Original Article

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Astaxanthin compound nutrient improved insulin resistance, hormone levels, embryo quality and pregnancy outcomes in polycystic ovary syndrome patients undergoing *in vitro* fertilization/ intracytoplasmic sperm injection

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SUMMARY This study aimed to evaluate the effect of astaxanthin compound nutrient (ACN) complementary therapy on pregnancy outcomes in polycystic ovary syndrome (PCOS) patients undergoing *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI). This study enrolled 92 patients with PCOS who were continuously supplemented with ACN for three months prior to IVF/ICSI treatment from 2021 to 2023, and selected 92 patients who did not receive the treatment during the same period as controls. Baseline characteristics, ovulation induction outcomes, and pregnancy outcomes were compared between the two groups. In addition, the body mass index (BMI), anti-Müllerian hormone (AMH), antral follicle counting (AFC), fasting blood glucose (FBG), fasting insulin (FINS), homeostasis model assessment of insulin resistant (HOME-IR), and basal sex hormones of the supplementary group patients before and after treatment were compared. The results showed that there were no significant differences in the patient's duration of stimulation, total gonadotropin dose, peak E2 levels, and number of retrieved oocytes between the two groups. However, the number of 2 pronucleus (PN) fertilization, transferable embryos, and high-quality embryos was significantly higher in the ACN group compared with the control group. For both fresh and frozen embryo transplantation, positive pregnancy outcomes increased in PCOS patients who received supplementation of ACN for 3 months. In addition, after 3 months of supplementing with ACN, the patient's BMI, AMH, fasting insulin, HOME-IR, basal luteinising hormone (bLH), and basal testosterone (bT) decreased compared to before treatment. This study suggested that ACN improved insulin resistance, hormone levels, embryo quality and pregnancy outcomes in PCOS patients.

Keywords polycystic ovary syndrome, insulin resistance, oocyte quality, pregnancy outcomes

1. Introduction

Polycystic ovary syndrome (PCOS) is a highly heterogeneous disease featured by ovulation dysfunction, polycystic ovary morphology, and hyperandrogenism, often accompanied by endocrine disorders such as obesity and disorders of glucose and lipid metabolism. Seventy-five percent of underweight and 95% of overweight women with PCOS have insulin resistance (IR) (*1*). IR is not only related to ovulation disorders and hyperandrogenicity in PCOS patients, but also increases the risk of cardiovascular and other metabolic diseases in PCOS patients (*2*). In recent years, research has found that up to 80% of PCOS patients suffer from serious damage to their endocrine and reproductive health, leading to infertility as well as a younger age of occurrence (*3*), which has become a challenge in the reproductive field. Due to the complexity of the etiology and the diversity of symptoms, the cure of PCOS is relatively difficult. One of the treatment strategies recommended in the 2023 International Evidence-based Guideline of PCOS is healthy lifestyle, including diet and exercise interventions (*4*). Women with PCOS often lack some common vitamins, vitamin nutrients, and minerals. Supplementation of individual nutrients may improve the symptoms and severity of PCOS patients by influencing

key pathways, such as insulin signaling, IR, and lipid metabolism. Therefore, nutritional supplementation as an adjunct to traditional lifestyle treatments for PCOS can provide additional benefits (*5*).

Astaxanthin compound nutrient (ACN, Yunlike®) is a complex dietary supplement that contains D-chiro inositol (DCI), astaxanthin, L-carnitine, inulin, and α-linolenic acid (ALA). It is suitable for women with infertility, undergoing *in vitro* fertilization, or women of advanced maternal age preparing for pregnancy. Previous studies have shown the beneficial effects in improving PCOS condition of each ingredient (*6-13*). Our own practice also suggested that ACN is effective in improving insulin resistance, ovarian function, and oocyte quality. However, the clinical efficacy of ACN lacks the support of evidence-based medicine. Therefore, we conducted a single center study to evaluate the clinical efficacy of ACN supplementation in PCOS patients prior to IVF/ICSI (Figure 1).

2. Materials and Methods

2.1. Study population

PCOS patients who met the new diagnostic criteria of Rotterdam (*4*) and underwent IVF/ICSI-ET at Zhoushan Maternal and Child Health Hospital from 2021 to 2023 were screened. Patients with combined chromosomal abnormalities and those who have already taken other medications to treat PCOS, such as oral contraceptives, liver and kidney dysfunction, thyroid dysfunction, hyperprolactinemia, and any other endocrine disorder causing increased androgen were excluded from the study.

We enrolled 94 patients with PCOS who received ACN supplementation for 3 months prior to IVF/ICSI treatment as the supplement group, 2 of whom were lost to follow-up. For the control group, 92 patients with PCOS who did not receive any drug supplementation prior to IVF/ICSI treatment during the same period were selected. The study was approved by the Institutional Review Board of Zhoushan Maternal and Child Health Hospital and followed the guidelines established in the Declaration of Helsinki 2013 for research involving human participants (Reference No. 2024024).

2.2. Ovarian stimulation and oocyte retrieval

All enrolled patients were treated with an antagonist regimen for superovulation induction. Starting from the 3rd day of the menstrual cycle, gonadotropin (Gn, uniformly selected recombinant human follicle stimulating hormone injection, 450 IU/tube, Merck Serrano, Sweden) 150-225 IU was injected to stimulate ovulation. On the 6th to 8th day of the menstrual cycle or when serum LH > 5IU/L, GnRH-A (uniformly selected injection of cetuximab acetate, 0.25mg/tube, Merck Serrano, Sweden) 0.25 mg was injected. Regular monitoring of B-ultrasound and serum sex hormone levels were applied to adjust Gn dosage. When B-ultrasound detected 2 or more follicles with a diameter > 18mm, stop using Gn and inject HCG 5,000-10,000 U trigger. After 36.5 hours, puncture was performed for oocyte retrieval.

2.3. Embryo transfer plan

After oocyte retrieval, fertilization was performed through IVF or ICSI, depending on the patient's conditions (*e.g.* whether there was ovarian

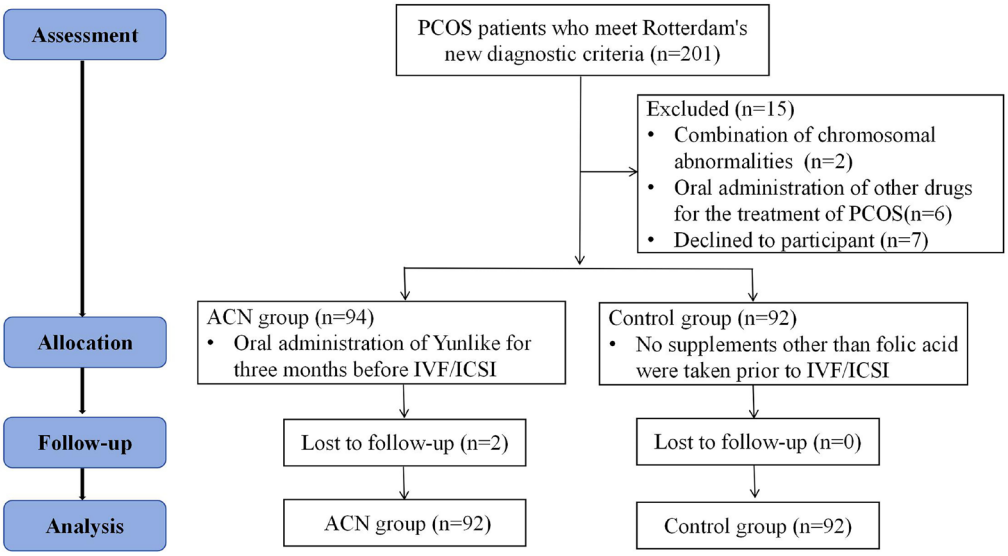

Figure 1. Study flowchart. A total of 201 patients met the diagnostic criteria of PCOS, of which 15 patients were excluded. Among included patients, 94 PCOS patients received continuous ACN supplementation for 3 months before IVF/ICSI treatment, of whom 2 patients were lost to follow-up. For the control group, 92 PCOS patients who did not supplement any nutrients except folic acid were included, and no one was lost to follow-up.

hyperstimulation syndrome, infection, hydrosalpinx, *etc.*). If transplanted, intramuscular injection of 60 mg/day progesterone injection was started after oocyte retrieval, and continued for three days to provide corpus luteum support. On the third day, two high-quality embryos were routinely selected and transplanted into the patient's uterine cavity. Luteal support was performed after transplantation.

If frozen embryo transfer was performed, hormone replacement regimen would be used to prepare the endometrium. Starting from the 3rd to 5th day of menstruation, 4 mg/day of estradiol valerate tablets (Bayer, Germany) were given for 5 consecutive days. From the 6th day onwards, the dosage was changed to 6 mg/day, and subsequently adjusted according to the patient's endometrial and blood hormone levels. When the thickness of the endometrium reached 8 mm, intramuscular injection of progesterone 60 mg/day was performed to transform the endometrium. After three consecutive days, two high-quality embryos were taken and transplanted into the patient's uterine cavity. Luteal support was performed post transplantation.

Urine pregnancy test or blood hCG quantification test was conducted 12 days after transplantation. For hCG positive patients, the first vaginal ultrasound examination should be performed 21 days after transplantation to rule out ectopic pregnancy. After 28 days of transplantation, ultrasound examination should be performed again to assess the number and development of embryos.

2.4. Data collection

Basic information, including age, height, weight, years of infertility, type of infertility, baseline sex hormones, AMH, AFC, FBG, and FINS levels was collected. The following variables were also collected after 3 months of ACN supplement: body weight, basal hormone levels, AMH, AFC, FBG, and FINS levels. BMI was calculated as weight (kg)/height $(m)^2$. HOME-IR was calculated as FBG (mmol/L) × FINS (μU/mL)/22.5.

The following variables of assisted reproductive technology (ART) were collected: Gn dosage, Gn days, total number of retrieved oocytes, 2PN fertilization rate, the number of transferable embryos, and the number of high-quality embryos. Pregnancy outcomes of patients undergoing frozen embryo transfer or fresh embryo transfer were followed-up.

2.5. Statistical analysis

Continuous variables conforming to the normal distribution were expressed in mean ± standard deviation and student *t*-test was used for comparison. Categorical variables were represented as numbers and percentages, and compared with chi-square tests. In addition, BMI, AMH, AFC, FBG, FINS, HOME-IR, and basal sex hormones of the experimental group patients after 3-months of ACN supplementation were compared using paired Student *t*-test. All data were analyzed by using SPSS 28.0 version, and the threshold of significance was bilateral $P < 0.05$.

3. Results

3.1. Participant characteristics

The 184 PCOS patients ranged in age from 24 to 42 years, and BMI ranged from 17.06 kg/m^2 to 32.27 kg/m^2. The ACN and control groups each contained 92 PCOS patients. In general, the baseline characteristics are comparable between the two groups ($P > 0.05$ for all), as presented in Table 1.

3.2. ART outcomes

As for the ART outcomes, the duration of stimulation, total gonadotropin dose, Peak E2 levels and the number of retrieved oocytes showed no significant differences between the two groups ($P > 0.05$ for all, Table 2). However, compared to the control group, the number of 2PN fertilization (10.85 ± 3.77 *vs.* 9.07 ± 3.06, $P = 0.04$), transplantable embryos (7.96 ± 2.88 *vs.* 6.01 ± 1.98, $P = 0.02$), and high-quality embryos (4.34 ± 2.51 *vs.* 3.16 ± 1.87, $P < 0.001$) were significant increased in the ACN group, as shown in Table 2.

3.3. Pregnancy outcomes

The pregnancy outcomes between the two groups are shown in Table 3. There were 25 cases in the ACN group underwent fresh embryo transfer, 67 cases underwent frozen embryo transfer; while the control group had 23 cases and 69 cases underwent fresh and frozen embryo transplantation, respectively. There was no significant difference in biochemical pregnancy, miscarriage, and ectopic pregnancy rates between the two groups, regardless of the embryo transfer methods. However, the positive pregnancy outcome was significantly higher than that in the control group (fresh embryo transfer, 44.00% *vs.* 34.78%, $P = 0.03$; frozen embryo transfer, 49.25% *vs*. 43.48%, $P = 0.04$).

3.4. Comparison of indicators in the supplementary group after 3 months of ACN supplement

After 3 months of ACN supplementation, the general indicators of the patients are compared, as shown in Table 4. Compared with before supplementation, there was no significant difference in AFC, FBG, bFSH, bE2, and bP among patients treated with ACN for 3 months. However, after 3 months of ACN supplementation, the patient's BMI (22.83 ± 3.11 kg/m^2 *vs.* 20.09 ± 3.13 kg/m^2, $P = 0.03$), AMH (7.13 ± 3.11 ng/mL *vs.* 5.88 ± 2.97 ng/mL, $P = 0.02$), fasting insulin (15.09 ± 5.84 μU/

Table 1. Clinical characteristics

Characteristics	ACN group (n = 92)	Control group (n = 92)	P value
Age, years	31.51 ± 3.03	31.79 ± 3.39	0.62
Years of infertility, years	3.17 ± 2.47	2.95 ± 2.53	0.61
Infertility type, n (%)			
Primary infertility	54 (58.7%)	61 (66.30%)	0.38
Secondary infertility	38 (41.3%)	31 (33.70%)	
BMI, kg/m^2	22.83 ± 3.11	22.37 ± 2.62	0.35
AMH, ng/mL	7.13 ± 3.11	7.80 ± 3.68	0.25
AFC, n	13.02 ± 2.01	13.43 ± 1.98	0.57
FBG, mmol/L	5.96 ± 1.87	5.88 ± 1.63	0.63
FINS, μU/mL	15.09 ± 5.84	16.55 ± 6.09	0.27
HOME-IR	2.71 ± 2.01	3.68 ± 2.43	0.16
bFSH, IU/L	5.67 ± 1.73	5.83 ± 1.49	0.36
bLH, IU/L	9.93 ± 2.08	10.06 ± 2.15	0.73
bE2, pg/mL	34.05 ± 7.95	32.60 ± 8.39	0.47
bT, nmol/L	2.81 ± 0.98	2.69 ± 1.08	0.18
bP, pmol/L	1.09 ± 0.58	0.96 ± 0.78	0.23

BMI: body mass index; AMH: anti-Müllerian hormone; AFC: antral follicle count; FBG: fasting blood glucose; FINS: fasting insulin; HOME-IR: homeostasis model asses sment of insulin resistant; bFSH: basal follicle-stimulating hormone; bLH: basal luteinising hormone; bE2: basal estradiol; bT: basal testosterone; bP: basal progesterone.

Table 2. Assisted reproductive technology (ART) outcomes

Parameters	ACN group (n = 92)	Control group (n = 92)	P value
Duration of stimulation, days	9.09 ± 2.60	9.73 ± 2.78	0.39
Total gonadotropin dose, IU	2179.15 ± 455.16	2236.97 ± 540.65	0.18
Peak E2 levels, pg/mL	4383.29 ± 899.17	4199.16 ± 815.67	0.14
Number of retrieved oocytes	14.88 ± 5.31	14.60 ± 5.38	0.71
Number of 2PN fertilization	10.85 ± 3.77	9.07 ± 3.06	**0.04**
Number of transferable embryos	7.96 ± 2.88	6.01 ± 1.98	**0.02**
Number of high-quality embryos	4.34 ± 2.51	3.16 ± 1.87	**< 0.001**

Table 3. Pregnancy outcomes

Pregnancy outcome	ACN group (n = 92)	Control group (n = 92)	P value
Fresh embryo transfer, n (%)	25 (27.17%)	23 (25.00%)	0.77
Biochemical pregnancy, n (%)	1 (4.00%)	1 (4.35%)	0.81
Positive pregnancy outcome, n (%)	11 (44.00%)	8 (34.78%)	**0.03**
Miscarriage, n (%)	1 (4.00%)	1 (4.35%)	0.81
Ectopic pregnancy, n (%)	1 (4.00%)	1 (4.35%)	0.81
Frozen embryo transfer, n (%)	67 (72.82%)	69 (75%)	0.77
Biochemical pregnancy, n (%)	4 (5.97%)	5 (7.25%)	0.08
Positive pregnancy outcome, n (%)	33 (49.25%)	30 (43.48%)	**0.04**
Miscarriage, n (%)	2 (3.00%)	3 (4.35%)	0.38
Ectopic pregnancy, n (%)	2 (3.00%)	2 (2.90%)	0.89

mL *vs.* 12.01 ± 4.13 μU/mL, P = 0.01), and HOME-IR (2.71 ± 2.01 *vs.* 1.91 ± 1.09 μU/mL, P = 0.02) decreased significantly. At the same time, after supplementation with ACN, the patient's basal LH (9.93 ± 2.08 IU/L *vs.* 7.14 ± 1.97 IU/L, P = 0.03) and basal T (2.81 ± 0.98 nmol/L *vs.* 1.84 ± 0.86 nmol/L, P = 0.01) levels were significantly decreased.

4. Discussion

As one of the main causes of female infertility, about 10% of PCOS patients seek ART treatment to aid in pregnancy (*3*). In this study, we retrospectively collected 92 PCOS patients who received ACN supplementation for 3 months prior to IVF/ICSI treatment, and selected 92 PCOS patients who did not receive any supplementation as controls. Our results showed that after complementary treatment with ACN, the number of 2PN fertilization, high-quality embryos, and transferable embryos significantly increased, and the positive pregnancy outcomes of fresh and frozen embryo transplantation also improved.

The etiology of PCOS is complex. Research has shown that excessive androgen, IR combined with hypothalamic pituitary dysfunction leading to ovarian dysfunction and ovulation disorders are the main

Table 4. Comparison of indicators in the supplementary group after 3 months of ACN supplementation

Parameters	Before treatment (n = 92)	After treatment (n = 92)	P value
BMI, kg/m^2	22.83 ± 3.11	20.09 ± 3.13	**0.03**
AMH, ng/mL	7.13 ± 3.11	5.88 ± 2.97	**0.02**
AFC, n	13.02 ± 2.01	12.86 ± 1.95	0.21
FBG, mmol/L	5.96 ± 1.87	5.04 ± 1.67	0.48
FINS, μU/mL	15.09 ± 5.84	12.01 ± 4.13	**0.01**
HOME-IR	2.71 ± 2.01	1.91 ± 1.09	**0.02**
bFSH, IU/L	5.67 ± 1.73	5.02 ± 2.11	0.45
bLH, IU/L	9.93 ± 2.08	7.14 ± 1.97	**0.03**
bE2, pg/mL	34.05 ± 7.95	32.79 ± 6.93	0.29
bT, nmol/L	2.81 ± 0.98	1.84 ± 0.86	**0.01**
bP, pmol/L	1.09 ± 0.58	0.99 ± 0.47	0.22

BMI: body mass index; AMH: anti-Müllerian hormone; AFC: antral follicle count; FBG: fasting blood glucose; FINS: fasting insulin; HOME-IR: homeostasis model assessment of insulin resistant; bFSH: basal follicle-stimulating hormone; bLH: basal luteinising hormone; bE2: basal estradiol; bT: basal testosterone; bP: basal progesterone.

mechanisms of infertility in PCOS patients (*14*). Previous studies have shown that supplementation of multiple nutrients can improve IR in PCOS patients (*5,15*). However, it is not clear whether the composite components are beneficial for superovulation and pregnancy outcomes in PCOS patients. To the best of our knowledge, our study is the first of its kind to investigate the synergistic effects of different nutrients (ACN) on PCOS patients.

ACN contains active ingredients which have been respectively proven to be beneficial in PCOS patients receiving ART. It was observed that decreased availability of DCI in plasma or increased excretion of DCI in urine was associated with IR, supporting the role of DCI as an insulin sensitizer (*16*). An early study about DCI on PCOS patients showed that the DCI group (n = 22) had increased insulin sensitivity, decreased free testosterone levels, and significantly higher ovulation rates than the placebo group (n = 22) (86% *vs.* 27%, $P < 0.05$ for all) (*7*). Astaxanthin is a ketocarotenoid, super antioxidant molecule (*8*). It has various biological activities such as clearing free radicals, antioxidation, enhancing immunity, and anti-aging. In animal experiments, astaxanthin can inactivate the Wnt/β-Catenin signal by upregulating Klotho and activate the MEK/ERK signal, thereby preventing inflammatory reactions, improving hormone levels in PCOS rats, and improving symptoms of obesity and polycystic ovary morphology (*9*). An *in vitro* experiment on primary culture of human granulosa cells showed that DCI can reduce the expression of cytochrome P450 family 19 subfamily A member 1 (CYP19A1), P450Side-chain cleavage (P450scc), and insulin-like growth factor-1R (IGF-1R), indicating that DCI can reduce the production of steroidogenic enzymes by antagonizing the action of insulin (*17*). A randomized clinical trial showed that after taking astaxanthin for 8 weeks, FBG, insulin, and HOMA-IR levels in PCOS patients were significantly reduced (*18*). It is reported that thin women with PCOS had lower levels of L-carnitine, which was mainly related to hyperandrogenism and IR (*19*). After supplementing PCOS patients with L-carnitine, their insulin sensitivity and glucose tolerance tests were significantly improved (*11*). Different aggregation levels of inulin can improve metabolic outcomes, androgen status, and clinical manifestations in PCOS patients by reducing total testosterone, free androgen index, BMI, fasting insulin, and HOMA-IR levels (*20*). Animal experiments have shown that supplementing with inulin can improve the estrus cycle and ovarian morphology of PCOS mice, reduce luteinizing hormone levels, increase serum levels of FSH and interleukin (IL)-22, and regulate gut microbiota and bile acid profile (*21*). Flaxseed oil rich in ALA improved the estrus cycle, ovarian morphology, steroid hormone imbalance, weight, lipid abnormalities, and IR in mice (*22*). It also improved plasma and ovarian inflammatory IL-1β, IL-6, IL-10, IL-17A, monocyte chemotactic protein-1 and tumor necrosis factor (TNF)-α (*22*). Consistent with previous findings, our study confirmed that after taking ACN for 3 months, BMI, AMH, fasting insulin, and HOME-IR improved significantly, while basal LH and T also decreased significantly compared to before the treatment.

Previous studies have also suggested that nutrients were beneficial for improving ART outcomes in PCOS patients. A study of seven oocyte quality markers in patients with PCOS receiving ICSI showed that high-dose DCI helped improve oocyte quality (*23*). It is speculated that DCI participates in oocyte activation and increases granulosa cell activity through phosphatidylinositol-3-kinase. Another study showed that supplementation with astaxanthin in PCOS significantly increased the expression of oxidative stress factors such as Nrf2, HO-1, and NQ-1 in its granulosa cells. In addition, the rates of metaphase II oocytes and high-quality embryos significantly increased, while there was no significant intergroup difference in biochemical pregnancy and clinical pregnancy rates (*24*). In a PCOS rat model, after adjuvant treatment with astaxanthin, the expression levels of IL-6, TNF-α and NF-κ in ovarian tissue decreased, as well as the malondialdehyde level, while the level of superoxide dismutase increased (*25*). Therefore, it is speculated that astaxanthin can protect ovary from oxidative stress damage. A study of 214 infertile women receiving oral L-carnitine during IVF cycles reported that the total number of oocytes retrieved, oocyte maturation rate, and fertilization rate before and after supplementing with L-carnitine showed no significant differences, but the embryo quality significantly improved (*26*). However, our results were slightly different. It is possible that the high number of 2PN insemination in our experimental group may be due to other components in pregnancy. Some studies have shown that antioxidant properties of L-carnitine can

improve and maintain mitochondrial activity in oocytes, obtain high-quality oocytes with high developmental ability, thus improving embryo quality (*27*).

In conclusion, ACN, a complex dietary supplement, can improve IR, hormone levels, embryo quality and pregnancy outcomes in PCOS patients. It is recommended that PCOS patients receive ACN supplementation for 3 months before entering the IVF/ICSI cycle to improve their pregnancy outcomes.

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References

1. Stepto NK, Cassar S, Joham AE, Hutchison SK, Harrison CL, Goldstein RF, Teede HJ. Women with polycystic ovary syndrome have intrinsic insulin resistance on euglycaemic-hyperinsulaemic clamp. Hum Reprod. 2013; 28:777-784.
2. Ding H, Zhang J, Zhang F, Zhang S, Chen X, Liang W, Xie Q. Resistance to the insulin and elevated level of androgen: A major cause of polycystic ovary syndrome. Front Endocrinol (Lausanne). 2021; 12:741764.
3. Joham AE, Norman RJ, Stener-Victorin E, Legro RS, Franks S, Moran LJ, Boyle J, Teede HJ. Polycystic ovary syndrome. Lancet Diabetes Endocrinol. 2022; 10:668-680.
4. Teede HJ, Tay CT, Laven JJE, Dokras A, Moran LJ, Piltonen TT, Costello MF, Boivin J, Redman LM, Boyle JA, Norman RJ, Mousa A, Joham AE. Recommendations from the 2023 international evidence-based guideline for the assessment and management of polycystic ovary syndrome. J Clin Endocrinol Metab. 2023; 108:2447-2469.
5. Rodriguez Paris V, Solon-Biet SM, Senior AM, Edwards MC, Desai R, Tedla N, Cox MJ, Ledger WL, Gilchrist RB, Simpson SJ, Handelsman DJ, Walters KA. Defining the impact of dietary macronutrient balance on PCOS traits. Nat Commun. 2020; 11:5262.
6. Unfer V, Nestler JE, Kamenov ZA, Prapas N, Facchinetti F. Effects of inositol(s) in women with PCOS: A systematic review of randomized controlled trials. Int J Endocrinol. 2016; 2016:1849162.
7. Nestler JE, Jakubowicz DJ, Reamer P, Gunn RD, Allan G. Ovulatory and metabolic effects of D-chiro-inositol in the polycystic ovary syndrome. N Engl J Med. 1999; 340:1314-20.
8. Kumar S, Kumar R, Diksha, Kumari A, Panwar A. Astaxanthin: A super antioxidant from microalgae and its therapeutic potential. J Basic Microbiol. 2022; 62:1064-1082.
9. Song Yu, Zhu Zhengyan, Huang Huan, Li Chun, Lu Jingquan, Liu Heyu, Hu Lina. Astaxanthin regulates Klotho expression and Wnt/ β - The improvement effect of the Catenin signaling pathway on polycystic ovary syndrome in rats. Tianjin Pharmaceutical. 2023; 51:1098-1103. (in Chinese)
10. Mohd Shukri MF, Norhayati MN, Badrin S, Abdul Kadir A. Effects of L-carnitine supplementation for women with polycystic ovary syndrome: A systematic review and meta-analysis. PeerJ. 2022; 10:e13992.
11. Tauqir S, Israr M, Rauf B, Malik MO, Habib SH, Shah FA, Usman M, Raza MA, Shah I, Badshah H, Ehtesham E, Shah M. Acetyl-L-Carnitine ameliorates metabolic and endocrine alterations in women with PCOS: A double-blind randomized clinical trial. Adv Ther. 2021; 38:3842-3856.
12. Xue J, Li X, Liu P, Li K, Sha L, Yang X, Zhu L, Wang Z, Dong Y, Zhang L, Lei H, Zhang X, Dong X, Wang H. Inulin and metformin ameliorate polycystic ovary syndrome *via* anti-inflammation and modulating gut microbiota in mice. Endocr J. 2019; 66:859-870.
13. Sun Lijun, Hu Jijun, Guan Yichun, Wu Wenbin, Ma Xiaojuan, Song Na, Qi Ruofan. The effect of α-linolenic acid on *in vitro* maturation of human immature oocytes. China Maternal and Child Health. 2015; 30:5002-5005.
14. Collée J, Mawet M, Tebache L, Nisolle M, Brichant G. Polycystic ovarian syndrome and infertility: Overview and insights of the putative treatments. Gynecol Endocrinol. 2021; 37:869-874.
15. Szczuko M, Kikut J, Szczuko U, Szydłowska I, Nawrocka-Rutkowska J, Ziętek M, Verbanac D, Saso L. Nutrition strategy and life style in polycystic ovary syndrome-narrative review. Nutrients. 2021; 13:2452.
16. Baillargeon JP, Nestler JE, Ostlund RE, Apridonidze T, Diamanti-Kandarakis E. Greek hyperinsulinemic women, with or without polycystic ovary syndrome, display altered inositols metabolism. Hum Reprod. 2008; 23:1439-1446.
17. Sacchi S, Marinaro F, Tondelli D, Lui J, Xella S, Marsella T, Tagliasacchi D, Argento C, Tirelli A, Giulini S, La Marca A. Modulation of gonadotrophin induced steroidogenic enzymes in granulosa cells by d-chiroinositol. Reprod Biol Endocrinol. 2016; 14:52.
18. Jabarpour M, Aleyasin A, Shabani Nashtaei M, Amidi F. Astaxanthin supplementation impact on insulin resistance, lipid profile, blood pressure, and oxidative stress in polycystic ovary syndrome patients: A triple-blind randomized clinical trial. Phytother Res. 2024; 38:321-330.
19. Fenkci SM, Fenkci V, Oztekin O, Rota S, Karagenc N. Serum total L-carnitine levels in non-obese women with polycystic ovary syndrome. Hum Reprod. 2008; 23:1602-1606.
20. Ziaei R, Shahshahan Z, Ghasemi-Tehrani H, Heidari Z, Nehls MS, Ghiasvand R. Inulin-type fructans with different degrees of polymerization improve insulin resistance, metabolic parameters, and hormonal status in overweight and obese women with polycystic ovary syndrome: A randomized double-blind, placebo-controlled clinical trial. Food Sci Nutr. 2023; 12:2016-2028.
21. Li T, Zhang Y, Song J, Chen L, Du M, Mao X. Yogurt enriched with inulin ameliorated reproductive functions and regulated gut microbiota in dehydroepiandrosterone-

induced polycystic ovary syndrome mice. Nutrients. 2022; 14:279.
22. Wang T, Sha L, Li Y, Zhu L, Wang Z, Li K, Lu H, Bao T, Guo L, Zhang X, Wang H. Dietary α-linolenic acid-rich faxseed oil exerts beneficial effects on polycystic ovary syndrome through sex steroid hormones-microbiota-inflammation axis in rats. Front Endocrinol (Lausanne). 2020; 11:284.
23. Mendoza N, Galan MI, Molina C, Mendoza-Tesarik R, Conde C, Mazheika M, Diaz-Ropero MP, Fonolla J, Tesarik J, Olivares M. High dose of d-chiro-inositol improves oocyte quality in women with polycystic ovary syndrome undergoing ICSI: a randomized controlled trial. Gynecol Endocrinol. 2020; 36:398-401.
24. Gharaei R, Alyasin A, Mahdavinezhad F, Samadian E, Ashrafnezhad Z, Amidi F. Randomized controlled trial of astaxanthin impacts on antioxidant status and assisted reproductive technology outcomes in women with polycystic ovarian syndrome. J Assist Reprod Genet. 2022; 39:995-1008.
25. Toktay E, Selli J, Gurbuz MA, Alaca R. Investigation of the effects of astaxanthin in experimental polycystic ovary syndrome (PCOS) in rats. Iran J Basic Med Sci. 2023; 26:1155-1161.
26. Kitano Y, Hashimoto S, Matsumoto H, Yamochi T, Yamanaka M, Nakaoka Y, Fukuda A, Inoue M, Ikeda T, Morimoto Y. Oral administration of l-carnitine improves the clinical outcome of fertility in patients with IVF treatment. Gynecol Endocrinol. 2018; 34:684-688.
27. Sheida A, Davar R, Tabibnejad N, Eftekhar M. The effect of adding L-carnitine to the GnRH-antagonist protocol on assisted reproductive technology outcome in women with polycystic ovarian syndrome: A randomized clinical trial. Gynecol Endocrinol. 2023; 39:1878135.

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