidant and ω-3 supplements within the previous 3 months; 3) A history of receiving radiation and/or chemotherapy, testosterone, and antiandrogens; 4) Genital tract infection or use of medication for this condition within the previous 3 months; 5) Being a candidate for ICSI owing to severe sperm motility failure; 6) Exposure to extreme heat and/or pollutants such as pesticides, chemical solvents, heavy metals, and/or radioactive agents; and 7) Enrollment or planned enrollment in other research that might conflict with full participation in the current study or confound the observation or interpretation of the study findings.
Kopets et al [38]	2020	RCT	Age 21–50 years, idiopathic male infertility (absence of conception in a couple having regular unprotected intercourse for 12 months with a woman without evident pathology), OAT.	Genetic, anatomical, inflammatory, trauma, testicular cause of male infertility, cause of female infertility, STIs, inflammatory bowel disease, alcohol or drug addiction.
Steiner et al [42]	2020	RCT	Couples with at least 12 months of infertility were included in the study. Male partners were 18 years of age or older with at least one abnormal semen parameter in the preceding 6 months: sperm concentration ≤15 million/mL (oligospermia), total motility ≤40% (asthenospermia), normal morphology ≤4% (teratospermia), or DNA fragmentation ≥25%. Female partners were between 18 and 40 years of age with regular menstrual cycles, defined as 25–35 days in duration, and evidence of ovulation by biphasic basal body temperature, ovulation predictor kits, or luteal serum progesterone level ≥3 ng/mL; and a normal uterine cavity with at least one patent Fallopian tube. Women over the age of 35 had normal ovarian reserve, defined as an early follicular phase FSH ≤10 IU/L, AMH ≥1.0 ng/mL, or antral follicle count >10.	Male partners were excluded if they had a sperm concentration less than 5 million/mL or if they were taking fertility medication or testosterone. Men were required to be off all vitamins for 4 weeks prior to randomization.
Ardestani Zadeh et al [27]	2019	RCT	Infertile patients with varicocele who underwent sub-inguinal varicocelectomy.	Usage of supplements, vitamins or alcohol, tobacco smoking, addiction to opium or using the opium during the follow-up period, diabetes mellitus, peptic ulcer history, hormonal disorders (based on clinical history and medical examination), chronic or active genitourinary infection (according to the history, medical examination, urine and semen analysis) and previous reaction to folic acid, selenium or vitamin E. As well, patients with missed follow-up, incorrect usage of drugs, presenting side effects, and delayed complications of varicocelectomy including recurrent varicocele, hydrocele or testicular atrophy were excluded from the study.
Kızılay et al [28]	2019	RCT	Infertile (>12 mo) patients with OAT with grade I–III varicocele, spouses <35 years, no female infertility.	Genitourinary system and/or varicocele surgery, idiopathic infertility, disease affecting fertility, medical treatment affecting fertility, undescended testis, testicular cancer, testicular trauma, post-pubertal mumps, endocrine disorder, obstructive urogenital disease, HIV, acute infection, fertility-specific diet, alcohol, drugs, cigarettes, opioids and hallucinogens.

Supplement Table 3. Continued 1

Reference	Year	Study design	Inclusion criteria	Exclusion criteria
Nouri et al [60]	2019	RCT	Infertile men aged between 25–45 years, a sperm count of less than 20 million per milliliters, normal sperm of <65% and sperm volume of <3.0 mL, and average motility of <60% while receiving no treatments.	History of disorders (urinary tract infection, testicular atrophy, testicular torsion, azoospermia, asthezoonospermia, inguinal and genital surgery, genital trauma, and other genital diseases, such as current genital inflammation and cryptorchidism), anatomical disorders, endocrinopathy, previous hormonal therapy, use of androgens, antiandrogens, anticoagulants, cytotoxic drugs, or immunosuppressants. Patients with physiological and psychiatric disorders that could affect sperm and sexual performance, alcohol and drug abuse, and BMI of ≥30 kg/m^2 were also excluded.
Blomberg Jensen et al [69]	2018	RCT	Impaired semen quality (determined by WHO criteria) and vitamin D insufficiency (25-OHD level <50 nmol/L).	Serious comorbidities.
Busetto et al [36]	2018	RCT	Age 18–50 years, oligo-, astheno- and/or teratozoospermia, with or without varicocele, having a history of infertility for more than 12 months. Varicocele patients were not surgically treated before and during the treatment, patients without varicocele were suffering from idiopathic male infertility, no other previous history of diseases affecting fertility. Fertile female partners were required with regular menstrual cycles, age <40 years and couples not looking for fertility-related procedures (IVF/ICSI/IUI) for the next 90 days.	Known hypersensitivity to any of the treatment compounds, history of undescended testes or cancer, endocrine disorders, history of 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 within last 3 months, excessive consumption of alcohol or regular use of illicit or "recreational" drugs, positive serology for HIV, participants following any special diet, any condition which in the opinion of the investigator might put the participant at risk by participating in this study, participants involved in any other clinical trials.
Lu et al [55]	2018	RCT	The patients were diagnosed with a left-sided clinical varicocele in the urology clinic. Fifty-four patients who were mildly oligospermic (sperm count: 5–15 million) and could not have a child for at least 1 year were included.	Not specified.
Stenqvist et al [43]	2018	RCT	Normal reproductive hormone levels and high SDF (≥25%).	Smoking, use of anabolic steroids, AOX, anti-hypertensive drugs, statins, obesity and hypogonadism.
Busetto et al [35]	2017	RCT	Infertile patients with oligo- and/or astheno- and/or teratozoospermia, history of infertility for more than 12 months.	Subjects with known hypersensitivity to any of the treatment compounds, history of undescended testes or cancer, endocrine disorders, history of 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 within last 3 months, excessive consumption of alcohol or regular use of illicit or "recreational" drugs, positive serology for HIV, subjects following any special diet, any condition which in the opinion of the investigator might put the subject at risk by participating in this study and subjects involved in any other clinical trials. Female factor excluded.

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Supplement Table 3. Continued 2

Reference	Year	Study design	Inclusion criteria	Exclusion criteria
Barekat et al [29]	2016	RCT	Age <45 years, primary infertility, left-sided varicocele (grade 2–3) diagnosed by palpation and Doppler duplex ultrasound. Female partner with age <35 years, normal ovulatory cycles and patent tubes (confirmed by hysterosalpingography or laparoscopy)	Varicocele grade I, azoospermia, recurrent varicocele, leukocytospermia, urogenital infections, testicular size discrepancy, abnormal hormonal profile, anatomical disorders, Klinefelter's syndrome, cancer, fever in the 90 days prior to surgery, seminal sperm antibodies, excessive alcohol and drug consumption, previous history of scrotal trauma or surgery and occupational exposure. Female partner with endometriosis, cycle irregularity, or gross anatomical abnormalities was also excluded.
Ener et al [30]	2016	RCT	Males diagnosed with a left-sided clinical varicocele in the urology outpatient clinic, and for whom subinguinal varicocelectomy was planned.	The use of alcohol, tobacco or any drugs including vitamins.
Boonyarangkul et al [46]	2015	RCT	Abnormal semen analysis of at least one parameter according to WHO criteria 2010 (concentration <15 million/mL, motility <40%, or morphology <4%), failure of the female partner to conceive after one year of regular unprotected sexual intercourse, no history of tamoxifen and folate allergy.	Use of tamoxifen and folate within three months before recruitment, use of other medicines or vitamin during the study period.
Cyrus et al [31]	2015	RCT	A palpable varicocele in physical examination and accompanying abnormalities in count, motility, or morphology of sperm in two separate semen analyses (according WHO criteria 1999), age range between 18 and 50 years, weight between 50 and 100 kg and being married.	Missed follow-up, incorrect usage of the capsules, demonstrating side effects due to vitamin C, commencement of smoking or opium addiction during the follow-up period, delayed complications of varicocelectomy such as: hydrocele, recurrence of varicocele, and testicular atrophy. Azoospermia, diabetes mellitus, hormonal disorders (according to medical history and clinical examination), tobacco smoking, opium or recreational drugs addiction, regular usage of vitamins or nutritional supplements, active or chronic genitourinary infection (based on medical history, physical examination, semen and urine analysis), history of peptic ulcer and previous reaction or intolerance to vitamin C.
Haghighian et al [53]	2015	RCT	Unwilling childlessness at least 24 months in duration with a female partner, no medical condition that could account for infertility, normal fertile female partner according to investigations, all patients were needed to have stopped all medical therapy 12 weeks before the study initiation.	History of epididymo-orchitis, prostatitis, genital trauma, testicular torsion, inguinal or genital surgery, urinary tract infection, or previous hormonal therapy, another genital disease (cryptorchidism, current genital inflammation or varicocele), severe general or central nervous system disease and endocrinopathy, use of cytotoxic drugs, immunosuppressants, anticonvulsants, androgens, or antiandrogens, recent history of STI, psychologic or physiologic abnormalities that would impair sexual performance or the ability to provide semen samples, drug or alcohol abuse, hepatobiliary disease, significant renal insufficiency, occupational and environmental subjections to possible reproductive toxins, BMI of >30 kg/m^2, participation in another investigational study, unlikely availability for follow-up.
Moslemi Mehni et al [57]	2014	RCT	Age 25–40 years, infertile men with OAT, healthy fertile wives.	Existence of genital abnormalities (undescended testes, varicocele, atrophy of testes), occupational chemical exposure history, systemic diseases, abnormal semen volume, pH, agglutination or viscosity, serum hormonal abnormalities (FSH, LH, testosterone, estradiol, PRL), wives with known fertility risk factors confirmed by gynecologist.

Supplement Table 3. Continued 3

Reference	Year	Study design	Inclusion criteria	Exclusion criteria
Azizollahi et al [32]	2013	RCT	The presence of grade III varicocele assessed by clinical parameters and was confirmed by Doppler ultrasound scanning.	Evidence of leukocytospermia, low testicular volume <15 mL, congenital urogenital abnormalities and urogenital infections.
da Silva et al [49]	2013	RCT	Subfertile male patients who did not take any additional supplement and/or vitamin other than the supplement provided, and who did not change eating habits during the follow-up period.	All the men were required to be between the ages of 20 and 55 years at the time of enrollment. Patients with high FSH serum levels, azoospermia and patients treated with clomiphene citrate were excluded.
Gopinath et al [37]	2013	RCT	Age 21–50 years, infertility >1 year, sperm count less than 15 million/mL, sperm total motility <40%, no history of taking therapy for infertility, no history of OAT, regular sexual intercourse with a potentially normal fertile female, willing to sign informed consent and likely to be available for all visits during follow-up period.	Primary testicular disease, any organic cause for infertility including varicocele, prostate-vesiculo-epididymitis, genital infectious disease, planning for any other ART during study period, serum FSH >15 mIU/mL, abnormal serum levels of LH, testosterone, estradiol and PRL, presence of antispermatozoa antibodies, severe oligospermia (<2 million sperm/mL), azoospermia, seminal WBCs more than 10^6/mL, major hepatic and renal disease, myopathy, history of allergy to any ingredient of the formulation, not likely to be available for follow-up, have participated in another clinical trial in the past 3 months, female partners with anatomic or physiological alterations causing subfertility.
Safarinejad et al [64]	2012	RCT	History of primary infertility of more than 2 years, abnormal sperm count and motility according to WHO criteria, wife's age between 20 and 40 years, documentation of fertile female partner, no known medical or surgical condition which can result in infertility.	History of cancer chemotherapy or radiotherapy, history of genital disease such as cryptorchidism and varicocele, history of genital surgery, BMI 30 kg/m^2 or greater, any endocrinopathy, Y chromosome microdeletion or karyotype abnormalities, leukocytospermia (more than 10^6 WBC per mL), drug, alcohol or substance abuse, tobacco use, use of anticonvulsants, androgens or antiandrogens, significant liver (serum bilirubin greater than 2.0 mg/dL) or renal dysfunction (serum creatinine greater than 2.0 mg/dL), occupational and environmental exposure to reproductive toxins, severe oligozoospermia (less than 5×10^6/mL), azoospermia and testicular volume less than 12 mL.
Nadjarzadeh et al [59]	2011	RCT	OAT, infertility (having been trying for pregnancy for >1 year unprotected intercourse).	Seminal WBC >1,000,000 /mL, presence of anatomical abnormalities of the genital tract, presence of infectious genital diseases or systemic diseases, presence of treatment with other drugs and dietary supplement during the 3 months before enrolling in the study, currently smoking, using drug, or alcohol use or occupational chemical exposure.
Safarinejad [65]	2011	RCT	Unwanted childlessness of at least 24 months.	Female infertility, abnormal testis, cryptorchidism, varicocele, genital surgery, Y chromosome microdeletion, abnormal karyotype, azoospermia, hormonal abnormality, history of cancer chemotherapy, thymidine/thymidine, anti-androgens and AOX usage, smoking, medical problems associated with decreased fertility, hepatobiliary disease, renal insufficiency, BMI ≥30 kg/m^2, occupational and environmental exposure.
Dimitriadis et al [50]	2010	RCT	Unclear	Unclear

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Supplement Table 3. Continued 4

Reference	Year	Study design	Inclusion criteria	Exclusion criteria
Martinez-Soto et al [56]	2010	RCT	Men suffering from male factor infertility, according to the WHO guidelines (WHO 1999), and who were undergoing infertility evaluation during the period 2009 to 2011.	Oncological patients, those suffering from metabolic disease, chromosomal or genetic alterations, and patients on anticoagulant treatment.
Morgante [58]	2010	RCT	Age 28–45 years, sperm concentration <20×10^6 spermatozoa/mL, sperm progressive motility <30%, normal morphology <30%, leucocyte <1×10^6/mL, no infections.	Men younger than 28 and over 45, sperm concentration >20×10^6 spermatozoa/mL, sperm progressive motility >30%, normal morphology >30%, leucocyte >1×10^6/mL, current infections, history of testicular pathology: cryptorchidism, varicocele, surgical operations, radiotherapy or chemotherapy, use of anabolic steroids, deficiency of hypothalamic-pituitary-gonadal axis, genital tract infections.
Peivandi et al [62]	2010	RCT	At least two abnormal sperm analyses based on WHO criteria with a two-week interval during four weeks, normal range of gonadotropins, testosterone and PRL concentrations.	Varicocele, testicular atrophy, ejaculatory disorders, use of medications, azoospermia, endocrinological disorders, ICSI candidacy or other causes of infertility.
Balercia et al [34]	2009	RCT	Age 20 to 40 years, infertility >2 years, regular sexual intercourse with a potentially fertile female, normal rheologic characteristics (appearance, consistency and liquefaction) of semen and volume and pH in normal range, sperm count >20×10^6/mL, sperm motility <50% (WHO 1999), normal morphology >30%, seminal WBC <1×10^6/mL and a negative sperm culture, normal levels of gonadotropins.	Genital disease and anatomical abnormalities of the genital tract including varicocele, systemic disease, treatment with other drugs within 3 months of being enrolled in the study. Smoking, alcohol and drug addiction and exposure to occupational chemicals.
Ciftci et al [47]	2009	RCT	Patients with idiopathic infertility with normal sperm parameters.	Infertile patients with well-known pathologic features such as varicocele, leukospermia, hormonal abnormalities, and/or obstruction were excluded from the study. The additional exclusion criteria included the presence of cryptorchidism, vasectomy, abnormal liver function, cigarette smoking, and alcohol consumption.
Safarinejad and Safarinejad [66]	2009	RCT	Sperm count >5×10^6/mL, over 2 years of failed conception, no female fertility problems, no history of possible cause for male infertility.	Abnormal testes, history of cancer or chemotherapy, testosterone or antiandrogen usage, usage of selenium or N-acetylcystein supplements, abnormal hormone levels, genital disease, genital inflammation or varicocele, history of genital surgery, major surgery, central nervous system injury, a known sperm defect or retrograde ejaculation. Y chromosome abnormalities, sexually transmitted disease, genitourinary infection, leukocytospermia, smoking, any environmental exposures to reproductive toxins. Medical, neurological or psychological problems. A history of drug or alcohol abuse, hepatobiliary disease or significant renal insufficiency. Any endocrine abnormality, BMI of 30 kg/m^2 or over, participation in another investigational study and a likelihood of being unavailable for follow-up.

Supplement Table 3. Continued 5

Reference	Year	Study design	Inclusion criteria	Exclusion criteria
Safarinejad [67]	2009	RCT	Minimum 2 years unprotected intercourse with 2 years unwilling childlessness. Male infertility diagnosed if 1 or more standard semen parameters were below cutoff levels accepted by WHO. A fertile female partner. No known medical condition that could account for infertility, testicular volume 12 mL or greater. No medical therapy for at least 12 weeks before the study begins. Only patients seeking medical attention for infertility were included.	Azoospermia or severe oligozoospermia (sperm count less that 5 million/ml). A history of epididymal-orchitis, prostatitis, genital trauma, testicular torsion, inguinal or genital surgery. Any genital or central nervous system disease, endocrinopathy, usage of cytotoxic drugs, immunosuppressants, anticonvulsive, androgens, antiandrogens, a recent history of sexually transmitted disease. Psychological or physiological abnormalities that would impair sexual functioning or ability to produce sperm samples. Drug, alcohol or substance abuse. Liver disease, renal insufficiency or chromosome abnormalities. Occupational and environmental exposures to reproductive toxins. A BMI of 30 kg/m^2 or over, participation in another investigational study and a likelihood of being unavailable for follow-up.
Stanislavov et al [68]	2009	RCT	The patients were aged between 30 and 50 years. They had been in a stable sexual partnership for the past 6 months persisting over the whole period. The duration of infertility was more than 2 years and had different deviations in sperm concentration, motility and morphology in terms of seminological nomenclature: oligo-, astheno, teratozoospermia or a combination of these deviations, according WHO Laboratory Manual (WHO, 1999).	Testicular maldescent, varicocele, orchitis, globozoospermia, infections, disturbances of semen deposition (hypospadias), severe cardiovascular disease, severe hypertension d ≥90 mmHg ; s ≥150 mmHg, renal failure, hepatic insufficiency, endocrine hypogonadism abnormality, psychiatric disorders, testicular tumors, treatment of ED with any drugs during the past 4 weeks.
Omu et al [61]	2008	RCT	Asthenozoospermia with normal sperm concentration (20 to 250 million/mL) but with 40% or more immotile sperm.	Sperm concentration of <20 million/mL.
Paradiso Galatioto et al [40]	2008	RCT	Having performed a retrograde embolization with concomitant oligospermia, persistent oligospermia and infertility >12 months.	Smoking, alcohol consumption, taking any fertility drugs within 3 months prior to the study, serious medical or psychiatric condition, abnormal hormonal profile, sperm infection.
Sigman et al [71]	2006	RCT	Males 18 to 65 years with infertility of at least six months duration, sperm concentration of at least 5 million sperm/mL, motility of 10% to 50%, absence of pyospermia and normal FSH and testosterone levels.	History of post-pubertal mumps, cryptorchism, vasal or epididymal surgery, history of medication or chemotherapy. Alcohol and chronic marijuana usage. Usage of testosterone or steroids. Exposure to environmental toxins. Recent history of fever. Having a history of diabetes, liver failure, renal failure, endocrine disorder, untreated varicocele, urogenital infection, or prior vasectomy reversal.
Balercia et al [33]	2005	RCT	Primary infertility >2 years after regular intercourse with a fertile woman, 20 to 40 years of age, normal rheologic characteristics, sperm count $>20\times10^6$/mL, sperm motility <50%, normal sperm morphological features >30%, seminal WBC $<1\times10^6$/mL, negative sperm culture and Chlamydia trachomatis and Ureaplasma urealyticum infection, normal serum gonadotropins, testosterone, E2 and PRL.	Infectious or genital disease, anatomic abnormalities of the genital tract, systemic diseases or treatment with other drugs within the 3 months before enrolment in the study, smoking, alcohol or recreational drug use or occupational chemical exposure.
Greco et al [52]	2005	RCT	Men consulting for infertility in whom previously performed TUNEL assay showed the presence of fragmented DNA in more than or equal to 15% of ejaculated spermatozoa.	Varicocele, genitourinary inflammation, or infection, and smokers.

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Supplement Table 3. Continued 6

Reference	Year	Study design	Inclusion criteria	Exclusion criteria
Lenzi et al [54]	2003	RCT	OAT, age between 20 to 40 years, infertility >2 years with regular intercourse, without a history of endocrine disease, cryptorchidism, genital infections or obstructions, varicocele or testicular hypertrophy and anti-sperm antibodies.	None
Závaczki et al [45]	2003	RCT	Unsuccessful pregnancy attempts for over one year. A healthy female partner examined by a gynecologist. Sperm volume <2 mL and/or sperm concentration <20 million/mL and/or morphology ratio <30% and/or motility <50%. No genital tract infection, no bacteria or fungi in urine or semen. Hormones are within physiological range. Intact renal function. No excessive magnesium intake.	Unclear
Conquer et al [48]	2000	RCT	Asthenozoospermic, sperm motility <50% of total sperm.	Unclear
Rolf et al [63]	1999	RCT	Asthenozoospermia (<50% motile) diagnosed after 2 examinations, normal or reduced sperm concentration (>20×10^6 per ejaculate) and without infection of accesory glands.	Unclear
Omu et al [39]	1998	RCT	Asthenozoospermia with normal sperm concentration (20 to 250 million/mL) but with 40% or more immotile sperm.	Sperm concentration of < 20 million/mL.
Scott et al [41]	1998	RCT	Low sperm motility.	Not specified
Suleiman et al [44]	1996	RCT	Asthenospermia (≥20×10^6 /mL). Sperm motility ≤40%, normal sperm count, leucocyte concentration <5%, normal fructose concentration, normal female.	Unclear
Dawson et al [70]	1990	RCT	Sperm agglutination over 25%, negative sperm antibodies, physically normal, no inflammatory disease.	Unclear

AMH: anti-Müllerian hormone, AOX: antioxidant, ART: assisted reproductive technique, BMI: body mass index, E2: 17β-estradiol, ED: erectile dysfunction, FSH: follicle-stimulating hormone, HIV: human immunodeficiency viruse, ICSI: intracytoplasmic sperm injection, IUI: intrauterine insemination, IVF: *in vitro* fertilization, LH: luteinizing hormone, OAT: oligo-astheno-teratozoospermia, PRL: prolactin, RCT: randomized controlled trial, SDF: sperm DNA fragmentation, STI: sexually transmitted infection, TT: thymidine/thymidine, TUNEL: terminal deoxynucleotidyl transferase dUTP nick-end labeling, WBC: white blood count, WHO: World Health organization, 25-OHD: 25-hydroxy vitamin D.