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Exploratory pilot trial of astaxanthin supplementation in PCOS patients at risk of OHSS with focus on RAGE–NFκB pathway

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Polycystic ovary syndrome (PCOS) is a major risk factor for ovarian hyperstimulation syndrome (OHSS) during controlled ovarian stimulation (COS). Oxidative stress and inflammation, mediated by the advanced glycation end products (AGE)–receptor for AGE (RAGE)–nuclear factor kappa-B (NFκB) pathway, contribute to OHSS development and impaired oocyte quality. Astaxanthin (AST), a potent antioxidant, may modulate this pathway. In this exploratory pilot trial using a triple-blind, randomized, placebo-controlled design, 44 PCOS patients at high risk for OHSS were assigned to receive AST ($n = 22$) or placebo ($n = 22$) adjunct to the COS regimen. COS was performed using a gonadotropin-releasing hormone (GnRH) antagonist protocol with individualized gonadotropin dosing. Stimulation characteristics, gonadotropin dose, and follicle distribution were comparable between groups. The mean number of retrieved oocytes was slightly higher with AST, and the oocyte maturity rate (OMR) was significantly greater. Estradiol and progesterone levels on trigger day were lower in the AST group, though not statistically significant. Molecular analyses showed reduced RAGE expression, a lower phosphorylated inhibitor of kappa-B (pIκB)/inhibitor of kappa-B (IκB) ratio in granulosa cells (GCs), and significantly decreased interleukin-6 (IL-6) in follicular fluid (FF), with vascular endothelial growth factor (VEGF) showing a downward trend. These findings suggest that AST supplementation may improve COS outcomes and favorably modulate inflammatory pathways, with potential to reduce OHSS risk in high-risk PCOS patients. However, this pilot trial was not powered enough to confirm the primary OHSS endpoint, and larger studies are required for validation.

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Polycystic ovary syndrome (PCOS) is the leading cause of anovulatory infertility[1]. Standard treatment protocols for these patients, which typically rely on various ovulation induction strategies, are frequently complicated by an elevated risk of ovarian hyperstimulation syndrome (OHSS)[2]. OHSS is a severe iatrogenic complication of controlled ovarian stimulation (COS) characterized by an exaggerated ovarian response, increased vascular permeability, and fluid imbalance. Paradoxically, despite the presence of a large follicular cohort, evidenced by high antral follicle counts (AFC) and anti-mullerian hormone (AMH) levels, PCOS patients who develop OHSS often exhibit suboptimal outcomes, such as empty follicle syndrome, diminished oocyte quality, and a predominance of immature oocytes[3,4]. These adverse outcomes likely reflect the detrimental effects of oxidative stress and inflammation[5,6].

Despite decades of research, OHSS remains a potentially life-threatening complication of COS, particularly in women with PCOS. Although several preventive approaches have been proposed, including gonadotropin-releasing hormone (GnRH) agonist triggering, dopamine agonists, and cycle segmentation, no universally

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effective strategy has emerged, and treatment options remain largely supportive[7,8]. This underscores the need for novel interventions targeting the underlying inflammatory and vascular mechanisms of OHSS.

Recent investigations have shed light on the role of reactive molecules, including advanced glycation end-products (AGEs), in driving the poor reproductive outcomes and endocrinologic disturbances seen in PCOS[9,10]. AGEs are formed through non-enzymatic reactions between reducing sugars and free amino groups on proteins, lipids, or nucleic acids. Under normal conditions, cellular mechanisms efficiently clear these products[11]. In PCOS, excessive metabolic stress leads to an overproduction of AGEs that exceeds clearance capacity, thereby resulting in AGE accumulation, oxidative stress, and activation of inflammatory cascades[9,12].

Additionally, hyperandrogenism in PCOS has been shown to increase receptor for advanced glycation end products (RAGE) expression and promote further AGE accumulation by inducing endoplasmic reticulum (ER) stress[13,14]. AGEs exert their pathogenic effects primarily via receptor-dependent mechanisms[11]. Numerous studies have demonstrated that these receptors are present in a variety of cell types within the female reproductive system, and that heightened activity of this signaling pathway is linked to disrupted folliculogenesis and impaired ovulation[15,16]. Binding of AGEs to RAGE activates intracellular cascades, most notably the Nuclear Factor-kappa B (NF-κB) pathway[17], leading to the production of pro-inflammatory cytokines and reactive oxygen species that disrupt normal ovarian functions such as folliculogenesis and steroidogenesis[18,19].

Vasoactive mediators such as vascular endothelial growth factor (VEGF) and various inflammatory cytokines play crucial roles in the pathogenesis and severity of OHSS by promoting the enhanced vascular permeability characteristic of the syndrome[20,21]. Moreover, activation of the AGE-RAGE signaling pathway has been shown to increase Vascular endothelial growth factor (VEGF) secretion into follicular fluid (FF)[22]. Conversely, the soluble form of the AGE receptor (sRAGE), which counteracts membranous AGE signaling, can downregulate VEGF expression in ovarian granulosa cells (GCs) in OHSS[23]. These observations suggest that dysregulation of the AGE-RAGE axis may influence OHSS development. Therefore, targeting the AGE-RAGE system, whether through the use of RAGE inhibitors, ER stress modulators, or anti-inflammatory agents, represents a promising therapeutic approach for reducing the risk or severity of OHSS in PCOS patients[13].

Astaxanthin (AST) is a red-orange oxycarotenoid from the xanthophyll family with a unique polar–nonpolar–polar structure that confers potent antioxidant and anti-inflammatory properties. AST can reduce proinflammatory cytokine production, alleviate ER stress, and inhibit non-enzymatic glycation, mechanisms that ultimately suppress RAGE signaling[24–27]. Although previous clinical trials have demonstrated that AST can improve reproductive outcomes by reducing oxidative stress in infertile women[28–32], no study to date has investigated its impact on modulating the AGE-RAGE, inflammation axis in relation to ovarian steroidogenesis and the risk of OHSS in PCOS patients.

Given the limitations of existing therapeutic strategies in fully mitigating the risk of OHSS without compromising COS success, there is an urgent need for alternative approaches. Moreover, scant data exist regarding the efficacy of complementary interventions in reducing OHSS risk while preserving the favorable dynamics of COS. To address this gap, we undertook a pilot-like exploratory clinical trial to assess whether AST can reduce OHSS risk during COS and correct imbalances in estradiol and progesterone levels. We also evaluate the effects of AST supplementation on the RAGE–NFκB signaling pathway in ovarian GCs and the level of its downstream inflammatory mediators in FF, including Interleukin-6 (IL-6) and VEGF.

Results

Study flow and dropout analysis

Recruitment was conducted from July 1, 2024, to October 6, 2024. The trial was completed on November 10, 2024, when follow-up and data collection for the last enrolled participant were finalized. Of 44 randomized participants, 37 initiated COS and completed follow-up (AST: 18; placebo: 19). Seven participants (AST: $n=3$; placebo: $n=4$) discontinued before COS initiation due to spontaneous pregnancy or cycle cancellation and were therefore not evaluable for OHSS or related outcomes (Fig. 1). As all participants who initiated COS completed the study, the modified ITT and per-protocol populations were identical.

No non-OHSS adverse events (e.g., gastrointestinal, allergic, or systemic reactions) were reported in either group. This is consistent with previous clinical studies indicating that AST supplementation is generally safe and well tolerated. A balancedness test was performed to compare baseline characteristics between completers ($n=37$) and dropouts ($n=7$). No statistically significant differences were observed in age, BMI, ovarian reserve markers, endocrine profiles, or PCOS phenotype distribution (Supplementary Table 2, Supplementary File). These findings suggest that attrition was balanced and unlikely to introduce bias.

Baseline characteristics

As presented in Table 1, both groups were well balanced in demographic and clinical characteristics. The mean age of participants was comparable between groups (30.33 ± 3.88 years in the AST group vs. 29.89 ± 4.44 years in the placebo group; $p=0.751$). Likewise, BMI values were similar (28.33 ± 3.21 vs. 27.38 ± 4.10; $p=0.440$), and the distribution of BMI categories did not differ significantly ($p=0.476$). There were also no significant differences in reproductive history. The number of infertility treatment cycles, whether overall, the more minimally invasive MAR cycles, or ART cycles, was similar between groups. Likewise, the prevalence of OHSS history and the distribution of PCOS phenotypes (with phenotype A representing 61.1% versus 57.9% in the AST and placebo groups, respectively; $p=0.751$) were comparable.

Paraclinical parameters are presented in Table 2. Ultrasound parameters, including ovarian volume (10.14 ± 3.06 cm^3 vs. 10.41 ± 3.11 cm^3; $p=0.793$) and AFC (16.11 ± 6.17 vs. 14.21 ± 6.04; $p=0.350$), were also well matched between groups. Endocrine profiles measured on days 1–3 of the menstrual cycle (including AMH, FSH, LH, Estradiol, progesterone, TSH, prolactin, and fasting blood sugar) did not reveal any significant intergroup differences, although there was a near-significant trend in LH levels ($p=0.066$).

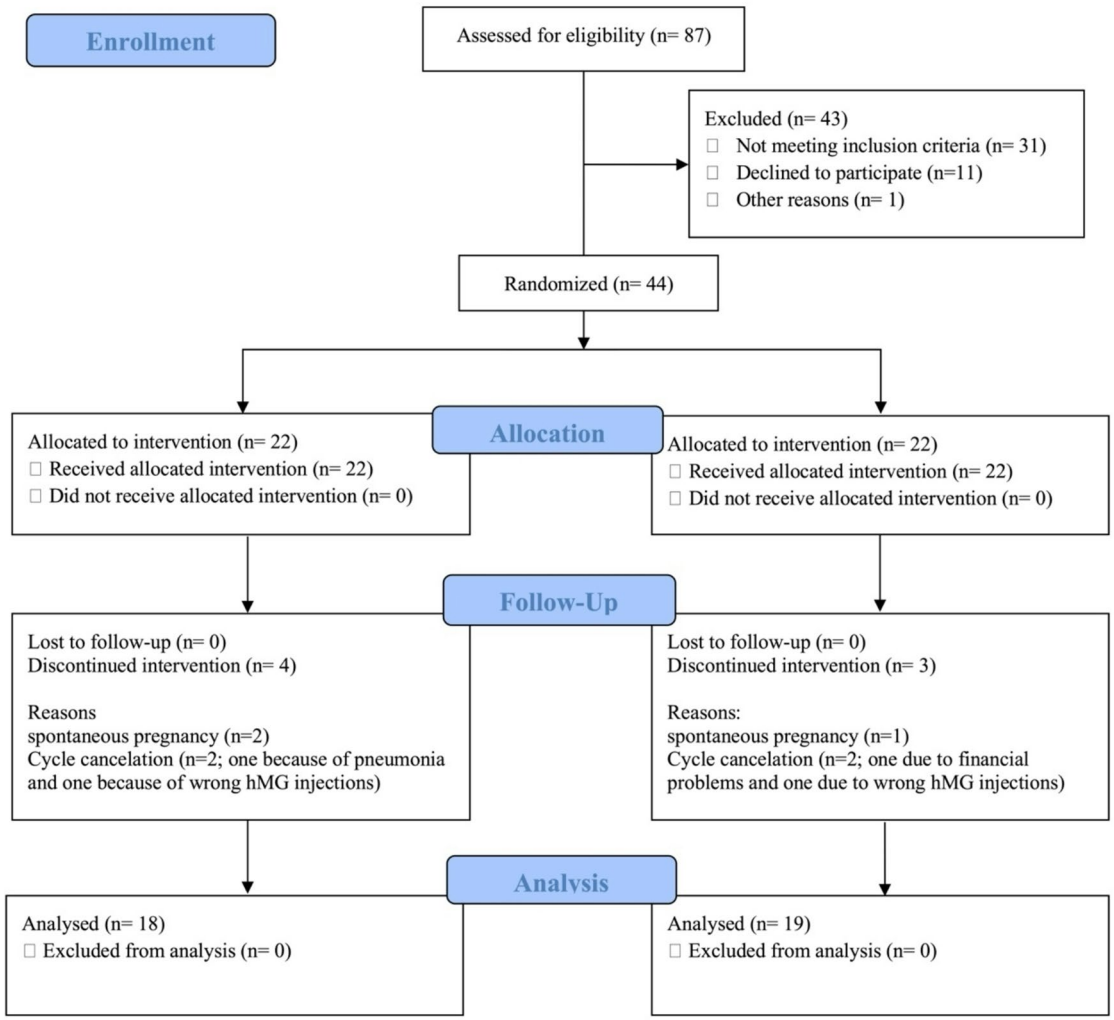


Fig. 1. CONSORT 2010 flow diagram.

Ovarian response outcomes

Ovarian response outcomes are presented in Table 3. During the COS cycle, both treatment groups exhibited similar characteristics. The distribution of the initial Gn dose (with most patients receiving 225 IU, followed by lower proportions receiving 150 or 300 IU) did not differ statistically ($p = 0.274$). Similarly, the duration of Gn administration was nearly identical (12.56 ± 1.42 days for AST vs. 12.47 ± 1.71 days for placebo, $p = 0.717$), and the choice of trigger agent (with approximately 70% receiving a GnRH agonist and the balance hailing from hCG triggering) was comparable ($p = 0.999$).

Ultrasonographic assessments on trigger day, such as the prevalence of a high number of small follicles and the distribution of follicles larger than 10 mm, showed similar frequencies, with no significant differences (both p values = 0.999). Although the categorization of total retrieved oocytes (< 20, 20–30, or > 30) revealed a slight numerical difference (with the proportion of cycles yielding < 20 oocytes tending to be higher in the placebo group), this did not reach statistical significance ($p = 0.220$). In addition, the mean number of retrieved oocytes was slightly higher in the AST group (30.89 ± 9.19 vs. 26.26 ± 7.15, $p = 0.095$), and the oocyte maturity rate (OMR) was significantly greater with AST supplementation (71.52 ± 12.26% vs. 61.17 ± 13.95%, $p = 0.044$).

Endocrinological measurements on the trigger day indicated that AST may modulate the endocrine disruptions linked to OHSS. The AST group showed lower mean levels of estradiol (4224 ± 2895 vs. 5443 ± 2864 in the placebo group) and reduced progesterone levels (2.71 ± 1.14 vs. 3.22 ± 1.40). However, these differences were not statistically significant ($p = 0.206$ and $p = 0.236$, respectively).

Finally, the incidence of OHSS was slightly lower in AST group compared to placebo group (55.5% vs. 68.4%) but this finding was not statistically significant. In a stratified analysis by final trigger agent, 72.2% of participants in the AST group and 68.4% in the placebo group received a GnRH agonist trigger; the remainder received hCG. Within the GnRH agonist stratum, OHSS incidence was 53.8% (7/13) in the AST arm versus 69.2% (9/13) in the placebo arm ($P = 0.69$). In the hCG stratum, OHSS rates were 60.0% (3/5) and 66.7% (4/6) in the AST and placebo arms, respectively ($P = 1.00$). These findings indicate that trigger type did not confound the comparison of OHSS risk between treatment arms (Supplementary Table 3, Supplementary File).

		Groups		P
		Astaxanthin ($n=18$)	Placebo ($n=19$)	
Demographic characteristics				
Age	Mean ± SD	30.33 ± 3.88	29.89 ± 4.44	0.751 *
	Median (range)	31.5 (23 to 35)	31 (20 to 35)	
BMI	Mean ± SD	28.33 ± 3.21	27.38 ± 4.10	0.440 *
	Median (range)	28.05 (23.22 to 33.67)	27.18 (20.48 to 37.18)	
	< 18.5	0	0	0.476 †
	18.5–24	2 (11%)	5 (26.4%)	
	24.1–28	6 (33.4%)	6 (31.6%)	
	>=28	10 (55.6%)	8 (42%)	
Infertility treatment hx				
Number of total treatment cycles	Mean ± SD	1.94 ± 2.55	2.05 ± 2.75	0.943 ‡
	Median (range)	1 (0 to 8)	1 (0 to 8)	
Number of MAR (IO/IUI) cycles	Mean ± SD	1.38 ± 2.40	1.31 ± 2.60	0.584 ‡
	Median (range)	0 (0 to 8)	0 (0 to 8)	
Number of ART (IVF/ICSI/FET) cycles	Mean ± SD	0.55 ± 0.98	0.73 ± 1.28	0.671 ‡
	Median (range)	0 (0 to 3)	0 (0 to 4)	
OHSS hx	Yes	5 (27.7%)	7 (36.8%)	0.728 #
	No	13 (73.3%)	12 (63.2%)	
PCOS phenotype	A	11 (61.1%)	11 (57.9%)	0.751 †
	B	4 (22.3%)	5 (26.3%)	
	C	1 (5.5%)	2 (10.5%)	
	D	2 (11.1%)	1 (5.3%)	

Table 1. Baseline characteristics of participants before intervention. ART (IVF/ICSI/FET): Assisted Reproductive Technology (In Vitro Fertilization/IntraCytoplasmic Sperm Injection/Frozen Embryo Transfer); BMI: Body Mass Index; MAR (IO/IUI): Medically Assisted Reproduction (Intrauterine Insemination); SD: Standard Deviation. *Based on Unpaired T test. ‡Based on Mann-Whitney test. †Based on Chi-square test. #Based on Fisher's exact test.

Also, severity of OHSS, the rates of hospitalization, and the distribution of subsequent management strategies (conservative, medical, or a combination including paracentesis), was comparable between the groups.

Exploratory molecular analyses

In an effort to probe deeper into potential biochemical mechanisms underlying the clinical outcomes, several markers relevant to inflammation and cell signaling were assessed.

RAGE expression

RAGE mRNA expression in GC, quantified by PCR, was significantly lower in the AST group (mean 0.66) compared to the placebo group (mean 1.03; $p=0.010$) (Fig. 2). The difference between groups reinforces the notion that AST may modulate expression of AGE receptor in ovarian GCs.

NF-κB signaling

To confirm that the Western-blot subset reflected the overall cohort, we compared age, BMI, estradiol on trigger day, OHSS incidence, and trigger-agent distribution between the six WB samples and the remaining 31 participants. No statistically significant differences were observed (Supplementary Table 4), indicating that our molecular subset is representative of the full study population.

Total IκB protein levels in GCs exhibited a trend toward being higher in the AST group (mean 1.58) compared to placebo (mean 1.00), although this difference was not statistically significant ($p=0.179$). Similarly, levels of pIκB in GCs tended to be lower in the AST group (mean 1.17) versus the placebo group (mean 1.40; $p=0.086$). Importantly, when these two measures were integrated as the pIκB/IκB ratio, a surrogate marker for NF-κB pathway activation, a statistically significant difference emerged. Participants receiving AST had a significantly lower ratio (1.28) than those on placebo (2.59; $p=0.049$), suggesting a reduction in the phosphorylation-driven activation of the NF-κB pathway in ovarian GCs with AST supplementation (Fig. 3).

Inflammatory cytokine and angiogenic factor levels in FF

IL-6 levels were markedly different between FF derived from two groups. The AST group demonstrated a lower mean IL-6 level (4.764) compared to the placebo group (6.846), and this difference was statistically significant ($p=0.004$), in line with an anti-inflammatory profile potentially attributable to AST.

VEGF levels, while numerically lower in the AST group (mean 12.84) than in the placebo group (mean 15.24), did not differ significantly between the two ($p=0.168$) (Fig. 4).

		Groups		P
		Astaxanthin ($n=18$)	Placebo ($n=19$)	
Ultrasound findings[b]				
Ovarian volume (cm3)	Mean ± SD	10.14 ± 3.06	10.41 ± 3.11	0.793 *
	Median (range)	9.45 (5.66 to 16.32)	10.25 (5.28 to 16.02)	
AFC[a]	Mean ± SD	16.11 ± 6.17	14.21 ± 6.04	0.350 *
	Median (range)	15.5 (6 to 28)	13 (6 to 25)	
Endocrine profile[b]				
AMH level (ng/ml)	Mean ± SD	8.69 ± 2.51	8.80 ± 3.43	0.911 *
	Median (range)	8.5 (3.8 to 14.5)	8.3 (4.6 to 16.5)	
FSH level (IU/l)	Mean ± SD	4.61 ± 0.94	4.30 ± 1.87	0.536 *
	Median (range)	4.4 (3.5 to 6.2)	4.2 (1.5 to 8.9)	
LH level (IU/l)	Mean ± SD	8.12 ± 1.00	6.91 ± 2.53	0.066 *
	Median (range)	8.2 (5.9 to 10)	6.6 (2.3 to 12)	
FSH/LH	Mean ± SD	1.81 ± 0.40	1.78 ± 0.85	0.892 *
	Median (range)	1.8 (1.3 to 2.5)	1.6 (0.8 to 4.3)	
Estradiol level (pg/ml)	Mean ± SD	66.04 ± 23.33	71.97 ± 6.35	0.486 *
	Median (range)	71 (11.8 to 103)	72 (32.7 to 120)	
Progesterone level (ng/ml)	Mean ± SD	0.96 ± 0.76	1. 39 ± 1.73	0.351 *
	Median (range)	0.65 (0.1 to 2.3)	0.6 (0.1 to 5.4)	
TSH level (μIU/ml)	Mean ± SD	2.59 ± 1.20	2.54 ± 1.32	0.893 *
	Median (range)	2.2 (1.2 to 5.3)	2.1 (1.1 to 5.5)	
Prolactin level (mIU/l)	Mean ± SD	339 ± 152.2	414 ± 198.0	0.206 *
	Median (range)	339 (91.3 to 689)	356 (201 to 890)	
FBS level (mg/dl)	Mean ± SD	95.19 ± 10.52	92.71 ± 6.71	0.422 *
	Median (range)	94 (80 to 112)	92 (81 to 103)	

Table 2. – Baseline paraclinical parameters of participants before Intervention. AFC: Antral Follicle Count; AMH: Anti-Müllerian Hormone; FBS: Fasting Blood Sugar; FSH: Follicle Stimulating Hormone; LH: Luteinizing Hormone; SD: Standard Deviation; *Based on Unpaired T test. ‡Based on Mann-Whitney test. †Based on Chi-square test. #Based on Fisher's exact test.

Discussion

Our study investigated the impact of AST supplementation during COS on both clinical outcomes and molecular markers associated with OHSS. To date, no clinical studies have evaluated AST or other nutraceutical agents for OHSS prevention, and while pharmacologic strategies such as GnRH agonist trigger, dopamine agonists, and metformin have been investigated, our trial represents the first exploratory effort to assess a non-pharmacological antioxidant in this context. Clinically, both treatment groups exhibited similar COS characteristics. Key parameters, such as the duration of Gn administration, and the selection of trigger agents, were statistically indistinguishable between groups. Ultrasonographic assessments on trigger day, including the distribution of small follicles and follicles larger than 10 mm, and the categorization of total retrieved oocytes, also revealed no significant differences. Beyond overall stimulation dynamics, our analysis revealed that AST supplementation was associated with a significantly higher OMR. This is a clinically meaningful observation, as being an "over-responder" with a high follicle count does not necessarily translate into optimal oocyte maturation or quality. Patients at risk of OHSS often develop numerous follicles, but a substantial proportion may remain immature. The improvement in OMR suggests that AST may enhance both the quantity and quality of oocytes, potentially counterbalancing the tendency toward immature follicle development in high responders.

Although women with PCOS are generally considered highly sensitive to gonadotropins, their ovarian response is heterogeneous, particularly across phenotypes A–D. A uniform low-dose approach may risk inadequate follicular recruitment in some patients, whereas higher doses can increase OHSS risk. In our study, we therefore adopted a moderate starting range of 150–300 IU rFSH, with the majority of patients beginning at 225 IU, and individualized the regimen after five days based on ultrasound and hormonal monitoring. Importantly, the GnRH antagonist protocol and the use of GnRH agonist triggering provided additional safeguards against OHSS. The comparable stimulation characteristics and OHSS incidence between groups suggest that this dosing strategy achieved effective follicular development without compromising safety.

Endocrine measurements taken on the trigger day revealed trends that suggest the potential modulatory effects of AST. The AST group exhibited lower mean levels of estradiol and progesterone compared to the placebo group. Although these differences did not reach statistical significance, they indicate that AST may have a subtle influence on the ovarian endocrine environment, which could contribute to a reduced risk of OHSS. Supporting this observation, the incidence of OHSS was lower in the AST group (55.5%) compared to the placebo group (68.4%), although this difference was not statistically significant. Importantly, the significantly higher OMR in

		Statics	Groups		P
			Astaxanthin (*n* = 18)	Placebo (*n* = 19)	
COS cycle characteristics					
Initial dose of Gn (IU/d)		150	4 (22.3%)	5 (26.3%)	0.274 †
		225	11 (61%)	10 (52.7%)	
		300	3 (16.7%)	4 (21%)	
Duration of Gn (days)		Mean ± SD	12.56 ± 1.42	12.47 ± 1.71	0.717 ‡
		Median (range)	12 (11 to 16)	12 (10 to 16)	
Type of trigger		HCG	5 (28%)	6 (32%)	0.999 #
		GnRHa	13 (72%)	13 (68%)	
A high number of small follicles (=< 10 mm)[a, b]		Yes	10 (55.5%)	11 (58%)	0.999 #
		No	8 (44.5%)	8 (42%)	
Number of follicles sized > 10 mm[a]		=<25	9 (50%)	10 (52.5%)	0.999 #
		> 25	9 (50%)	9 (47.5%)	
Total retrieved oocytes		Mean ± SD	30.89 ± 9.19	26.26 ± 7.15	0.095 *
		Median (range)	28 (19–52)	26 (12–42)	
		< 20	1 (5.5%)	5 (26.3%)	0.220 †
		20–30	10 (55.5%)	9 (47.4%)	
		> 30	7 (39%)	5 (26.3%)	
OMR (%) [b]		Mean ± SD	71.52 ± 12.26	61.17 ± 13.95	0.044 ‡
		Median (range)	71.13 (40.82 to 95.45)	63.64 (37.50 to 83.33)	
Endocrine profile[c]					
Estradiol level (pg/mL)		Mean ± SD	4224 ± 2895	5443 ± 2864	0.206 *
		Median (range)	3325 (1025 to 10560)	5340 (1200 to 10560)	
Progesterone level (ng/mL)		Mean ± SD	2.71 ± 1.14	3. 22 ± 1.40	0.236 *
		Median (range)	2.45 (1.2 to 5.5)	3.3 (1.2 to 5.9)	
Ovarian response					
OHSS [d]		Yes	10 (55.5%)	13 (68.4%)	0.507 #
		No	8 (44.5%)	6 (31.6%)	
Among those with OHSS	OHSS severity	Mild	5 (50%)	6 (46.2%)	0.929 †
		Moderate	4 (40%)	5 (38.4%)	
		Sever	1 (10%)	2 (15.4%)	
		Critical	0	0	
	Hospitalization	Yes	1 (10%)	3 (23%)	0.603 #
		No	9 (90%)	10 (77%)	
	Management strategy[e]	Conservative	7 (70%)	7 (54%)	0.733 †
		Medical	2 (20%)	4 (31%)	
		Paracentesis + Medical	1 (10%)	2 (15%)	

Table 3. Ovarian response outcomes. Gn: Gonadotrophins; GnRHa: Gonadotropin-Releasing Hormone agonist; HCG: Human Chorionic Gonadotropin; LMWH: Low Molecular Weight Heparin; SD: Standard Deviation; OHSS: Ovarian Hyperstimulation Syndrome. *Based on Unpaired T test. ‡Based on Mann-Whitney test. †Based on Chi-square test. #Based on Fisher's exact test. a) The OMR is calculated for each participant by dividing number of M II oocyte to total retrieved oocytes. b) All ultrasonic and endocrine parameters are evaluated on trigger day. c) A high number of small follicles was defined as the presence of more than 10–15 small follicles (< 10 mm) on trigger day. d) Stratified analysis based on triggering strategies (hCG vs. GnRH agonist) in COS and their correlation with OHSS risk is provided in "Supplementary Table 3" in Supplementary File. e) Medical interventions include vaginal cabergoline and thromboprophylaxis with LMWH.

the AST group indicates that AST may reduce OHSS risk without compromising, and perhaps even improving, oocyte maturation.

We found that, compared to the placebo group, the AST group exhibited a significant reduction in RAGE mRNA expression in GCs and a significantly lower pIκB/IκB ratio, indicating reduced activation of the NF-κB pathway. However, we acknowledge that the protein-level findings on the pIκB/IκB ratio are preliminary, as they were derived from a limited subset and therefore require validation in a larger cohort. Additionally, FF analysis revealed significantly lower IL-6 levels in the AST group. Although VEGF levels were numerically lower with AST, this difference did not reach statistical significance. Clinically, trends toward reduced incidence and severity of OHSS were observed, although these differences were not statistically significant.

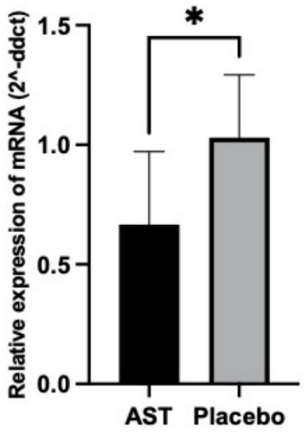


Fig. 2. Comparison of the expression of the *RAGE* gene in GCs between the Astaxanthin and placebo groups. The results revealed that the expression of *RAGE* gene was significantly lower in the Astaxanthin group ($p = 0.010$). The data is presented as the means ± SD. * Indicates $P < 0.050$. *RAGE*: Receptor for Advanced Glycation End-Products; GCs: Granulosa cells.

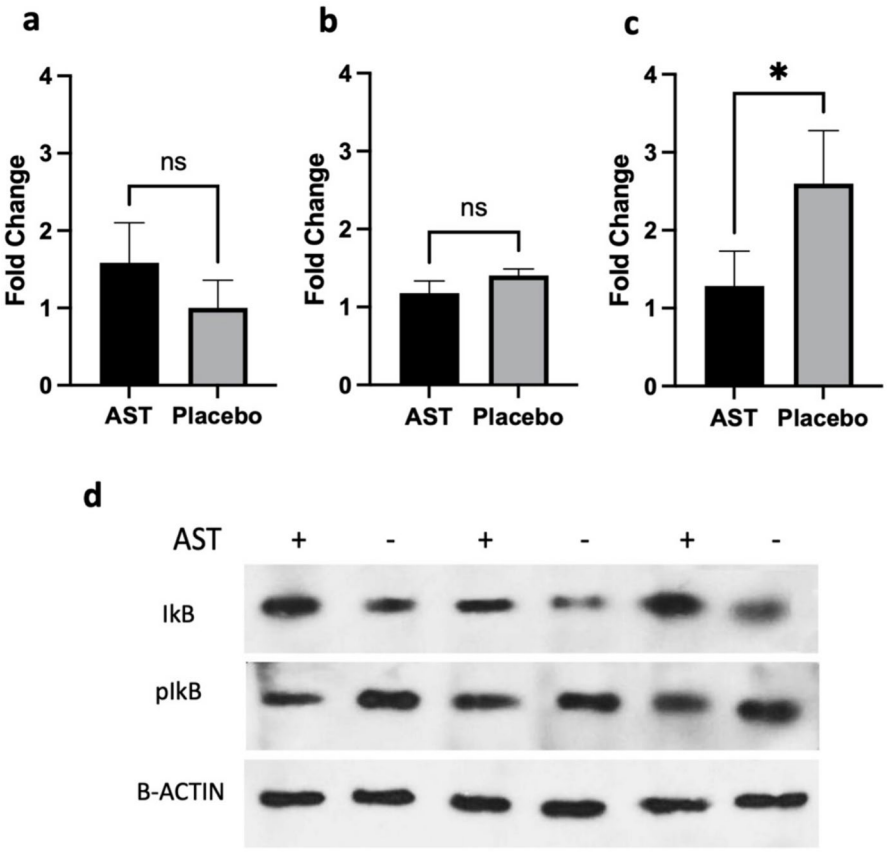


Fig. 3. Comparison of protein expression levels of IκB, pIκB, and pIκB to IkB ratio in GCs between the Astaxanthin and placebo groups (**a**) The protein expression of IκB was higher in astaxanthin group, although not statistically significant ($p = 0.179$) (**b**) The protein expression of pIκB was slightly higher in placebo group, although this difference was not statistically significant ($p = 0.086$). (**c**) The ratio of pIκB to total IκB was significantly lower in the Astaxanthin group compared to the placebo group ($p = 0.049$), indicating decreased IκB degradation in Astaxanthin group and eventually decreased NFκB signaling in this group (**d**) Blots of IκB, pIκB, and β-ACTIN. The data are presented as the means ± SD. "*" symbol indicates $P < 0.05$ and "ns" symbol indicates non-significant change. IκB: Inhibitor of Nuclear Factor kappa B; pIκB: phosphorylated form of IκB; GCs: Granulosa cells.

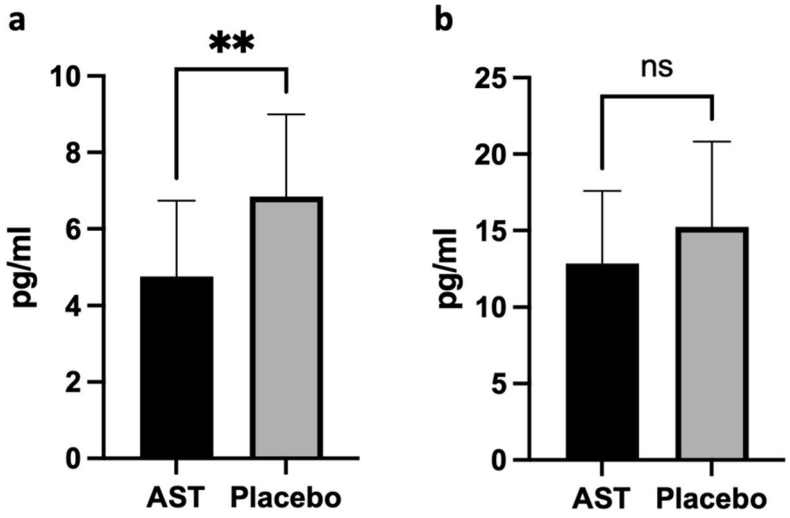


Fig. 4. Comparison of the IL-6 and VEGF levels in FF (**a**) The results revealed that the FF level of IL-6 was significantly lower in the Astaxanthin group ($p = 0.004$) (**b**) The results revealed that the FF level of VEGF was lower in the Astaxanthin group but this difference was not statistically significant ($p = 0.168$). The data are presented as the means ± SD. "**" symbol indicates $P < 0.01$ and "ns" symbol indicates non-significant change. IL-6: interleukin-6; VEGF: vascular endothelial growth factor; FF: Follicular fluid.

The alignment between these clinical observations and molecular findings supports the hypothesis that AST can attenuate pro-inflammatory and endocrine disruptions associated with OHSS. The lack of statistically significant differences on clinical parameters might be related to the sample size. It is conceivable that the subtle endocrine modulations observed may become more pronounced or clinically relevant in larger cohorts or well-selected patient subpopulations.

The pathophysiology of OHSS is predominantly driven by hCG-induced stimulation of the ovaries, leading to the overproduction of proinflammatory mediators, including VEGF, a key molecule responsible for enhanced vascular permeability and third-space fluid accumulation. Also, abnormally elevated RAGE activity in PCOS patients leads to NF-κB signaling, which in turn facilitates the transcription of several inflammatory cytokines, including IL-6, and promotes the expression of VEGF. Our findings that AST appears to attenuate this pathway by reducing RAGE mRNA expression, thereby diminishing the downstream activation of NF-κB, as elucidated by the significant decrease in the pIκB/IκB ratio. The resultant reduction in IL-6 levels corroborates the anti-inflammatory effects of AST observed in our study. Although VEGF was not significantly altered, its trend toward reduction further supports the notion that AST has the potential to modulate angiogenic factors indirectly through upstream inflammatory pathways.

In summary, while most clinical outcomes did not show statistically significant differences between the AST and placebo groups, the observed trends in endocrine profiles and OHSS incidence, together with the significant improvement in OMR, are consistent with the beneficial molecular effects of AST. These findings suggest that AST supplementation may not only attenuate inflammatory and endocrine disruptions associated with OHSS but also support oocyte maturation in high-risk patients. Future studies with increased power and focused endpoints are necessary to determine whether these combined effects can translate into definitive improvements in reproductive outcomes.

Several clinical studies have examined the effects of antioxidants, including AST, on inflammatory cytokines in FF, demonstrating that it can modulate these pathways in various reproductive contexts[28,29,31,33]. However, there is a paucity of research specifically addressing the role of AST or any kind of natural antioxidants in the prevention of OHSS. An animal study by Darabi et al.[34] demonstrated that Coenzyme Q10 supplementation in a rat model of OHSS significantly reduced ovarian VEGF and Cyclooxygenase-2 expression, suggesting that antioxidants can mitigate the inflammatory cascade induced by ovarian stimulation. In a similar vein, resveratrol has been shown to attenuate OHSS severity by modulating the NF-κB signaling pathway. Specifically, a study by Ozgur et al.[35] reported that resveratrol treatment led to decreased VEGF activity, thereby reducing endothelial permeability and ovarian edema.

Notably, regarding the RAGE pathway as one of the critical upstream regulator of NF-κB activation and VEGF expression, only one experimental study by Wang et al.[23] has directly investigated its role in OHSS. In that study, the use of soluble RAGE (sRAGE) was shown to downregulate VEGF expression in ovarian GCs derived from an OHSS model, suggesting that suppression of RAGE signaling may attenuate the angiogenic and inflammatory cascades implicated in OHSS. To date, no interventional studies have evaluated the effect of directly suppressing RAGE in OHSS prevention.

Our study extends the literature in two important ways. First, it provides human data on the use of a natural antioxidant, AST, in the context of OHSS prevention. We demonstrate that AST supplementation is associated with significant downregulation of the RAGE/NF-κB/IL-6 axis in ovarian GCs and FF. Second, by focusing

on RAGE expression, a relatively unexplored target in OHSS prevention, our findings offer novel mechanistic insights that complement previous research on antioxidant effects in FF. The observed reduction in RAGE mRNA, coupled with a lower pIκB/IκB ratio indicating decreased NF-κB activation, underscores the potential of targeting this pathway to reduce downstream cytokine production and VEGF-mediated vascular permeability.

Collectively, these molecular findings not only parallel the anti-inflammatory effects observed with other antioxidants but also highlight the unique benefit of AST in modulating the RAGE pathway, a therapeutic target that has not been clinically addressed for OHSS prevention. This novel evidence supports the rationale for further clinical studies to explore natural antioxidant supplementation as an adjunct strategy for mitigating OHSS risk.

Clinically, if AST supplementation can consistently reduce inflammatory markers associated with OHSS, it may serve as a useful adjunct in the management of OHSS risk during ART cycles. By dampening the inflammatory milieu, AST could potentially reduce the severity of ovarian responses, thereby ameliorating the clinical manifestations of OHSS. Importantly, while our molecular findings are promising, the trends in clinical endpoints (such as the incidence and severity of OHSS) require further validation in larger patient cohorts to ascertain the true clinical benefit.

A primary limitation of our study is the relatively small sample size, which may have limited the power to detect significant differences in clinical outcomes. Importantly, this study represents a preliminary exploration, a pilot trial, in an area where no similar clinical investigations have been conducted. Estimating the baseline risk of OHSS using pre-COS variables alone is inherently challenging due to limited reported incidence data and the absence of prior trials evaluating antioxidant interventions like AST. For this reason, our sample size calculation relied on conservative, exploratory assumptions, which is consistent with the design of early pilot studies intended to generate hypotheses for larger confirmatory trials.

Another limitation of this study is that the scope of molecular analyses was confined to a subset of inflammatory and angiogenic markers. Furthermore, establishing the mechanistic role of AST in modulating the RAGE–NFκB pathway would require in vitro studies using cultured GCs, which were beyond the scope of this pre-planned clinical trial. Future experimental studies are therefore warranted to confirm and expand upon these preliminary findings. We also acknowledge that analyzing only six GC samples by Western blot limits statistical power and may miss modest protein-level effects. Future studies should include larger sample subsets to validate these findings.

Additionally, because this study exclusively enrolled patients at high risk for OHSS based on PCOS diagnosis and elevated ovarian reserve markers, the findings may not be generalizable to broader IVF populations or PCOS patients with lower baseline risk. Future studies should assess the effects of AST supplementation across more diverse patient profiles to determine its applicability beyond high-risk cohorts.

Also, we did not evaluate implantation or clinical pregnancy, leaving uncertainty about the effect of astaxanthin on final assisted reproductive technology (ART) outcomes in high-risk PCOS patients. Although these parameters have been investigated in previous studies involving general PCOS populations, they have not been specifically evaluated in high-risk patients, which remains an important gap.

The study's strength lies in its integrative design, combining clinical and molecular endpoints to provide a comprehensive overview of AST's potential effects. This dual approach enhances the biological plausibility of our findings and lays the groundwork for future mechanistic and clinical studies. Importantly, to our knowledge, this is the first randomized trial to explore a nutraceutical antioxidant such as AST for OHSS prevention in high-risk PCOS patients, underscoring the novelty of our investigation within the broader landscape of OHSS management strategies.

The lack of statistical significance in OHSS incidence and endocrine outcomes may reflect insufficient statistical power rather than a true absence of effect. So, future research should build on our findings by conducting larger, multicenter randomized controlled trials to confirm the clinical efficacy of AST in reducing the incidence and severity of OHSS. In addition, further studies are warranted to determine the optimal dosage and timing of AST administration during COS, and to explore whether extending supplementation beyond oocyte retrieval could provide additional protection against OHSS. Finally, it will be important to investigate potential synergistic effects when AST is combined with established preventive strategies, such as GnRH agonist triggering, dopamine agonists, or cycle segmentation, to better define its role within a comprehensive prevention framework.

In conclusion, reduced IL-6 levels in FF, downregulation of the RAGE–NFκB pathway, and enhanced oocyte maturation provide preliminary mechanistic support for AST as a potential adjunctive strategy to mitigate OHSS risk while maintaining an adequate ovarian response to COS. These findings should be interpreted with caution, as this was an exploratory pilot trial with a limited sample size and protein-level data derived from a small subset of patients. Larger, adequately powered studies incorporating extended molecular profiling and reproductive outcome assessment are required to validate these observations and clarify the clinical relevance of AST supplementation in OHSS context.

Materials and methods

Study design and settings

The trial was designed and reported in accordance with the CONSORT guidelines for randomized controlled trials[36]. This randomized, triple-blind, placebo-controlled, pilot clinical trial was conducted at the Omid Fertility Center in Tehran. Forty-four eligible women were enrolled and randomized in a 1:1 ratio to receive either AST or placebo. An independent researcher generated the randomization list using an online tool (sealedenvelope.com) with block sizes of 4 and 6, and assignment was secured using sequentially numbered, sealed envelopes. All study personnel, patients, outcome assessors, and the statistician, remained blinded throughout the trial. Both AST and placebo capsules were identical in appearance, taste, and packaging; an independent party coded each bottle and all sample containers (including those for blood, FF, and cellular material) to maintain blinding

during outcome assessments. Outcome assessments, encompassing both clinical and molecular outcomes, were conducted by assessors who were completely unaware of the participants' group assignments.

Sample size calculation

Estimating the baseline risk of OHSS using only pre-COS variables is challenging due to limited reported incidence data and the absence of previous trials evaluating antioxidant interventions similar to AST. To account for these uncertainties, the sample size was calculated using an exploratory assumption of a 70–80% baseline OHSS incidence in high-risk PCOS patients, with an anticipated 30–40% relative risk reduction with AST supplementation. This assumption was not derived from published incidence data but was chosen conservatively to ensure adequate power. Historically, moderate-to-severe OHSS has been reported in approximately 1%–5% of IVF cycles, while the overall incidence (mild, moderate, and severe) in PCOS patients can be even up to 13.9 times higher than in women without PCOS[37–40]. In addition to the diagnosis of PCOS itself, which is a well-established risk factor, our study specifically recruited women with very high-risk baseline characteristics such as markedly elevated ovarian reserve markers (AMH ≥ 3.4 ng/mL and/or AFC ≥ 24). These features are recognized by both ASRM and the OHSS risk assessment index as strong predictors of excessive ovarian response, and therefore may further increase the likelihood of OHSS beyond the average incidence reported for unselected PCOS populations. Given the absence of prior clinical trials investigating AST for OHSS prevention, this study was designed as a pilot-like exploratory trial, consistent with the common practice of conducting preliminary studies on smaller patient cohorts before planning larger confirmatory trials.

Using these assumptions, a significance level (α) of 0.05, and a desired power (1-β) of 0.80, we calculated the necessary sample size according to standard formulas for comparing two proportions. After accounting for an anticipated 5% dropout rate, the final sample size was determined to be 44 participants, with 22 subjects randomized to each group. Sample size calculation formula is provided in Supplementary File.

Ethics

This study was designed and performed in accordance with the principles of the Declaration of Helsinki. Also, all participants provided written informed consent before participating in the study. The study protocol was assessed and approved by the Research Ethics Committees of the School of Medicine, Tehran University of Medical Sciences, Tehran, Iran (approval date: 2024-03-04; code: IR.TUMS.MEDICINE.REC.1402.724). Moreover, the protocol was prospectively registered at the Iranian Registry of Clinical Trials (IRCT20231028059882N2) on June 15, 2024, prior to initiation of participant enrollment.

Participants

An infertility specialist evaluated potential participants for eligibility.

Inclusion criteria were as follows:

(1) Age ≤ 35 years.
(2) Diagnosis of PCOS (based on the Rotterdam criteria: at least two of the following, oligo-/anovulation, clinical/biochemical hyperandrogenism, and polycystic ovarian morphology defined as ≥ 12 follicles measuring 2–9 mm and/or ovarian volume > 10 mL in one ovary).
(3) High ovarian reserve: AFC ≥ 24 or AMH level ≥ 3.4 ng/mL.
(4) Medium to high risk for OHSS prior to commencing a COS cycle, as determined according to criteria from the American Society for Reproductive Medicine (ASRM)[40] and an established OHSS risk assessment index[41].

Note

According to the American Society for Reproductive Medicine (ASRM) guideline, women are considered at high risk for OHSS if they present with baseline factors such as PCOS), AFC > 24, or serum AMH > 3.4 ng/mL. Additional stimulation-related factors include > 17 follicles ≥ 10 mm at trigger, estradiol > 3,500 pg/mL, or retrieval of > 15 oocytes.

In parallel, the OHSS risk assessment index proposed by Cao et al. (2021) stratifies risk dynamically across four stages of the IVF cycle. At baseline (Stage 1), risk is assessed using female age, AMH, BMI, and cycle number. Later stages incorporate stimulation protocol, gonadotropin dose and duration, estradiol level, follicle number at trigger, type of trigger, number of oocytes retrieved, and embryo transfer strategy. In our study, participants were recruited before COS and classified as medium-to-high risk primarily based on Stage 1 criteria (age, AMH, BMI, cycle number), in addition to ASRM thresholds (PCOS diagnosis, AFC ≥ 24, AMH ≥ 3.4 ng/mL).

Exclusion criteria included a history of pregnancy or abortion within the past year, untreated or uncontrolled endocrinopathies (including thyroid dysfunction, hyperprolactinemia, diabetes mellitus, adrenal or Cushing's syndrome, and hypothalamic disorders), obesity (BMI > 30), other causes of infertility (severe male factor, endometriosis, uterine malformations, pelvic inflammatory disease, premature ovarian insufficiency), recent use (within 3 months) of any antioxidants (herbs, vitamins, minerals, flavonoids, polyphenols), hormonal or metabolic medications (e.g., metformin), and adherence to specialized diets (fasting or ketogenic).

Interventions

Participants were randomized into two parallel groups:

Intervention group
Received 12 mg/day of AST (administered as three 4-mg capsules; AstaZine®, BGG Europe SA, Italy) for six weeks, continuing until the day before ovum pick-up (OPU).

Placebo group
Received three identical capsules per day filled with edible paraffin, formulated for research purposes by the same company.

This dose and duration were selected based on established safety profiles and prior evidence of efficacy in improving reproductive outcomes in women with PCOS or endometriosis[28–31,33,42].

Safety considerations
Participants were monitored for adverse events, including gastrointestinal discomfort, allergic reactions, or any other unexpected symptoms.

COS protocol

Due to elevated OHSS risk in PCOS, all participants underwent a GnRH antagonist regimen for COS. On the third day of the menstrual cycle, a daily dose of 150–300 IU recombinant follicle-stimulating hormone (r-FSH; Gonal-F, Merck Serono SA, Switzerland) was initiated. Starting dose was selected from institutional range according to baseline ovarian reserve and BMI, with the modal dose at 225 IU. This range was chosen to ensure adequate follicular recruitment across heterogeneous PCOS phenotypes, while allowing early dose adjustment after day 5 to minimize OHSS risk within the GnRH antagonist framework. After five days, doses were adjusted in a step-up/step-down manner based on ultrasound and hormone monitoring to balance follicular recruitment with OHSS mitigation. GnRH antagonist (Cetrotide, Merck Serono SA, Switzerland) was introduced once at least two follicles measuring 10–12 mm was identified.

Final triggering was determined by assessing OHSS risk on the day mature follicles reached 18 mm. Patients with low risk received human chorionic gonadotropin (hCG), while those with moderate to high risk (≥ 25 follicles > 10 mm or Estradiol levels > 3,500 pg/mL) were triggered with a GnRH agonist (Buserelin; CinnaFact®, Iran). Oocyte retrieval was scheduled approximately 36 h post-hCG trigger or 34 h after the GnRH agonist injection. Patients fasted for 6 h before the procedure, which was performed under general anesthesia with ultrasound guidance.

FF processing and GCs isolation

Immediately following oocyte retrieval, the FF was collected and processed for GCs isolation. Approximately 30 mL of FF was layered into 50-mL conical tubes pre-loaded with Ficoll (Sigma-Aldrich, F5415) and then centrifuged at 400×g for 20 min at room temperature. The buffy coat containing GCs was transferred to microtubes and washed by centrifugation at 600×g for 5 min with 1 mL phosphate-buffered saline (PBS; Sigma-Aldrich, P4474). Residual red blood cells were eliminated by treatment with 4 mL of red blood cell (RBC) lysis buffer (formulated with 2 M Tris-HCl [Sigma-Aldrich, 648313] at pH 7.6, 1 M $MgCl_2$ [Sigma-Aldrich, 208337], and 3 M NaCl [Sigma-Aldrich, S6150]), incubated at room temperature for 2–5 min with gentle agitation, followed by centrifugation at 300×g for 3 min. A final wash with PBS (centrifuged at 700×g for 5 min at 4 °C) was performed, and the resultant GC pellets were snap-frozen and stored at − 80 °C for subsequent analyses.

Study variables

Variables and outcomes were selected based on an existing OHSS risk assessment tool as established by Cao et al.[43] and include:

Demographic variables
Age and body mass index (BMI), reported as continuous variables (mean ± SD) and, when applicable, categorized by pre-defined thresholds.

Reproductive history and infertility treatment
Data on the total number of previous treatment cycles (including minimally invasive medically assisted reproduction, such as IUI and ovulation induction, and advanced reproduction techniques such as IVF, ICSI, and FET), as well as a history of OHSS.

PCOS phenotyping
Classification of PCOS into phenotypes A, B, C, and D based on the presence of hyperandrogenism (HA) (clinical and/or biochemical), ovulatory dysfunction (OD), and polycystic ovarian morphology (PCO). Phenotype A included HA + OD + PCO, phenotype B included HA + OD, phenotype C included HA + PCO, and phenotype D included OD + PCO.

Ultrasonographic measurements
Ovarian volume and AFC were recorded on days 8–10 of the preceding menstrual cycle (prior to the intervention) to ensure a stable assessment of the recruitable follicular pool.

Baseline endocrine profiles
Fasting serum concentrations (collected on days 1–3 of the menstrual cycle) for hormones including AMH, FSH, LH, estradiol, progesterone, thyroid-stimulating hormone (TSH), prolactin (PRL), and fasting blood sugar (FBS).

Study outcomes

Primary outcomes

OHSS Incidence The primary clinical outcome was the occurrence of early OHSS, defined as onset within 7 days after the final trigger drug administration. Diagnosis was based on a combination of clinical presentation, laboratory data, and ultrasound findings in accordance with the Royal College of Obstetricians and Gynaecologists (RCOG) guidelines[43]. In line with this guideline, OHSS severity is categorized into four levels (mild, moderate, severe, critical); however, as severity was designated a secondary outcome in this study, the detailed diagnostic criteria are provided in the Secondary Outcomes section.

Ovarian response indicators *COS characteristics*

Initial gonadotropin (Gn) dose (150, 225, or 300 IU/day),
Duration of Gn administration (days), and
Trigger type (human chorionic gonadotropin [hCG] versus GnRH agonist).

High number of small follicles

A high number of small follicles was defined as > 10–15 follicles ≤ 10 mm,
The count of follicles > 10 mm was categorized as ≤ 25 or > 25 based on established OHSS risk cutoffs[40].

Oocyte yield

Mean number of total retrieved oocytes that susequently categorized into three groups: <20, 20–30, or > 30.

Oocyte maturity rate (OMR)

Number of MII (mature) oocytes per total retrieved oocytes.

Endocrine markers on trigger day: Serum levels of estradiol and progesterone measured on the day of the trigger were recorded as surrogate markers of follicular maturation and endocrine response.

Secondary outcomes

OHSS severity The severity of OHSS was classified into four categories (mild, moderate, severe, critical) according to the diagnostic criteria outlined in the RCOG guideline[43], which incorporate clinical, ultrasound, and laboratory parameters:

Mild OHSS Abdominal bloating, mild abdominal pain; ovarian size < 8 cm.

Moderate OHSS Moderate abdominal pain, nausea ± vomiting; ascites detectable by ultrasound; ovarian size 8–12 cm.

Severe OHSS Clinically evident ascites ± hydrothorax; ovarian size > 12 cm; oliguria (< 300 mL/day); hematocrit > 0.45; hypoproteinemia; osmolality < 282 mOsm/kg; potassium > 5 mmol/L; serum albumin < 35 g/L.

Critical OHSS Tense ascites or large hydrothorax; hematocrit > 0.55; WBC > 25,000/mL; anuria (< 100 mL/day); thromboembolism; acute respiratory distress syndrome.

Clinical management Details on subsequent interventions were documented, including:

Management strategy type (conservative measures, medical therapy, or combined approaches such as paracentesis plus medical treatment), and
Hospitalization rates.

Exploratory molecular outcomes *RAGE expression in ovarian GCs*

RAGE mRNA levels were quantified by real-time polymerase chain reaction (RT-PCR). Briefly:

RNA was extracted using the TRIzol method, and its quality and quantity were checked using a NanoDrop spectrophotometer.

cDNA synthesis was performed with the 2-step 2xRT-PCR premix kit (Biofact, DQ116-250) according to the manufacturer's protocol.

Real-time PCR was executed using SYBR Green with specific primers for RAGE and GAPDH (as a housekeeping gene). Primer sequences are listed in Supplementary Table 1 (see Supplementary File). A melting curve analysis (65 °C to 95 °C) confirmed product specificity.

NF-κB regulatory activity in ovarian GCs

Expression levels of the inhibitor of Nuclear Factor kappa B-alpha (IκB-α) and its phosphorylated form (pIκB) were measured via western blot analysis. The pIκB/IκB ratio served as an index of NF-κB activation. Briefly:

Sample selection and preparation

A subset of 6 GC samples (3 from the AST group and 3 from the placebo group) was randomly selected by an independent technician from all participants who completed COS. Selection was blind to OHSS outcome, hormone levels, and embryo data.

Western blot procedure

Protein concentration was determined using the Bradford assay. Following SDS-PAGE, proteins were transferred onto PVDF membranes. The membranes were blocked with 2% non-fat dry milk in TBS-T for 1 h 15 min at room temperature, then incubated overnight (16–18 h) at room temperature with primary antibodies against β-ACTIN (Santa Cruz Biotechnology, sc-517582), IκB-α (Elabscience, E-AB-63526), and phosphorylated IκB-α (Ser32/S36; Elabscience, E-AB-20911). After washing with TBS-T, membranes were incubated with the appropriate secondary antibodies, and chemiluminescence was used for detection. Protein

bands were quantified using IMAGE J software. The complete uncropped blots are provided as supplementary Figs. 1–3 (see Supplementary File).

Inflammatory cytokine and angiogenic factor levels in FF

Levels of interleukin-6 (IL-6) and vascular endothelial growth factor (VEGF) in FF were measured using ELISA kits (Karmania Pars Gene; KPG-HIL6 for IL-6 and KPG-HVEGF for VEGF). These kits, with a sensitivity of 2 pg/mL and inter-assay variability < 8–10% (intra-assay < 3–4%), were used according to the manufacturer's instructions.

IL-6 was selected because NFκB-dependent upregulation of IL-6 in GCs is a hallmark of the inflammatory cascade in OHSS, and IL-6 levels correlate with OHSS severity. VEGF was measured as the primary mediator of vascular permeability in OHSS and a direct NFκB target downstream of RAGE signaling. Together, these markers provide a focused readout of RAGE–NFκB–mediated inflammation and endothelial activation in response to AST supplementation [44–46].

Statistical analysis

Data were analyzed using SPSS software (version 20), and graphs were generated with GraphPad Prism (version 9.5.1). A two-tailed P value < 0.05 was considered statistically significant. Continuous variables were summarized as mean ± standard deviation (SD) or median (range), according to distribution, and categorical variables as frequencies and percentages. Normally distributed continuous variables were compared using independent t-tests, while non-normally distributed variables were analyzed with Mann–Whitney U tests. Categorical variables were compared using Chi-square or Fisher's exact tests, as appropriate.

All efficacy and safety outcomes were analyzed according to a modified intention-to-treat principle, defined as including all randomized participants who initiated COS and were therefore at risk of OHSS. Participants who discontinued before COS initiation (due to spontaneous pregnancy or cycle cancellation) were excluded from outcome analyses, as no OHSS or related data could be obtained. Because all participants who initiated COS completed the study without major protocol deviations, the per-protocol population was identical to the modified ITT set.

To evaluate whether attrition introduced bias, baseline demographic and clinical characteristics were compared between completers and dropouts (Supplementary Table 2, Supplementary File).

Data availability

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions

AM-H: Data collection, statistical analyses, interpretation of data, visualization, writing manuscript original draft; AA: Data collection, supervision, interpretation of data, and manuscript review and revision; FA: Study design, methodology, supervision, interpretation of data, and manuscript review and revision.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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