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NARRATIVE REVIEW

Antioxidant therapy in endometriosis treatment: systematic review

Antioxidant therapy in endometriosis treatment

Laura **Pivazyan** [1], Eva **Nakhapetyan** [2], Sapiyat **Isaeva** [1], Veronika **Tarlakyan** [3,*], Anastasia **Laevskaya** [4], Eduard **Ayryan** [3], Valeriia **Seregina** [1]

[1] FSBI «National medical research center for obstetrics, gynecology and perinatology named after academician V.I.Kulakov» Ministry of health of the Russian Federation, Moscow, Russia.

[2] Pirogov Russian National Research Medical University, Moscow, Russia.

[3] I.M. Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia.

[4] Botkin Hospital, Moscow, Russia.

***Corresponding author:** Veronika **Tarlakyan** MBBS – I.M. Sechenov First Moscow State Medical University (Sechenov University), 119991, Moscow, Russia.

Email: vtarlakyan00@mail.ru.

ORCID: 0000-0003-1163-2874.

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ABSTRACT

Objective. To summarize current knowledge on the effectiveness of antioxidant therapy in endometriosis treatment.

Materials and Methods. A systematic review was conducted per PRISMA guidelines and registered in PROSPERO 2023 CRD42023454705. Studies published until November 2024 were identified through PubMed, The Cochrane Library, ClinicalTrials.gov, Google Scholar, and MEDLINE. COVIDENCE software was used for screening. Risk of bias was assessed using the Cochrane Handbook.

Results. Out of 512 studies, 11 were included in the systematic review. Endometriotic cysts weight and volume are dose-dependent parameters that are significantly lower in antioxidant treatment groups ($p < 0.05$) Neither histological cell scores nor trichrome fibrosis scores showed statistically significant differences among treatment and control groups ($p > 0.05$) in 2 out of 3 studies. Significantly lower levels of TOS, NO and OSI are evaluated in the antioxidant group compared to control. However, no significant differences were observed in MDA, SOD and CAT levels. The number of follicles was significantly increased, and the atretic follicles number was significantly decreased after therapy ($p < 0.05$). The IVF, cleavage, blastocyst formation rates and blastocyst number were significantly higher in treatment group compared the control.

Conclusions. Antioxidants may be considered as a possible component of endometriosis therapy to potentially enhance fertility outcomes and slow disease progression, though current evidence is preliminary and requires further validation.

Key words

Endometriosis; antioxidants; treatment; oxidative stress.

Abbreviations: TOS, total oxidant status; NO, nitric oxide; OSI, oxidative stress index; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; IVF, In vitro fertilization.

Introduction

Endometriosis is an estrogen-dependent inflammatory gynecological disease defined by the presence of endometrial-like mucosa outside the uterine cavity. The pathogenesis of endometriosis is supported by several theories, such as immunological, implantation (Sampson's theory), dysontogenetic, dissemination, metaplastic, genetic, hormonal, etc. [1] Endometriosis has an impact on fertility, affecting the ovarian reserve, embryo quality, implantation and normal anatomical structure of the reproductive organs and surrounding tissues . However, the mechanisms leading to endometriosis-associated infertility are not fully understood. Currently, the role of oxidative stress (OS) leading to iron metabolism disorders in the pathogenesis of endometriosis is widely discussed. Endometriotic lesions are resistant to ferroptosis - iron-mediated programmed non-apoptotic cell death, which allows their implantation in the peritoneal cavity [2]. There is a need for further research in this area due to its high relevance, theoretical and practical importance.

A microenvironment with a high level of reactive oxygen species (ROS), free radicals and iron is created as a result of cyclic changes in ectopic endometriotic lesions, which increases their adhesion and the progression of the disease.

The presence of ROS in cells is a physiological process due to their formation in normal oxidative metabolism. They control the ovarian cycle, steroidogenesis and ovulation [3]. However, the imbalance between free radicals and the antioxidant system causes oxidative stress, leading to a reduction in oocyte quality [4].

In this systematic review, we observe the efficiency of antioxidants supplementation in endometriosis treatment.

Materials and methods

Study design

Our systematic review was conducted and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [5].

The present systematic review has been registered in the PROSPERO international prospective registry of systematic reviews by the National Institute of Health Research (NIHR). The registration number is PROSPERO 2023 CRD42023454705.

Search strategy

To identify relevant articles, we conducted an electronic database search using several databases: PubMed, Google Scholar, ClinicalTrials.gov, Cochrane library to identify studies using key words and MeSH terms. The date of the last screening was November 26, 2024. Using the advanced search tool on PubMed, the following combination of key words was used: ((endometriosis) OR (endometrioma)) AND (antioxidants) AND (oxidative stress) AND (treatment). No filters or limits were used. Additionally, the search was conducted using MeSH terms (endometriosis [MeSH Terms]) AND ((antioxidants[MeSH Terms]) AND (treatment [MeSH Terms]) AND (oxidative stress [MeSH Terms]).

The Cochrane Library electronic database search strategy was conducted. The combination of the search was as follows: ((endometriosis) OR (endometrioma)) AND (antioxidants) AND (oxidative stress) AND (treatment). No filters or limits were used. MeSH terms were also screened (MeSH descriptor: [Endometriosis] explode all trees and with qualifier(s): [antioxidants - MeSH]).

The search was also conducted in the ClinicalTrials.gov electronic database using an advanced search combination: endometriosis | antioxidants.

Study selection

For search conducting and further screening COVIDENCE software was used. To ensure the quality and accuracy of the search results, two investigators performed the search independently. After the initial search, all articles were reviewed based on their titles and abstracts. The full texts of the studies that appeared to be appropriate according to their titles and abstracts were reviewed. Potential trials were also identified by searching the reference lists of the eligible trials. We included randomized (RCTs) and non-randomized clinical trials. Only articles written in

English were included. Abstracts from congresses and unpublished articles were not included. As this is a review of published studies, Institutional Review Board (IRB) approval was not sought.

Two investigators (E.N., I.S) independently read the full texts of the preselected articles to verify their eligibility. Any studies with duplicate records were excluded. To minimize potential bias during the review process, any disagreements about the inclusion or exclusion of preselected studies were resolved with the help of a third author (A.L).

Inclusion criteria

The inclusion criteria specified autograft endometriosis mice or rat models and women with endometriosis related infertility, receiving antioxidant therapy.

Studies that described high levels of oxidative stress markers due to non endometriosis-related reasons, phytoalexins, antioxidant decoctions as a therapy were excluded.

Data Extraction and quality assessment

The studies included were independently collected by two authors (E.N., I.S) using a standardized data extraction procedure. We obtained the following characteristics from our studies: study design, type of animal model, types of antioxidants and regimens used, and the number of patients in each groups and the follow-up duration.

The analysis in animal models was aimed to evaluate the level of oxidative stress markers, embryo and oocyte quality, implant weight, volume and histological cell scores of endometriotic lesions after antioxidant therapy

The analysis of human studies was aimed to establish the pregnancy outcomes in addition to previously mentioned parameters.

Risk of bias was assessed for each included study using the Cochrane Handbook for Systematic Reviews of Interventions [6]. Two reviewers (V.T., A.L) independently assessed the quality of the selected studies. A third investigator (L.P) was involved in the case of inconsistencies. In accordance with the Cochrane Handbook for Systematic Reviews of Interventions, the RoB 2 tool [7] was used to assess the risk of bias for randomized controlled trials and ROBINS-I [8] for non-randomized trials, SYRCLE's RoB tool for animal model studies [9].

Results

Summary of Included Studies

The study selection process is illustrated by the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow chart diagram (Figure 1).

A total of 512 publications were identified through an electronic database search of PubMed, Google Scholar, and ClinicalTrials.gov., Cochrane library.

Of these, 425 studies were screened for title and abstract. 388 were excluded, 38 were selected for eligibility assessment. After screening the full-text articles, 27 were excluded for failing to meet the eligibility criteria. The references of the selected studies were additionally searched for other eligible studies and 7 studies were identified. Finally, 11 trials that met the criteria were included in the systematic review. A total of 73 rats, 14 mice and 759 women from ten trials were analyzed. The detailed summary of the studies analysis is shown in Table 1-2.

Antioxidants effectiveness in animal population

In 4 studies, endometriosis was induced by transplanting autologous uterine tissue onto the peritoneal wall of female Wistar albino rats [10,11] and female NMRI mice [12,13].

The vitamin C effectiveness in animal population

Ozlem Ulas Erten et al [10] and Yildirim Durak et al [11] evaluated the efficacy of vitamin C in the prevention and regression of endometriotic implant development in an experimentally induced autografted endometriosis rat model. Implant weight and volume, histological cell scores and trichrome fibrosis scores were measured. Both authors suggest that histological scores are independent of vitamin C dosage, in contrast to implant volume. However, the exact reason for their insensitivity is not mentioned. It could either be due to the small number of animals included or the lack of direct effects.

Hayedeh Hoorsan et al [12] conducted a study on the efficacy of vitamin C endometriosis treatment in the NMRI mouse model. In contrast to the previous two studies, the authors found a significant difference not only in the volume of endometriotic implants but also in the trichrome fibrosis scores ($p=0.03$). Follicle, atretic follicle and corpus luteum counts were also measured. The number of follicles was significantly increased ($p=0.0005$) and the number of atretic follicles was significantly decreased ($p=0.006$) after vitamin C therapy.

L-arginine, L-carnitine effectiveness in animal population

The other study based on the induced endometriosis mouse model was conducted by Eshrat Kalehoei et al [13]. The authors compared the effects of L-arginine (LA), L-carnitine (LC), bone morphogenetic stem cells (BMSC-CM) on endometriosis-induced oocyte quality and levels of oxidative stress markers. In the endometriosis group, mice treated with LC, LA or BMSC-CM had significantly lower levels of oxidative stress markers compared to control. In normal and endometriosis treatment groups In vitro fertilisation (IVF), cleavage and blastocyst formation rates were significantly improved compared to the control ($p < 0.05$).

Antioxidants effectiveness in human population

Other 7 studies were based on the follicular fluid and plasma samples collected from women with endometriosis related infertility.

The vitamin C effectiveness in human population

In human population, the efficacy of vitamin C supplementation was analyzed by Xiang Lu et al [14]. There was no significant difference in fertilization, implantation and pregnancy rates between all participants. The number of oocytes and frozen embryos in endometriosis groups was significantly lower than in control ($P<0.05$). Treatment with vitamin C for 2 months improved its serum and follicular fluid concentration in patients with endometriosis, however did not affect oxidative stress markers rate. The results could be as follows because of several limitations: small sample size and only one time point measurement (2 months of vitamin C supplementation): comparison of different time points was not performed.

N-acetyl-cysteine, L-carnitine effectiveness in human population

Vanessa S. I. et al. conducted two studies [15,16] in which the percentage of meiotically normal oocytes in metaphase II, presumptive zygotes, cleavage rate, blastocyst formation rate and hatching rate were measured. The number of meiotically abnormal and normal metaphase II oocytes was similar in all 9 groups. The authors suggest that follicular fluid (FF) from infertile women with endometriosis increases the percentage of meiotically abnormal oocytes. There was no significant difference between groups in cleavage ($p=0.54$) and blastocyst formation ($p=0.4349$) rates. However, the hatching rate was higher in the control follicular fluid group than in the endometriosis follicular fluid (EFF) group. The addition of antioxidants in CFF groups did not affect the hatching rate. The addition of N-acetylcysteine reduces the destructive effects of FF on the oocyte meiotic spindle and increases hatching rate. The addition of L-carnitine completely prevents this destructive effect on the meiotic spindle, but is less effective in terms of hatching rate.

The vitamin C and vitamin E combination effectiveness in human population.

The effect of combined vitamin C + vitamin E treatment was evaluated by Jennifer Mier-Cabrera et al [17], Nalini Santanam et al [18] and Leila Amini et al [19].

Jennifer Mier-Cabrera et al [17] analyzed the lipid hydroperoxide (LOOH) and malondialdehyde (MDA) rate in plasma and peritoneal fluid. There was a statistically significant difference in plasma LOOH and MDA concentrations between control and treatment groups. Leila Amini et al [19] held randomized, triple-blind placebo-controlled clinical trial, where statistically reduced MDA ($p=0.002$) and reactive oxygen species (ROS) ($p < 0.001$) levels in treatment group compared to placebo were evaluated.

Anti- myeloperoxidase therapy human population

Nalini Santanam et al [18] suggested that the level of myeloperoxidase (MPO) - one of the oxidative stress markers - depends on the severity of endometriosis. Mean MPO levels in follicular fluid collected from women with severe endometriosis were significantly higher than in control and mild endometriosis groups. Combination antioxidant treatment did not significantly reduce MPO levels in both groups.

Astaxanthin therapy in human population

Sahar Rostami et al. conducted a study on the efficacy of astaxanthin (AST) on oxidative stress markers, cytokine levels and associated reproductive technology (ART) outcomes in infertile women with endometriosis [20]. All parameters were reduced after antioxidant therapy

except serum catalase (CAT), IL-1b, IL-6 levels. Embryo quality, number of metaphase II oocytes improved significantly after therapy. However, the number of embryos transferred, fertilisation rate and pregnancy rate were similar in both groups.

Visualisation tools were provided by the ROBVIS application [21]. According to the ROBINS-I tool, the overall risk of bias for non-randomised trials was 66,7 % low and 33,3% serious (Figure 2). Based on the RoB 2 tool (Figure 3), randomized trials had a 75% chance of low risk of bias and an 25% chance of some concern regarding the overall risk of bias. The SYRCLE's RoB tool was used to assess the quality of included animal studies (Figure 4). The risk of allocation concealment and random housing could not be confirmed because none of the studies offered complete information.

Discussion

There is increasing evidence to suggest that specific diet patterns and nutrients may modulate the pathophysiological processes underlying endometriosis.

In this systematic review, we evaluated the efficiency of antioxidants supplementation in endometriosis treatment. We found out that antioxidants reduce the severity of endometriosis symptoms by affecting the pathogenesis of the disease.

Oxidative stress occurs when the balance between reactive oxygen species production and antioxidant capacity is disturbed, either by insufficient antioxidant protection or by increased ROS production. The relationship between ROS production and the progression of endometriosis has been studied previously [22]. Due to dysregulation of iron metabolism, these abnormal endometriotic lesions are thought to be resistant to ferroptosis. Ferroptosis is a form of regulated, iron-catalyzed cell death caused by excessive lipid peroxidation in cell membranes. This process was first described by Dixon in 2012 [23].

Li B et al [24] found out that there was an excess expression of ferroptosis-associated genes in the ectopic and eutopic endometrium in patients with endometriosis, showing a general trend towards inhibition of the ferroptosis pathway. Increased transferrin receptors (TFR1) and Ras gene mutations in abnormal endometriotic cells directly affect ferroptosis resistance. A local imbalance in iron homeostasis leads to oxidative stress in the intraperitoneal cavity, inflammation and ferroptosis in intact peripheral tissues. Iron-dependent ROS synthesis is based on the Fenton reaction: $Fe2+ + H2O2 \rightarrow Fe3+ + OH- + OH-$. As a result, a hydroxyl radical (-OH) is formed, leading to lipid peroxidation and accumulation of lipid LOOH, which damages the membrane. This is why ectopic endometriotic tissue has higher levels of lipid peroxidation products than normal endometrial tissue. This is also confirmed by other studies [17, 25].

In addition, we observe changes in enzyme levels - SOD and indicators such as TAC, TOS and OSI in serum and FF. The decrease in TAC and SOD between patients with and without endometriosis is confirmed. It is noted that there was a significant difference in this indicator in FF as opposed to serum between both groups [14]. Total antioxidant response (TAR) is also lower in patients with endometriosis, leading to an excess of OSI [26]. Treatment with AST improved TAC and SOD levels [19]. LC and LA administration also improved the TAC, reduced TOS, NO and OSI ($P<0.05$) [16]. However, vitamin C treatment showed no difference in oxidative stress markers and enzyme levels [14]. But there is evidence that vitamin C prevents the progression of endometriotic lesion development by reducing their weight, size and volume [10-12].

Vitamin C and vitamin E combination significantly suppressed levels of MPO (a neutrophil marker that is increased due to oxidative stress and depends on the severity of endometriosis) in FF [18]. These findings support previous data [26].

It should be mentioned that immune cells play a crucial role in ectopic endometriotic lesions detection and elimination. It is known that oxidative stress impairs the efficiency of the immune system, leading to reduced recognition of abnormal endometrial tissue, allowing its invasion, accumulation and growth in the pelvic and abdominal cavity. Antioxidants are known to stimulate the whole process of phagocytosis [27]. Yildirim Durak et al [11] found that NK cell (Natural killer cells) levels were significantly lower in control groups than in those on antioxidant therapy ($P < 0.01$). Similar results have been reported in other studies [28,29]. It should be noted that the decrease in cellular immunity correlates with the severity of the disease. Whether this decrease in NK cells is a cause or a consequence of the severity of endometriosis remains unclear. The reduction in cellular immunity is related to the "endometriotic disease theory", also known as Sampson's theory, according to which the most important factor in the development of endometriosis is not the initial implantation in the peritoneal cavity, but cell mutations that cannot be eliminated due to the reduced number of immune cells. It is these ectopic endometriotic cells, ignored by regulatory factors in the peritoneal fluid, that trigger the disease [30].

All of the above factors affect fertility in women with endometriosis. The granulosa cells and the surrounding cumulus cells in the follicle are involved in the maturation of the oocyte. This process depends on the intrafollicular environment. If it is damaged, the developmental competence of the oocytes, the quality of the embryos and the clinical pregnancy rate are reduced. In the case of antioxidant therapy, IVF, cleavage and blastocyst formation rates are increased compared to no treatment. It is also important to highlight that this finding is potentially helpful for translation into clinical practice [13-16,19]. High-level antioxidant diet can significantly influence inflammatory processes, which are directly related to the pathophysiology of endometriosis.

Strengths and limitations of the study

The limitations we encountered were mainly related to the available data sources. The patients were not similar between studies: rats and mice with induced endometriosis, human. The heterogeneity of the antioxidants should also be mentioned. A total of 6 antioxidants were included in this review, but it is difficult to compare them because of differences in regimen and dosage in each study. Studies in animal models have a lower quality of evidence than those in humans. It is important to emphasize that more research is needed in human to assess the clinical relevance and to establish the efficacy of antioxidants, as clinical trials evaluating their effects on endometriosis are still relatively limited.

The main strength of this study is that we observed antioxidant supplementation as a therapy that affect oxidative stress – the main aspect of endometriosis pathogenesis. All previously published reviews were aimed to analyze the types of oxidative stress markers and their levels in patients with endometriosis, but did not observe and summarize any medications for their reduction.

Implications for future studies may include investigating the development of targeted antioxidant treatment, the possibility of delivering antioxidants directly to endometriotic lesions. This could potentially increase the efficacy of antioxidant therapy and minimize potential side effects.

Conclusion

Antioxidants may be considered as a possible component of endometriosis therapy to potentially enhance fertility outcomes and slow disease progression, though current evidence is preliminary and requires further validation. This type of treatment reduces oxidative stress markers concentration, suspend endometriotic lesions progression and improve oocyte developmental competence. However, there is still controversy about the antioxidant treatment as a monotherapy of endometriosis. That is why more clinical trials to make stronger recommendations is needed.

Compliance with Ethical Standards

Authors' contribution

L.P., E.N., S.I., V.T., A.L., and V.S. contributed to **Conceptualization**. E.N., I.S., and A.L. were responsible for **Data curation** and **Writing – original draft**. L.P., V.T., and V.S. contributed to **Writing – review & editing**. All authors read and approved the final version of the manuscript.

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Study registration

PROSPERO CRD42023454705.

Disclosure of interests

The authors declare that they have no competing interests.

Ethical approval

Not applicable. This article is a narrative review and does not involve human participants, animal subjects, or medical records.

Informed consent

Not applicable.

Data sharing

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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Table 1. Description of selected studies included in the review (rats and mice)

First author, year of publication	Title	Population	Follow up period	Intervention	Comparison	Outcomes
Ozlem Ulas Erten et al., 2016 [10]	Vitamin C is effective for the prevention and regression of endometriotic implants in an experimentally induced rat model of endometriosis	Female Wistar Albino Rats (n=33) Weight=209g-270g A group (n=11) B group (n=11) C group (n=11)	42 days	Surgical induced endometriosis (autograft model) A group: 1st operation + intravenous vitamin C 500mg/kg every 2 days B group: 1st operation; 2nd operation + intravenous vitamin C 500 mg/kg every 2 days C group (1st operation; 2nd operation)	Vitamin C vs no vitamin C in all groups	*Implant volume* at the 2nd operation (mm^3) *Implant volume* at the 3rd operation (mm^3) *Weight 1 (initial) (g)* *Weight 2 (final) (g)* *Histological cell scores* *Trichrome fibrosis scores*
Yildirim Durak et al., 2013 [11]	Effect of vitamin C on the growth of experimentally induced endometriotic cysts.	Female Wistar Albino Rats (n=40) V1 group (n=10) V2 group (n=10) V3 group (n=10) C group (n=10)	6 weeks	Surgical induced endometriosis (autograft model) V1 group: 0.5 mg (2mg/kg) vit C/1 mL water for 6 weeks V2 group: 1.25 mg (5 mg/kg) vit C/1 mL water for 6 weeks V3 group: 2.5 mg (10 mg/kg) vit C/1 mL water for 6 weeks C group: distillated water 1 mL for 6 weeks Final surgical assessment	Vitamin C vs no vitamin C in all groups	*Implant volume* after opertaion (mm^3) *Weight of cyst (mg)* *Histological cell scores* *Trichrome fibrosis scores* *NK cell contents*
Eshrat Kalehoei et al., 2023 [13]	Therapeutic effects of L-arginine, L-carnitine, and mesenchymal stem cell-conditioned medium on endometriosis-induced oocyte poor quality in an	Adult female NMRI mice (6–8 weeks old). (n=not stated)		1. EMS induction 2. IVF	1. control 2. 250 mg/kg LA 3. 250 mg/kg LC	1. In vitro maturation of immature oocytes: *GV (%)*

	experimental mouse model	1. Normal group 2. EMS-induced group		LA: 250 mg/kg LC: 250 mg/kg LC BMSC-CM: 100 µL of CM/mouse	4. 100 µL BMSC-CM	*GVBD* (%) *MII* (%) *DEG* (%) 2. Blastocysts cell number *N. Blast* *N. total cells* *N. TE* *N. ICM* *TE/ICM* 3. The percentage of different steps of mice emb development *N. MII* *IVF (%)* *Cleavage (%)* *Morula (%)* *Blastocyst (%) Degenerated (%)* 4. blood serum antioxidant capacity *TAC (nmol/mL)* *NO (nmol/mL)* *TOS (nmol/mL)* *OSI* All these outcomes are assessed in both EMS a normal groups and according to different antioxi therapy (CO, LA, LC, BMSC-CM).

Hayedeh Hoorsan et al., 2022 [12]	The effectiveness of antioxidant therapy (vitamin C) in an experimentally induced mouse model of ovarian endometriosis	Mature, virgin female NMRI mice (n=14) A group (n=7) B group (n=7)	Not stated	Surgical induced endometriosis (autograft model) 2nd surgery (assessment of the endometriotic implants) A group: 50 mg/kg (0.5 mL) vit C every 2 days for 4 weeks B group: a 0.5 mL mix of water and starch Final surgical assessment	Vitamin C vs no vitamin C in all groups	*Implant volume* at the 2nd operation (mm^3) *Implant volume* at the 3rd operation (mm^3) *Weight 1 (initial) (g)* *Weight 2 (final) (g)* *Histological cell scores* *Trichrome fibrosis scores* *Follicle number* *Atretic follicle number* *Corpus luteum number*

Abbrevations: NK, natural killer; vit C, vitamin C, EMS, endometriosis; IVF, in vitro fertilization; LA, L-arginine; LC, L-carnitine; BMSC-CM, bone morphogenetic stem cells GV: Germinal vesicle; GVBD: Germinal vesicle break down; MII, metaphase II; DEG: degenerate oocytes; GVBD: Germinal vesicle break down; TOS, total oxidant status; NO, nitric oxide; TAC, total antioxidant capacity; OSI, oxidative stress index; CO, control.

Table 2. Description of selected studies included in the review (patients - human)

First author, year of publication	Title	Population	Follow up period	Intervention	Comparison	Outcomes
Vanessa S. I. et al., 2015 [15]	N-Acetyl-Cysteine and L-Carnitine Prevent Meiotic Oocyte Damage Induced by Follicular Fluid From Infertile Women With Mild Endometriosis	FF samples from infertile women (n=22) 1. EMS-associated infertility (n=11) 2. other infertility (n=11)	February 2009 - February 2011	1. Laparoscopic surgery in women with EMS. 2. FF-collection 3. Bovine oocyte collection 4. In Vitro Maturation 1.NAC 1.5 mmol/L 2. LC 0.6 mg/mL 3.NAC 1.5 mmol/L + LC 0.6 mg/mL	1. (No-FF) 2. (CFF) 3. (C + NAC 1.5 mmol/L) 4. (C + LC 0.6 mg/mL) 5. (C + 2Ao); 6. (EFF) 7. (E + NAC 1.5 mmol/L) 8. (E + LC 0.6 mg/mL) 9. (E + 2Ao).	*MI*, n (%) *TI*, n (%) *PA*, n (%) *Total no. of MII*, n (%) *Analyzable MII*, n (%) *Normal MII*, n (%)
Vanessa S. I. et al., 2021 [16]	Follicular Fluid from Infertile Women with Mild Endometriosis Impairs In Vitro Bovine Embryo Development:	FF samples from infertile women (n=22)	February 2009 - February 2011	1. Laparoscopic surgery in women with EMS. 2. FF-collection 3. Bovine oocyte collection	1. (No-FF) 2. (CFF) 3. (C + NAC 1.5 mmol/L)	*Presumptive zygotes* (n) *Cleavage rate %* (n)

	Potential Role of Oxidative Stress	1. EMS-associated infertility (n=11) 2. other infertility (n=11)		4. In Vitro Maturation 5. In Vitro Fertilization 6. n Vitro Embryo Culture 1.NAC 1.5 mmol/L 2. LC 0.6 mg/mL 3.NAC 1.5 mmol/L + LC 0.6 mg/mL	4. (C + LC 0.6 mg/mL) 5. (C + 2Ao); 6. (MEFF) 7. (MEFF + NAC 1.5 mmol/L) 8. (MEEF + LC 0.6 mg/mL) 9. (MEFF + 2Ao).	*Blastocysts formation rate %* (n) *Hatching rate %* (n)
Xiang Lu et al., 2018 [14]	Effects of vitamin C on the outcome of in vitro fertilization–embryo transfer in endometriosis: A randomized controlled study	Patients with EMs (n=280) ✓ Group 1 – Vit C treatment (n=160) ✓ Group 2 – no vit C (n=120) Patients with no EMs (n=150)	June 2013 - December 2016.	1. IVF-ET procedure 2. Vit. C treatment Group 1 (n=160) received 1000 mg/day from 2 months before IVF-ET treatment until 2 weeks after ET	EMS patients vs no EMS patients. EMS patients treated with vit C/ not treated with vit C	1. Laboratory and pregnancy outcomes in EMS patients/ no EMS patients *Total Gn dosage* *No. of retrieved oocytes* *Fertilization rate* (%) *High-grade embryo rate* (%)

						Implantation rate (%) *Clinical pregnancy rate* (%) No. of frozen embryos 2. Changes in serum levels of VitC and oxidative stress markers in EMS patients with vit C/ no vit C *Serum levels of VitC* (μmol/L) *Serum levels of SOD* (U/L) *Serum levels of TAC*(mmol/L) *Serum levels of MDA*(μM) *Serum levels of ROS*(cps)

Jennifer Mier-Cabrera et al., 2008 [17]	Effect of vitamins C and E supplementation on peripheral oxidative stress markers and pregnancy rate in women with endometriosis	Patients with EMs (n=34) Group 1 – Vit C and Vit E treatment (n=16) Group 2 – placebo (n=18)	15 months	Group 1 - 343 mg of vitamin C and 84 mg of vitamin E	Vit C and vit E patints group vs placebo group	1. Oxidative stress marker levels in women with endometriosis ((Baseline, at 2 months, at 4 months, at 6 months in PF, plasma). *LOOH* (µmol/L) *MDA* (µmol/L) 2. Pregnancy rate
Nalini Santanam et al., 2016 [18]	Myeloperoxidase as a Potential Target in Women With Endometriosis Undergoing IV	Patients (n=117) Complete data (n=68). No EMs group (n=41) Mild EMs group (n=20)	Not stated	1. IVF 2. Collection of FF 3. Collection of Blood Plasma Patients received 800 IU of vit E and 1000 IU of vit C for a minimum of 8 weeks:	Vit C and vit E patints group vs placebo group	*MPO level* (ng/ml)

		Moderate/severe EMs group (n=7)		No EMs group vit C+E (n=5) Mild EMs group vit C+E (n=5) Moderate/severe EMs group vit C+E (n=4)		
Sahar Rostami et al., 2023 [20]	Astaxanthin ameliorates inflammation, oxidative stress, and reproductive outcomes in endometriosis patients undergoing assisted reproduction: A randomized, triple-blind placebo-controlled clinical trial	Infertile patients (n=73) with EMs. Complete data (n=50). AST group (n=25) Placebo group (n=25)	December 2021 - September 2022.	1. IVF 2. Blood and FF collection AST group: 6 mg daily of oral AST for 12 weeks Placebo group: 6 mg daily of placebo capsules for 12 weeks	AST treatment vs placebo	1. OS markers and cytokine levels *MDA* *SOD* *CAT* *TAC* *L-1b* *IL-6* *TNF-a* 2. ART outcomes

						Number of oocytes *GV* *MI* *MII* *Oocyte maturity rate* (MII %) *Fertilized* *Fertilization rate* (%) *Number of frozen embryos* *High-quality embryos* *Frozen embryos* *Number of transferred embryos*

Leila Amini et al., 2021 [19]	The Effect of Combined Vitamin C and Vitamin E Supplementation on Oxidative Stress Markers in Women with Endometriosis: A Randomized, Triple-Blind Placebo-Controlled Clinical Trial	Patients with endometriosis (n=60) A group (n=30) B group (n=30)	June 2017 - November 2017	A group: vitamin C 1000 mg/day (2 tablets/500 mg) + vitamin E 800 IU/day (2 tablets/400 IU) for 8 weeks. B group (placebo pills (mannitol and magnesium stearate polyvinylpyrrolidone)) for 8 weeks.	Vit C and vit E patints group vs placebo group	1. OS markers levels *MDA* *ROS* *TAC* 2. VAS score of dysmenorrhea, dyspareunia

Abbrevations: CFF, control follicular fluid; OS, oxidative stress; ROS, reactive oxygen species; LC, L-carnitine; NAC, N-Acetyl-Cysteine; BMSC-CM, bone morphogenetic stem cells; TOS, total oxidant status; NO, nitric oxide; TAC, total antioxidant capacity; OSI, oxidative stress index; IVF, In vitro fertilization; SOD, superoxide dismutase; MDA, malondialdehyde; FF, follicular fluid; EFF, endometriosis follicular fluid; CFF, control follicular fluid; LOOH, lipid hydroperoxide; MPO, myeloperoxidase; AST, astaxanthin; ART, associated reproductive technology; CAT, catalase; TAR, Total antioxidant response; NK, Natural killer; EMS, endometriosis; TNF-a, Tumor necrosis factor; IL-1b, interleukin 1b; IL-6, interleukin 6; GV, germinal vesicle; MI, metaphase I. PA, spontaneous parthenogenetic activation; TI, telophase

Figure 1. PRISMA flow chart diagram. The effectiveness of antioxidant therapy in women with endometriosis.

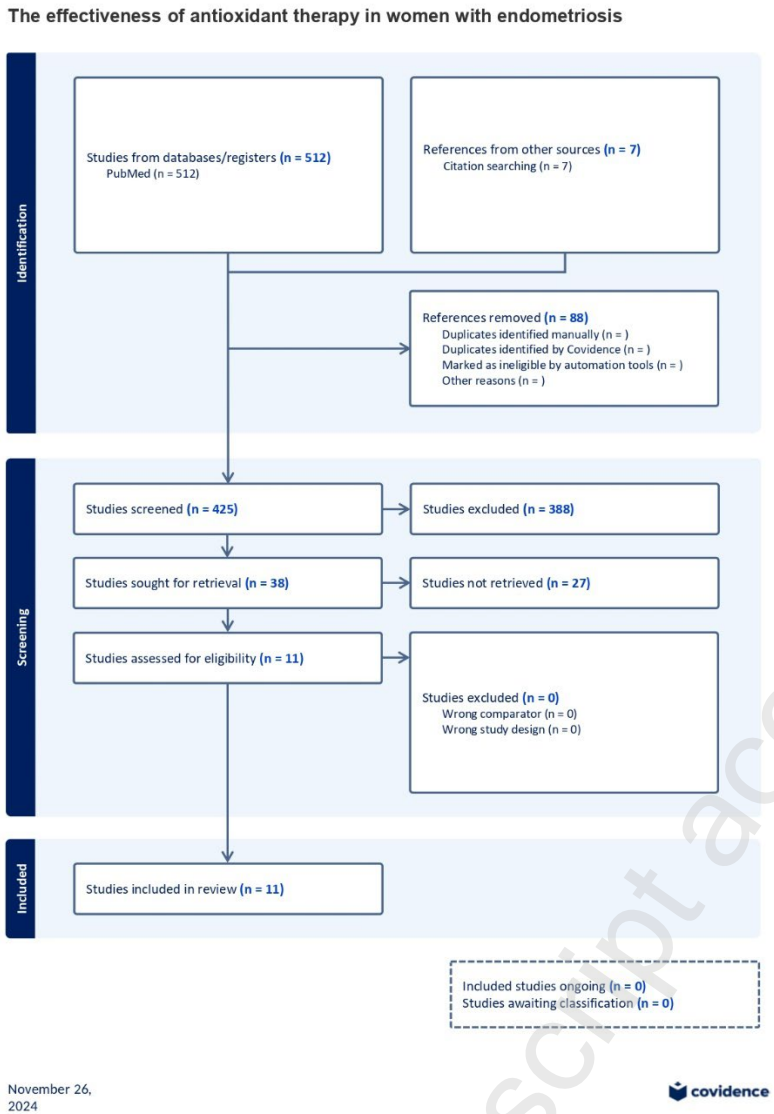
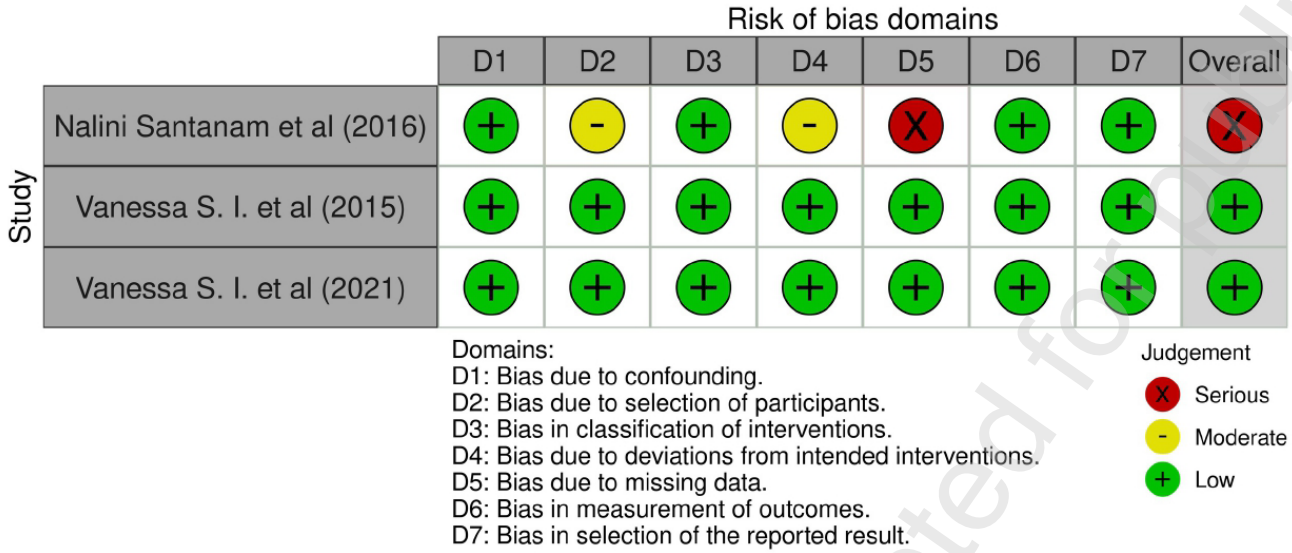
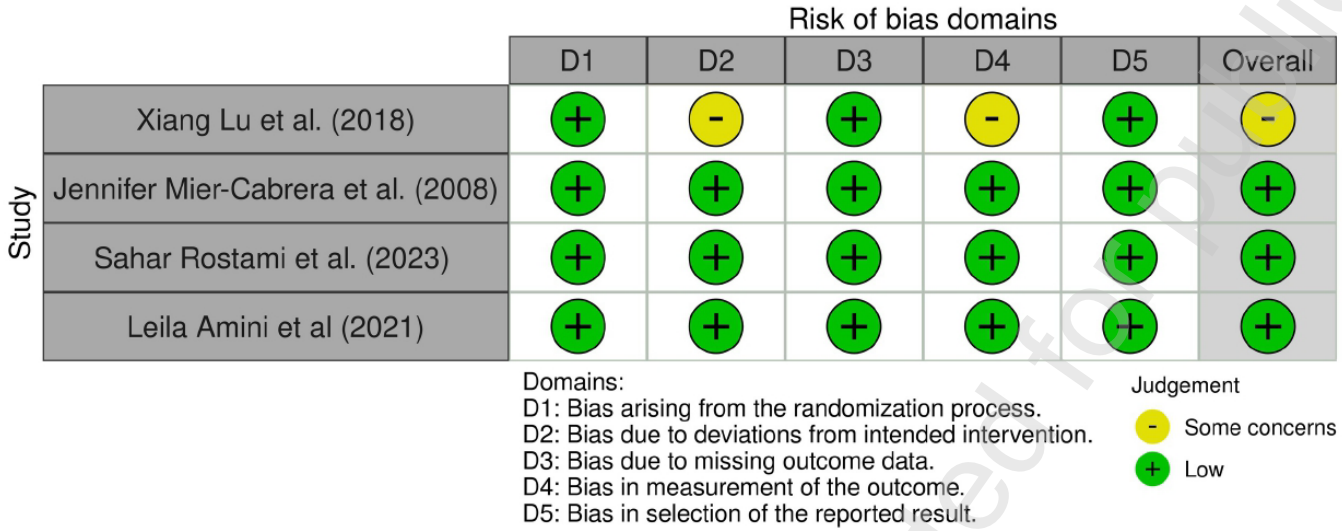


Figure 2. ROBINS-1 tool for non-randomized trials



Domains:
D1: Bias due to confounding.
D2: Bias due to selection of participants.
D3: Bias in classification of interventions.
D4: Bias due to deviations from intended interventions.
D5: Bias due to missing data.
D6: Bias in measurement of outcomes.
D7: Bias in selection of the reported result.

Figure 3. RoB2 tool for randomized trials



Domains:
D1: Bias arising from the randomization process.
D2: Bias due to deviations from intended intervention.
D3: Bias due to missing outcome data.
D4: Bias in measurement of the outcome.
D5: Bias in selection of the reported result