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ORIGINAL ARTICLE

Astaxanthin treatment decreases pro-inflammatory cytokines and improves reproductive outcomes in patients with polycystic ovary syndrome undergoing assisted reproductive technology: A randomized clinical trial

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Abstract

Research question In a randomized, triple-blind, placebo-controlled clinical trial (RCT), we investigated the effect of astaxanthin (AST) on pro-inflammatory cytokines, oxidative stress (OS) markers, and assisted reproductive technology (ART) outcomes in 44 infertile Polycystic Ovary Syndrome (PCOS) patients.

Design Patients with PCOS were randomly divided into two groups. The intervention group received 6 mg AST, and the control group received placebo daily for 8 weeks. Blood samples were obtained from all patients before and after intervention and follicular fluid (FF) was collected during the ART procedure. Interleukin (IL) -6, IL-1β were evaluated from serum samples and FF and OS markers (malondialdehyde [MDA], catalase [CAT], superoxide dismutase [SOD], and reactive oxygen species [ROS]) were measured from FF. The groups were compared for ART outcomes as well.

Results A significant decrease in IL-6 and IL-1β concentrations (both, $P = < 0.01$) serum levels was found following AST treatment. FF cytokine levels and OS markers did not differ significantly between the groups. Reproductive outcomes, including the number of oocytes retrieved ($P = 0.01$), the MII oocyte count ($P = 0.007$), oocyte maturity rate (MII %) ($P = 0.02$) and number of frozen embryos ($P = 0.03$) significantly improved after intervention. No significant differences were found in chemical, clinical and multiple pregnancies between the groups.

Conclusions AST pretreatment may modify inflammation and improve ART outcomes in PCOS infertile patients. Further investigations are recommended to verify these findings.

Keywords Polycystic ovary syndrome · Astaxanthin · ART · Inflammation · Oxidative stress · Infertility

Introduction

Polycystic ovary syndrome (PCOS), is an endocrine and metabolic disorder (Singh et al. 2023). The World Health Organization reports that PCOS has a prevalence rate of 2–26% worldwide (Jaripur et al. 2022). Because of its reproductive, metabolic, and psychological features, PCOS is a significant public health concern (Azziz et al. 2016).

Three major features distinguish PCOS: irregular menstruation, hyperandrogenism, and polycystic ovarian morphology (Islam, Masud, Islam, and Haque 2022). Despite decades of intensive research, pathology and etiology of this disease remain unknown. Various internal and external factors have been implicated, including insulin resistance (IR), hyperandrogenism, epigenetics, genetics, and environmental factors (Sadeghi et al. 2022). There is substantial evidence that chronic low-grade inflammation plays a crucial role in the pathogenesis of PCOS (Barrea et al. 2018; Li et al. 2019). There have been studies that indicate PCOS patients have higher levels of interleukin (IL)-6 and IL-1β than healthy women (Bednarska and Siejka 2017; Rostamtabar et al. 2021). In several studies, reactive oxygen species (ROS) production has been demonstrated to

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affect ovarian physiology and negatively affect reproductive function. The imbalance of ROS production stimulates inflammatory signaling pathways, resulting in cystic ovarian dysfunction and abnormal ovulation (Boots and Jungheim 2015).

Treatment options are determined by the target patient and her priorities. There are a variety of complications, such as infertility treatment, hormonal regulation, weight loss, or management of hyperandrogenic symptoms, such as acne, androgenic alopecia and hirsutism. Individuals should receive a customized approach to achieve the most effective results. Currently, there is no one ideal treatment for all women with PCOS, so physicians must treat them symptomatically (Helvaci and Yildiz 2023; Sadeghi et al. 2022). Metformin and thiazolidinediones were previously used, but both had side effects. (Kiani, Donato, Dhuli, Stuppia, and Bertelli 2022). Natural molecules from nutritional supplements and herbal medicines have recently achieved satisfactory results with no side effects (Günalan, Yaba, and Yılmaz 2018; Kiani et al. 2022).

However, supplementation with natural molecules has a significant impact on the oxidative stress (OS) associated with PCOS as well as its associated complications (Dubey et al. 2021). Herbal medicines may be effective treatment for PCOS patients with infertility and IR (Arentz, Abbott, Smith, and Bensoussan 2014; Brenjian et al. 2020).

Astaxanthin (3,3′-dihydroxy-β, β′-carotene-4,4′-dione) (AST) is a red fat-soluble carotenoid that is known as one of the strongest antioxidants in nature. It can be found in a variety of microorganisms and marine animals (Ambati, Phang, Ravi, and Aswathanarayana 2014; Tian et al. 2021). In addition to having strong antioxidant properties, AST has anti-inflammatory, anti-apoptotic, anti-proliferative, antidiabetic, anticancer, and skin-protective, DNA repair, cell regeneration, neuroprotective and immune modulatory effects, making it a valuable multi-target pharmacological agent (Chang and Xiong 2020; Si and Zhu 2022). Anti-inflammatory mechanisms of AST involved in targeting multiple signaling pathways in addition to inflammatory biomarkers (Chang and Xiong 2020). It can downregulate inflammatory cytokine, such as IL-1β, IL-6, and tumor necrosis factor-alpha (TNF-α) (Tian et al. 2021). Supplementing with AST has been found to reduce inflammation in young soccer players due to rigorous physical training (Baralic et al. 2015). AST reduced iNOS and COX-2 expression in human keratinocytes by reducing mRNA and protein expression (Yoshihisa, Rehman, and Shimizu 2014). This natural molecule significantly inhibited COX-2, iNOS, and ICAM-1 protein expressions in streptozotocin-induced diabetic rats, indicating that it inhibits inflammation resulting from diabetic liver dysfunction (Park et al. 2015). Moreover, this ketocarotenoid reduces circulating levels of IL-6 and MDA in type 2 diabetes patients (Shokri-Mashhadi, Tahmasebi, Mohammadi-Asl, Zakerkish, and Mohammadshahi 2021).

AST targets a variety of molecules and pathways, including nuclear factor erythroid 2-related factor 2 (Nrf2), nuclear factor-kappa B (NF-κB), phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt), Mitogen activated protein kinase (MAPK), Wnt/β-catenin, Janus kinase 1(JAK1)/transduction and the activators of the transcription-3 (STAT3) (Brasil et al. 2021; Cai et al. 2019; Song et al. 2012; Xu, Zhang, Jiang, and Zhang 2015; Zarneshan, Fakhri, Farzaei, Khan, and Saso 2020).

To counteract OS, AST inhibits extracellular signal-regulated kinase (p-ERK)/ERK and stimulates nuclear factor erythroid 2-related factor 2 (Nrf2)/antioxidant response element (ARE). This signaling pathway activates antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (Zarneshan et al. 2020). ROS stimulate NF-κB signaling pathway, which controls the production of pro-inflammatory chemokines and cytokines. AST reduces inflammation by preventing ROS-mediated induction of these transcription factors (Zarneshan et al. 2020).

Supplementation with AST for infertility management has been examined in many studies. An animal study showed that AST enhanced bovine oocyte quality and growth, while suppressing luteinization in granulosa cells (Chelenga et al. 2020). Furthermore, another study showed AST can increase the number of corpora lutea, implantation sites and fetuses (Patil, Kasabe, and Dandge 2022).Recently some research was also conducted clinical trials to examine the effect of AST on patients with infertility. The AST administration can reduce OS, inflammation and improve antioxidant response elements in female with infertility (Gharaei et al. 2022; Jabarpour, Aleyasin, Nashtaei, Lotfi, and Amidi 2023; Rostami et al. 2023). Thus, the present study examined the effects of AST supplementation on inflammation, OS, and reproductive outcomes in PCOS patients with infertility.

Materials and methods

The study design, randomization and blinding

There was a prospective randomized parallel triple-blind placebo-controlled study conducted from April 2022 to May 2023 at the Arash Hospital infertility clinic, Tehran, Iran. 44 Infertile women with PCOS aged 18–40 were enrolled according to Rotterdam criteria and recommended to undergo intracytoplasmic sperm injection (ICSI). This project registered at Iranian Registry of Clinical Trials and Tehran University of Medical Sciences' Ethics Committee approved this study (Reference number of the ethics committee: IR.TUMS.MEDICINE.REC.1400.1110).

The subjects did not have hyperprolactinemia, congenital adrenal hyperplasia, thyroid disease, Cushing's syndrome, ovarian tumors, autoimmune disease, systemic disorders, hyperlipidemia cardiovascular disease and severe male factor infertility. The participants did not use any type of oral contraceptive pill during the 3 month period prior to the study or any other medications that might affect their reproductive physiology.

The balanced block randomization method within a block size of 4 was used by an independent statistician to assign eligible patients to the AST or placebo groups (1:1). All researchers, patients, embryologists, statisticians and laboratory personnel were blinded to each patient's treatment plans (Fig. 1).

Intervention and controlled ovarian stimulation

Based on previous RCT studies, the therapeutic dose of AST is between 2 and 24 mg daily for at least 3 week(Brendler and Williamson 2019; Donoso, González-Durán, Muñoz, González, and Agurto-Muñoz 2021) In this study, treatment and placebo groups received 6 mg oral AST or placebo capsules (AstaReal® astaxanthin; AstaReal Co., Ltd., Tokyo, Japan) for 8 weeks before and during the routine gonadotropin-releasing hormone (GnRH) antagonist ovarian stimulation protocol. A placebo capsule had exactly the same color, size, shape, packaging, and taste as an AST capsule and made by the same company.

A flexible GnRH antagonist ovarian stimulation protocol was used in all patients during their IVF/ICSI cycles. Recombinant follicle stimulating hormone (rFSH) (150–300 IU/day, Gonal-F®, Merck Serono SA, Switzerland) was administered on day 2 or 3 of the menstrual cycle and

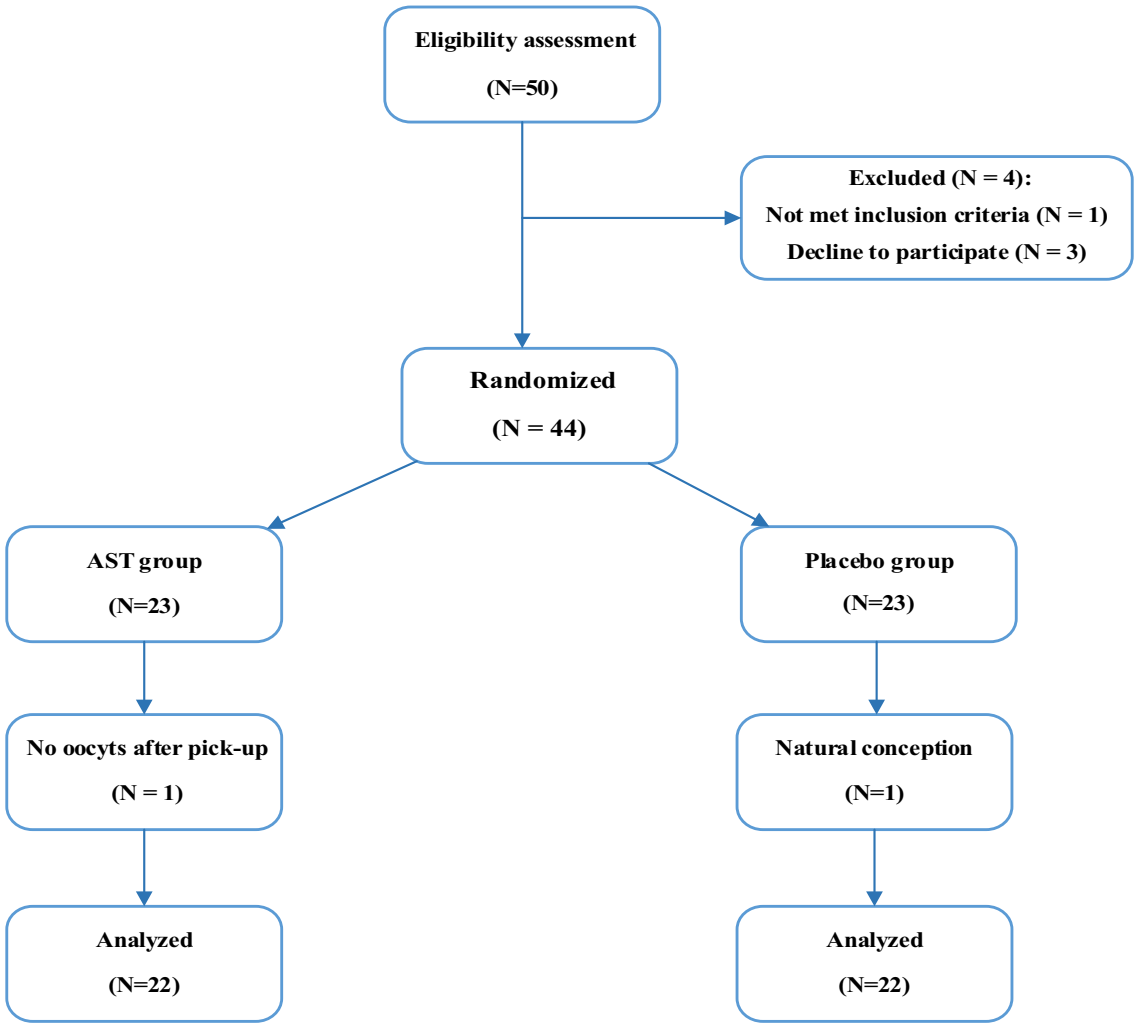

Fig. 1 Patient enrollment, randomization, follow-up and CONSORT diagram

continued until human chorionic gonadotropin (HCG) injection. In order to evaluate the ovarian response, serial transvaginal sonography (TVS) was performed. The GnRH-antagonist (Cetrotide®: 0.25 mg daily, cetrorelix acetate, Serono, Inc) was started when 2 or more follicles in size of ≥ 14 in diameter were observed. Cetrotide was stopped after at least two follicles reached a diameter of 18 mm. Final oocyte maturation was achieved by injecting 10,000 IU of HCG (Ovitrelle, Merck Serono SA, Switzerland). Under an ultrasound guide, the ovum pickup was planned 36 h after the trigger. All patients underwent ICSI.

Blood and FF collection and biochemical assessment

10 ml venous blood specimens were collected from all the patients before and after the intervention to assess pro-inflammatory cytokines (IL-1β, IL-6). After centrifuging the blood samples (1500 rpm for 10 min, 4 °C), the serum was separated and stored at – 80 °C until further analysis. During oocyte retrieval, FF was only aspirated from the first follicle to avoid blood contamination. The samples were centrifuged for 10 min at 3000 rpm(Kazemi et al. 2014; Pella et al. 2020), and the clarified supernatants were collected. The FF aliquots were also immediately stored at − 80 °C for OS marker measurement. FF was assessed for ROS, SOD, CAT activities and malondialdehyde (MDA) using human enzyme-linked immunosorbent assay (ELISA) kits (Zellbio GmbH, Germany).

The concentrations of pro-inflammatory cytokines in serum and FF were detected using human ELISA kits (Karmania Pars Gene Company; KPG, Iran). Pro-inflammatory serum cytokines were analyzed in duplicate, blinded, in pairs (pre/post-intervention) simultaneously, and in the same analytical run randomly.

ART outcomes

About 2 h after oocyte pick-up (OPU), cumulus-oocyte complexes (COCs) were denuded with hyaluronidase enzyme (Sigma®, USA). Under a stereo microscope (Olympus SZX7, Tokyo, Japan), oocytes were evaluated for quality and maturity. Based on maturity level oocytes were classified into germinal vesicles (GV), metaphase I (MI), and metaphase II (MII). The ICSI procedure was performed on MII oocytes, and the fertilization was assessed 17–20 h after insemination by observing two pronuclei (PN) and two polar bodies (PBs).

Oocyte number and quality were evaluated at the time of ICSI injection with regard to the oocyte maturity index (the ratio of MII oocytes to total oocytes collected (Uk et al. 2022)). Fertilization rate(FR) (Number of 2PN/2PB oocytes, 16-18 h post-insemination, divided by the number of MII oocytes injected (Uk et al. 2022)), number of frozen embryos, high-quality embryos (based on the ASEBIR criteria, grade A and grade B cleavage embryos ("The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting," 2011)), chemical and clinical pregnancy rate (the number of positive serum β-hCG per number of embryo transfers (ET) after 14 days from the day of ET and the number of observed gestational sacs per number of ET by ultrasonography six weeks after ET, respectively (Zafardoust, Ansaripor, Karimi, Hosseinirad, & Ataei 2022)) and multiple pregnancy rate (the number of multiple pregnancies × 100 per the number of clinical pregnancies) were also assessed.

Statistical analysis

Statistical analysis of the data was performed using SPSS (SAS Institute Inc, version 22, Cary, NC, USA). Results are presented as mean ± standard deviation (SD). Kolmogorov–Smirnov and Shapiro-Wilk tests were used to determine data normality. The independent and paired t-tests were used to compare continuous variables with normal distributions within and between groups. Additionally, the Mann-Whitney U test was used to analyze the data without normal distribution. For the evaluation of the correlation between the parameters, Pearson's correlation was used. Fisher's exact and Pearson's chi-squared tests were used to compare chemical, clinical, and multiple pregnancy rates. In order to test the correlation between serum and FF cytokines levels with ART outcomes, Pearson's correlation coefficient was used.

Results

A total number of 44 participants were randomly selected (Each group consists of 22 patients). The patients were enrolled between April 2022 to May 2023. Data on demographics and clinical characteristics are presented in Table 1. No statistically significant differences were found in age (29.86 ± 4.279 vs. 31.14 ± 6.213 P = 0.433), BMI (25.68 ± 2.603 vs. 25.52 ± 2.690 P = 0.841) and AMH (7.23 ± 1.494 vs. 8.04 ± 1.681 P = 0.1), duration of infertility (5.31 ± 2.798 vs. 6.23 ± 2.181 P = 0.234), FSH (4.75 ± 1.541 vs. 4.66 ± 0.849 P = 0.813), LH (8.47 ± 1.652 vs. 7.63 ± 2.481 P = 0.190) and TSH (2.56 ± 0.807 vs. 2.75 ± 0.647 P = 0.394) between the two groups at the start of the study. All 44 patients completed the research. Based on pill count-backs on returned bottles, the AST and placebo groups had adherence rates of 93% and 95%, respectively. During the intervention, no adverse effects or toxicity were reported by the patients. 80% of the AST group and 72% of the placebo group were diagnosed with primary Table 2 infertility.

Table 1 The demographics and hormonal characteristics of each group

	AST (n = 22)	Placebo (n = 22)	P-value
Age (years)	29.86 ± 4.279	31.14 ± 6.213	0.433
BMI (kg/m2)	25.68 ± 2.603	25.52 ± 2.690	0.841
AMH (µg/mL)	7.23±1.494	8.04 ± 1.681	0.1
Duration of infertility (years)	5.31 ±2.798	6.23 ± 2.181	0.234
FSH (mIU/mL)	4.75 ± 1.541	4.66 ± 0.849	0.813
LH (mIU/mL)	8.47 ± 1.652	7.63 ± 2.481	0.190
TSH	2.56 ± 0.807	2.75 ± 0.647	0.394

Data are presented as mean ± SD

AST Astaxanthin group, *BMI* body mass index [weight (kg)/height (m2)], *AMH* anti-müllerian hormone, *FSH* follicle-stimulating hormone *LH*, luteinizing hormone, *TSH* thyroid-stimulating hormone

A P value less than 0.05 is considered significant

Serum and FF cytokine parameters

The baseline and post-treatment values for the two groups (placebo and AST) are shown in Table2. Before AST supplementation, no statistically significant differences were found between the two groups in serum cytokine levels. A significant decrease in IL-6 (4.70 ± .794 vs 3.45 ± 1.112 P = <0.01) and IL-1β concentrations (5.64 ± .758 vs. 4.61 ± 0.561 P =<0.01) serum levels was found following AST treatment. After the intervention, the AST and placebo groups did not have significant differences in FF IL-1β and IL-6 levels (all P > 0.05).

FF oxidative stress profile

All the OS indicators are shown in Table 3. Assessment of OS parameters in FF showed that there was no statistically significant difference between the two groups (P > 0.05).

ART outcomes

After AST therapy, reproductive outcomes, including the number of oocytes retrieved (15.14 ± 4.794 vs. 11.36 ± 4.991 P = 0.01), the MII oocyte count (9.59 ± 3.459 vs. 6.50 ± 3.700 P = 0.007), oocyte maturity rate (MII %) (62.58 ± 12.246 vs. 53.63 ± 13.854 P = 0.02) and number of frozen embryos (7.41 ± 2.667 vs. 5.59 ± 2.806 P = 0.03) significantly improved. The high-quality embryos (P = 0.58) and fertilization rate (P =0.69) showed similar results in both groups. In addition, there were no significant differences in chemical (9/22 (40.9%) vs. 7/22 (31.8%) two-tailed P = 0.531), clinical (7/22 (31.8%) vs. 5/22(22.7%) two-tailed P = 0.498) and multiple (3/22

Table 2 Cytokine levels in each group

	AST, pre-intervention serum level	AST, post-intervention serum level	Paired P value	Placebo, pre-interventionserum levels	Placebo, post-intervention serum levels	Paired P value	P value-between-groups	AST, FF levels	Placebo, FF levels	t-test p value
IL-6	4.70 ±.794	3.45 ± 1.112	< 0.001	4.94 ± 1.186	4.90 ± 1.214	0.618	0.440	5.34 ± 1.784	5.65 ± 1.730	0.556
IL-1β	5.64 ±.758	4.61 ± 0.561	< 0.001	5.54 ± 1.191	9.13 ± 1.786	0.340	0.760	5.10 ± 1.162	4.63 ± 0.840	0.127

Data are presented as mean ± SD

AST Astaxanthin group, IL-1β, interleukin 1β; IL-6, interleukin 6

A P value less than 0.05 is considered significant

Table 3 OS markers in two groups

	AST	Placebo	P value between groups
MDA	1.06±0.330	0.97±0.294	0.317
SOD	0.36±0.046	0.38±0.052	0.187
ROS	9.32±1.09	9.56±1.549	0.554
CAT	0.57±0.099	0.61±0.103	0.213

Data are presented as mean ± SD

AST Astaxanthin group, *MDA* malondialdehyde, *SOD* superoxide dismutase, *ROS* reactive oxygen species, *CAT* catalase

A P-value less than 0.05 is considered significant

(13.6%) vs. 2/22 (9.1%) two-tailed P = 0.635) pregnancy rates between the groups.

Correlation analysis

According to correlation analysis, Serum IL6 correlated Table 4 positively with the number of oocytes retrieved (r_s = 0.481; P=0.02), MII count (r_s = 0.456; P = 0.03) and negatively with multiple pregnancy (r_s = − 0.356; P = 0.01). SerumIL-1β correlated positively with the number of oocytes retrieved (r_s = − 0.453; P = 0.03) and fertilization rate (r_s = − 0.418; P = 0.005). There was positive correlation between FF IL-1β and High-quality embryos (r_s = 0.320; P = 0.03). FF MDA and clinical pregnancy correlated negatively (r_s = − 0.312; P = 0.03). FF SOD positively correlated with chemical pregnancy rates (r_s = 0.395; P = 0.008) and clinical pregnancy rates (r_s = 0.379; P = 0.01).

Discussion

In this randomized, triple-blind, placebo-controlled clinical Table 5 trial investigating the antioxidant, anti-inflammatory, and fertility-promoting effects of AST supplementation on infertile patients with PCOS candidate for ART. The results showed that supplementation with 6 mg/day of AST for 8 weeks alleviated inflammation and improved ART outcomes.

The study indicated that AST reduced serum levels of IL-1β, IL-6; meanwhile, the concentration of these inflammatory markers in FF remains unchanged after AST administration.

Although there is growing prevalence of PCOS worldwide, the exact etiology and pathophysiology of PCOS remain unclear. There were two major factors responsible for this disorder. Insulin resistance, hyperandrogenism, inflammation, oxidative stress, and obesity are internal factors, whereas epigenetics, stress, environmental toxicants and diet are external factors (Sadeghi et al. 2022).

Inflammatory markers and low-grade inflammation have been associated with PCOS in recent studies (Rostamtabar et al. 2021).Women with PCOS have higher serum levels of IL6 and IL-1β than healthy women. Research has shown that IR and androgen levels are related to IL-6 levels. IL-1β regulates steroidogenesis, which is essential for ovulation and fertilization (Lin et al. 2011; Zafari Zangeneh, Naghizadeh, and Masoumi 2017).

A clinical trial demonstrated that AST supplementation could decrease plasma IL-6 levels in patients with type 2 diabetes(Shokri-Mashhadi et al. 2021). In a clinical trial study on women with endometriosis shown that serum IL-1β levels decreased after 6mg/day AST

Table 4 ART outcomes in AST and placebo groups

	AST	Placebo	P value between groups
Number of oocytes	15.14 ± 4.794	11.36 ± 4.991	0.014
GV	2.73 ± 1.486	2.65 ± 1.674	0.868
MI	2.74 ± 1.723	2.14 ± 1.390	0.218
MII	9.59 ± 3.459	6.50 ± 3.700	0.007
Oocyte maturity rate (MII %)	62.58 ± 12.246	53.63 ± 13.854	0.028
Fertilization rate (%)	76.25 ± 12.384	74.54 ± 16.383	0.698
Number of frozen embryos	7.41 ± 2.667	5.59 ± 2.806	0.033
High-quality embryos	4.68 ± 2.626	4.27 ± 2.334	0.588

Data are presented as mean ± SD

AST Astaxanthin group, Oocyte maturity rate, number of MII oocytes/ number of oocytes retrieved × 100; Fertilization rate, number of injected oocytes with 2 pronuclei (2 PN) and 2 polar bodies (2 PBs) 16–18 h post-insemination/ number of MII oocytes injected × 100; high-quality embryos, grade A/B cleavage embryos

A P value less than 0.05 is considered significant

Table 5 Correlations among pro-inflammatory cytokines and ART results

	number of oocytes		MII		Fertilization rate		Number of frozen embryos		High-quality embryos		Chemical pregnancies		Clinical pregnancies		Multiple pregnancies	
	r_s	P	r_s	P	r_s	P	r_s	P	r_s	P	r_s	P	r_s	P	r_s	P
Serum IL-6	0.481	0.023	0.456	0.033	0.089	0.565	0.254	0.096	0.187	0.225	− 0.016	0.918	− 0.094	0.544	− 0.356	0.018
Serum IL-1β	0.453	0.034	0.102	0.509	0.418	0.005	0.219	0.154	− 0.003	0.983	− 0.062	0.691	− 0.168	0.275	− 0.359	0.17
FF IL-6	0.013	0.932	− 0.053	0.732	− 0.054	0.728	− 0.124	0.422	− 0.114	0.463	0.009	0.953	0.023	0.884	0.074	0.633
FF IL-1β	0.289	0.057	0.252	0.099	0.019	0.905	0.246	0.108	0.320	0.034	0.186	0.226	0.145	0.348	− 0.062	0.687

MII, MII oocyte count; Fertilization rate, number of fertilized oocytes/ numbers of injected MII oocytes × 100; High-quality embryos, grade A/B cleavage embryos; Chemical pregnancies, pregnancies with a positive serum β-hCG; Clinical pregnancies, pregnancies with a definitive clinical sign. r_s, Pearson correlation coefficient

IL-1β interleukin 1β, *IL-6* interleukin IL-6

supplementation (Rostami et al. 2023). The mRNA expression levels of IL-1β, IL-6, CCL2, and CXCL2 decreased Table 6 significantly with AST in the colorectal carcinogenesis model of mice (Kochi et al. 2014). In addition, it has been reported that AST can block the NF-κB-dependent signaling pathway and inhibit the expression of downstream inflammatory mediators such as IL-1β, IL-6, and tumor necrosis factor-α (TNF-α) (Speranza et al. 2012).

OS is an internal factor that participates in PCOs etiology(Sadeghi et al. 2022; J. Zhang, Bao, Zhou, & Zheng 2019). Several studies reported that AST can ameliorate OS in several diseases (Kishimoto, Yoshida, and Kondo 2016; Wu, Xu, Chen, and Zhang 2020; X. S. Zhang et al. 2021);The present study did not find any significant changes in FF OS markers after AST supplementation. The results are in line with an RCT on PCOs which found no significant improvement in FF levels of the SOD and MDA after AST supplementation (Gharaei et al. 2022). Furthermore, another RCT showed that AST consumption had no effect on OS markers in the FF of endometriosis patients (Rostami et al. 2023). Also a study reported Growth hormone treatment did not significantly alter FF levels of TAC, MDA, and SOD in PCOS patients. However, treatment with growth hormone could reduce total oxidant status (TOS) and the OS Index (OSI) of the FF (Gong, Luo, et al., 2020). Therefore, Further studies will be required to determine TOS, OSI, total antioxidant status (TAS), pro-oxidant-antioxidant balance (PAB), and other biomarkers of OS following antioxidant therapy. Furthermore, the presence of AST and its oxidation fragments in FF also needs to be assessed in future studies. In addition, these findings can be explained by the following that FF serves as a complex microenvironment, derived from the diffusion of serum, plasma, and metabolites synthesized in the follicle wall. Granulosa cells and theca cells will modify this microenvironment later (Brinca et al. 2022). It is thus evident that the levels of OS markers in this fluid reflect the OS level of the ovaries and the systemic OS level (Bianchi et al. 2016). Furthermore, systemic OS occurs in association with hyperandrogenism, obesity, and IR. Recent studies have shown that PCOS patients with different phenotypes respond differently to various treatments and have different ART results (Clark et al. 2014; De Vos et al. 2018). Thus, the accuracy of the results may be increased by classifying the participants based on their PCOS phenotypic characteristics.

Despite the limited sample size, AST therapy resulted in a significant increase in number of oocytes, mature oocytes, and frozen embryos. The results of this study are consistent with those of some previous studies reporting that antioxidant supplementation improves ART outcomes in women with or without PCOS (Choi, Youn, and Shin 2011; Gharaei et al. 2022; Rostami et al. 2023). Thus, AST may provide

Table 6 Correlations among OS markers and ART results

	number of oocytes		MII		Fertilization rate		Number of frozen embryos		High-quality embryos		Chemical pregnancies		Clinical pregnancies		Multiple pregnancies	
	r_s	P	r_s	P	r_s	P	r_s	P	r_s	P	r_s	P	r_s	P	r_s	P
MDA	− 0.109	0.481	− 0.085	0.582	0.152	0.325	− 0.113	0.465	− 0.236	0.123	− 0.254	0.095	− 0.312	0.039	− 0.021	0.893
ROS	− 0.177	0.249	− 0.130	0.4	0.103	0.506	0.233	0.127	− 0.240	0.117	0.067	0.666	− 0.159	0.303	− 0.018	0.909
SOD	0.024	0.878	− 0.173	0.263	0.119	0.443	− 0.031	0.842	0.021	0.892	0.395	0.008	0.379	0.011	0.006	0.969
CAT	0.069	0.658	0.037	0.810	− 0.136	0.378	0.046	0.767	0.183	0.233	0.095	0.540	0.271	0.075	0.067	0.664

MII, MII oocyte count; Fertilization rate, number of fertilized oocytes/ numbers of injected MII oocytes × 100; High-quality embryos, grade A/B cleavage embryos; Chemical pregnancies, pregnancies with a positive serum β-hCG; Clinical pregnancies, pregnancies with a definitive clinical sign. r_s, Pearson correlation coefficient

MDA malondialdehyde, *ROS* reactive oxygen species, *SOD* superoxide dismutase, *CAT* catalase

promising protection against oxidative and inflammatory damage to oocytes and embryos.

High-quality embryo and FR were not significantly different between the groups. Despite excluding patients with male factors, fertilization may be affected by the quality of sperm. In addition, FR and High-quality embryo provide insight into the quality and maturity of gametes, as well as the competence of operators.

Moreover, in the current study, chemical, clinical, and multiple pregnancy rates were not significantly different between groups. A variety of factors can influence pregnancy outcome, such as male gamete quality, endometrial receptivity, the way embryos are handled, frozen and stored and Embryologist abilities in ET (Vlaisavljevic et al. 2021). There may be a need for further research comparing outcomes between fresh and frozen cycles with a larger sample size.

Based on a correlation analysis, serum IL-6 concentration was positively related to the number of retrieved oocytes as well as MII count, but negatively related to multiple pregnancy. Studies regarding serum IL-6 levels and the number of oocytes obtained from ART are rare. However, some authors suggested no relationship between serum level of pro-inflammatory factors and pregnancy achievement following ART(Fıçıcıoğlu et al. 2009; Ozgu-Erdinc et al. 2021). Serum IL-1β positively associated with number of retrieved oocytes and fertilization rate. According to the previous studies, an increase in serum IL-1β is associated with higher implantation and pregnancy (Karagouni, Chryssikopoulos, Mantzavinos, Kanakas, and Dotsika 1998; Kreines et al. 2018). Another study showed that IL-1β concentrations increased significantly in women who had a pregnancy through ART than in those who did not (Sequeira, Espejel-Núñez, Vega-Hernández, Molina-Hernández, and Grether-González 2015). We also found FF IL-1β was positively correlated with high-quality embryos. Previous studies suggested that serum and FF IL-1β concentrations associated with pregnancy success (Fıçıcıoğlu et al. 2009; Karagouni et al. 1998). However, the findings are controversial. In another study, it was found that FF IL-1β concentrations did not correlate with fertilization and embryo cleavage rates (Barrionuevo et al. 2000).

It was found that FF MDA was negatively correlated with clinical pregnancy. Study was reported that the quality of embryos in IVF-ET was strongly correlated with oxidative markers in FF, especially MDA and TOC. The quality of embryos is usually a good indicator of pregnancy success (Liu et al. 2021). In another study, females with negative IVF outcomes had significantly higher FF MDA (Kumar et al. 2018).

In our study, FF SOD was positively correlated with chemical and clinical pregnancy rates. SOD is an essential antioxidant in the body that protects against OS. Enzymes

are effective therapeutic agents against ROS-related diseases (Younus 2018). A study found that pretreatment with growth hormone reduced OS and increased SOD levels, improved oocyte quality and IVF outcomes (Gong, Zhang, et al. 2020a, b).

It is the first randomized, triple blind, placebo-controlled clinical trial to evaluate the effects of AST supplementation on OS markers, inflammatory cytokines, and ART outcomes in infertile PCO patients. Our study has some limitations that need to be considered. PCOS women have different phenotypic characteristics. As a result, a larger sample size is required to analyze different phenotypes separately. In addition, for more effective results different doses of AST and durations should be tried. We were also unable to assess late pregnancy outcomes, including live birth rate, the most relevant measure of ART success.

Conclusion

As a result, AST administration could be a promising supplement for improving PCO complications. Additionally, it may enhance ART outcomes in infertile PCO patients.

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Data availability The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Declarations

Conflict of interest The authors declare no conflict of interest

Informed Consent Informed consent was obtained from all subjects involved in the study

Institutional review board statement The experimental procedures used in this study were approved by Tehran University of Medical Sciences (Reference number of the ethics committee: IR.TUMS.MEDICINE.REC.1400.1110).

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