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Gushen Antai pill for expected normal ovarian responders undergoing IVF-ET (GSATP-FreET): interim analysis of a randomized controlled trial

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Abstract

Introduction Optimal luteal phase support (LPS) is essential for successful embryo transfer. In our previous study, we found that LPS combined with Gushen Antai pill (GSATP) could significantly improve ongoing pregnancy rate (OPR) and reduce threatened abortion rate after frozen-thawed embryo transfer (FET). Despite this, no high-quality studies have been performed on the combination of LPS and GSATP for the embryo transfer of fresh IVF cycles.

Methods A randomized, double-blind, placebo-controlled trial started on June 15, 2021 at 20 reproductive centers of public tertiary hospitals. In total, 371 eligible women with expected normal ovarian reserve (NOR) were age-stratified and randomly assigned to either the GSATP group or the placebo group at random in an equal ratio. The primary outcome is OPR. The report presents data from an interim analysis used for regulatory submissions.

Results In the per-protocol cohort, the OPR in the GSATP group and placebo group were 40.3% and 38.4%, respectively. Nevertheless, the GSATP group showed a significantly lower incidence of vaginal bleeding (11.3% vs. 21.6%, $p=0.046$), threatened miscarriage (16.0% vs. 29.4%, $p=0.021$), and functional constipation (16.0% vs. 29.4%, $p=0.021$) than the placebo group during the first trimester of pregnancy.

Discussion In the interim analysis, while GSATP did not result in better pregnancy outcomes for NOR patients undergoing fresh embryo transfers, it did improve common symptoms observed during the early pregnancy phase, such as vaginal bleeding, threatened miscarriage, and functional constipation.

Trial registration National Institutes of Health clinical trials database, NCT04872660. Registered 4 May 2021, https//clinicaltrials.gov/study/NCT04872660.

Keywords Traditional Chinese medicine, Gushen Antai pill, Embryo transfer, Ongoing pregnancy rate, Interim analysis

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Introduction

Infertility is a growing reproductive health problem, and it is estimated that approximately 15% of couples of reproductive age are affected [1]. However, in China, the prevalence of infertility is as high as 25% among couples of childbearing age who are actively trying to conceive [2]. Although in vitro fertilization and embryo transfer (IVF-ET) technology has improved sperm-egg encounter and fertilization barriers, implantation conditions or endometrial receptivity remain issues, and further optimization of pregnancy outcomes is necessary. The key to successful IVF-ET treatment is properly designed ovarian stimulation and LPS. Evidence shows that almost all patients who receive controlled ovarian stimulation have insufficient luteal function [3–5]. While natural progesterone (P_4) is the preferred option for LPS in IVF-ET cycles, there is controversy over the optimal route, dose, and duration of P_4 administration, as well as whether additional medications should be administered. A recent clinical study suggests that LPS with P_4, along with the addition of estrogen or human chorionic gonadotropin (hCG), does not appear to have any beneficial effect on improvement in live birth or OPR [6]. In contrast, LPS combined with hCG is associated with a higher incidence of ovarian hyperstimulation syndrome (OHSS) [6]. In our previous randomized controlled trial (RCT), we found that GSATP added to artificial cycle endometrial preparation in FET cycles significantly increased the OPR (56.62% vs. 44.44%, $p = 0.045$), and reduced the incidence of threatened miscarriage in early pregnancy (10% vs. 23.08%, $p = 0.032$), particularly in patients ≤ 35 years old [7]. As demonstrated in a prospective study by Lu and her colleagues, the OPR of LPS combined with GSATP was significantly higher than that of conventional LPS alone (63.83% vs. 42. 86%, $p = 0.039$) [8].

In traditional Chinese medicine (TCM) theory, "Kidney Governs Reproduction", and female infertility is closely related to kidney deficiency, and the main therapeutic principle of it involves tonifying the kidney. GSATP is widely used as an adjunctive therapy for pregnant women with threatened abortion in China, and the clinical effects reflected by the patients remained satisfactory [9]. GSATP is used in cases of early threatened abortion, which corresponds to the kidney yin deficiency syndrome of TCM. The function of GSATP is to nourish yin, tonify the kidney, strengthen Chong-Ren, and prevent miscarriage. Moreover, from a modern pharmacology perspective, it has the potential to enhance the hypothalamus-pituitary-ovarian axis' responsiveness to reproductive hormones, effectively regulate ovulation, and, at the same time, regulate hormone levels, notably estrogen-like effects as well as the ability to improve serum estrogen concentrations and estrogen receptor activity, which can enhance ovarian function, increase endometrial receptivity, and increase fertility.

Recently, it was demonstrated that LPS combined with GSATP could dramatically increase embryo implantation and clinical pregnancy rates, as well as reduce early pregnancy loss rates, and especially improve OPR in fresh IVF cycles [8]. However, the study's methodological deficiencies prevented it from reaching a definitive conclusion regarding GSATP's treatment effect. This was mostly attributed to the unspecified randomization process, the absence of distribution concealment and blinding, no placebo control, and vague inclusion and exclusion criteria. As a result, a well-designed RCT is essential to estimate the efficiency and safety of GSATP in NOR women during fresh IVF cycles.

Methods

Study design

The study is designed as a prospective, multicenter, randomized, double-blind, placebo-controlled trial, which conducted by the 20 reproductive centers of public tertiary hospitals. The first patient was enrolled on June 30, 2021, and the actual study start date was 15 June 2021. The trial is expected to conclude on 30 June 2025. As of the manuscript preparation, the study has enrolled 371 infertile women, with recruitment still ongoing. In total, 800 eligible patients will be enrolled in the study. Participants will be assigned to either the GSATP group or the placebo group at random in an equal ratio. All participants will receive conventional IVF-ET with GSATP or placebo as a complementary treatment. This RCT was registered at the National Institutes of Health clinical trials database (ClinicalTrials.gov, ID: NCT04872660).

Eligibility criteria

Inclusion criteria

1. Patients with NOR (5 ≤ AFC ≤ 15, 1.2 ng/ml ≤ AMH ≤ 3.5 ng/ml).
2. Patients with regular menstrual cycle (21–35 days) and normal ovulation.
3. Patients treated with initial IVF/ICSI treatment.
4. Patients have at least one embryo or blastocyst available for transfer.

Exclusion criteria

1. Patients aged ≥ 43 years.
2. Patients with body mass index (BMI) ≥ 28 kg/m^2.
3. "Freeze-all" strategy.
4. Patients who use the natural cycle or mild stimulation for IVF/ICSI treatment.
5. Patients with severe OHSS during controlled ovarian stimulation (COS).

6. Patients who accept donated oocytes or perform either in vitro Maturation (IVM), blastocyst biopsy for preimplantation genetic diagnosis (PGD), or preimplantation genetic testing for aneuploidies (PGT-A).
7. Patients with a history of two or more previous consecutive spontaneous abortions.
8. Patients with a history of two or more previous IVF-ET failures.
9. Patients with karyotype abnormalities.
10. Patients with polycystic ovary syndrome.
11. Patients with a non-surgically treated hydrosalpinx or uterine cavity fluid or endometrial polyp, or an ovarian endometriosis cyst requiring surgery, during ovarian stimulation.
12. Patients with congenital or acquired abnormalities of uterine anatomy.
13. Patients with combined contraindications to IVF or pregnancy.

Dropout and discontinuation criteria

1. Patients who do not take the medication as prescribed, or stop taking it privately.
2. Patients who take other prescription medications that interfere with study results during treatment.
3. Patients who experience adverse reactions to the drugs that make it difficult for them to continue treatment.
4. Insufficient follicle growth, premature follicle ovulation, failure to obtain a follicle, and cancellation of the cycle for patient-specific reasons.

Randomization, allocation, and blinding

Previous studies found that the OPR for the GSATP group was significantly higher in the < 35-year-old (yrs.) subgroup, while vaginal bleeding rates were significantly reduced in the ≥ 35-year-old subgroup compared to the placebo group. Due to the limitations of post-hoc analysis, we used stratified block randomization method to stagger according to their age (< 35 yrs. vs. ≥ 35 yrs.). In each age group, 400 cases will be arranged, with 200 cases in each GSATP and Placebo group. We generated random numbers using R software (version 3.5.1), performed stratified block randomization, and set the block length to 4, with a 1:1 ratio of participants in the two groups. Randomization will be performed by dedicated nurses who are not involved in patient recruitment as well as clinical management. The nurse will then prepare randomly grouped cards and place them in opaque envelopes. Finally, the nurses will hand the opaque envelopes to the patients on the day of ET.

The GSATP and placebo will be coded and correspond to the patients' code on a one-to-one basis, and the specific grouping details are mastered by the drug management center. GSATP and placebo are identical in appearance and taste. All clinicians, outcome assessors, statisticians, and patients will be blinded. Clinicians and patients will not be informed about their treatment groupings. When the clinical trial ends, the final blind-break process will be performed by authorized researchers.

Interventions

Drug Preparation and administration

The GSATP (Z20030144) is composed of 10 herbs, including radix-polygoni multiflori, radix rehmanniae praeparata, cistanche salsa, radix dipsaci, uncaria, semen cuscutae, rhizoma atractylodis macrocephalae, radix scutellariae, and radix paeoniae lactiflorae. The function of GSATP is nourishing yin and tonifying the kidney and spleen, invigorating qi and nourishing the blood and preventing miscarriage. GSATP is used in the early threatened abortion, which belongs to the kidney yin deficiency syndrome of TCM. Its production follows good manufacturing practice (GMP) standards and takes the form of water honey pills, each bag of 6 g.

Placebo Preparation and administration

Placebo is no different in appearance and taste from GSATP. This placebo is also produced by Beijing Boran Pharmaceutical Inc., which was mainly composed of soybean powder, starch, carbon melanin, and refined honey. The placebo simulate the appearance, color, and smell of GSATP, but does not have any active ingredients, so it has no clinical effect. Placebo is packaged in the same way as GSATP. On the one hand, it prevents participants from realizing that they are assigned to the placebo group, which may reduce participants' compliance; On the other hand, it prevents the potentially beneficial effects of using vitamins as a placebo.

COS and oocytes retrieval

Ovarian stimulation begins on day 2 or 3 of the menstrual cycle. All patients are given recombinant follicle stimulating hormone (r-FSH, Gonal-F, Merck Serono, Switzerland). The starting dose of r-FSH is determined by the doctor based on patient's age, AFC, basal FSH, estradiol (E_2) levels and BMI, typically ranging from 150 to 225 IU/day. The dose of FSH is adjusted every 2–3 days according to the ovarian response, E_2 level and follicle growth as monitored via transvaginal ultrasound. Then, from day 5 or 6 of the ovarian stimulation to trigger day, all patients are treated with gonadotrophin releasing hormone-antagonist (GnRH-ant, cetrorelix acetate, Cetrotide; Merck Serono, Germany). When more than 2 follicles reach at least 18 mm in size, 250 μg of recombinant human chorionic gonadotrophin (r-hCG, Ovidrel, Merck

Serono, Italy) is administered to trigger the final follicular maturation. After 35 to 37 h, transvaginal ultrasound-guided aspiration is performed. Intracytoplasmic sperm injection is performed only in cases of severe male factors of infertility.

Embryo transfer and luteal phase support

In all cases, cleavage-stage embryos were transferred on day 3 of oocyte retrieval, or blastocysts on day 5, except as follows: Serum $E_2 > 5000$ pg/mL on the trigger day; the number of oocytes retrieved ≥ 15; OHSS; uterine or endometrial abnormalities, such as endometriosis, uterine fibroids, endometrial polyps, or intrauterine adhesions; serum $P_4 > 1.5$ ng/mL before the trigger day; or patients refuse fresh embryo transfer. No more than two embryos will be transferred per cycle.

LPS begins from embryo transfer until the 10th gestational week. All participants take vaginal P_4 Vaginal Sustained-Release Gel (Selenone®8%, Merck Serono, Germany) 90 mg per day. Each participant will be randomly assigned to one of two groups, one group receiving GSATP (Beijing Boran Pharmaceutical Inc., China) 6 g, three times daily, and the other receiving placebo. It will be discontinued immediately if serum β-hCG is negative, there is biochemical pregnancy loss, or transvaginal ultrasound suggests embryo arrest.

Outcomes

Efficacy outcomes

The primary outcome is OPR at 10 weeks after the day of embryo transfer.

The following secondary outcomes will also be evaluated:

(1) Positive pregnancy: Serum β-hCG ≥ 10 mIU/mL 14 days after embryo transfer.
(2) Clinical pregnancy: An intrauterine gestational sac with fetal heartbeat detected by transvaginal ultrasonography at 4 weeks after the day of embryo transfer.
(3) Pregnancy loss: Clinically recognized spontaneous loss of pregnancy before the completion of twelve gestational weeks.
(4) Ectopic pregnancy: A pregnancy in which implantation takes place outside the uterine cavity at 4 weeks after the day of embryo transfer.
(5) Embryo implantation rate: The percentage of the number of the gestational sac to the total transferred embryos.
(6) Vaginal bleeding in early pregnancy: Vaginal bleeding that occurs within the first 12 weeks of pregnancy, without significant abdominal pain, and with ultrasound showing normal fetal development. This may appear as spotting or light to heavy bleeding, and the blood can be bright red, dark red, or brown in color. without significant abdominal pain, and with ultrasound showing normal fetal development.
(7) Threatened miscarriages in early pregnancy: Vaginal bleeding occurs before 28 weeks of pregnancy, especially in the early stages, with or without lower abdominal pain and lower back pain, with ultrasound showing abnormal fetal development or the risk of miscarriage.
(8) The prevalence of pregnancy constipation: The percentage of participants with pregnancy constipation compared to the total patients randomized. Functional constipation is defined as the presence of at least two out of the following six symptoms: straining, lumpy or hard stools, a sensation of incomplete evacuation, a sensation of anorectal obstruction/blockage, manual maneuvers to facilitate defecation, and fewer than three spontaneous bowel movements per week. Symptoms must be present in at least 25% of the defecations and last at least one month.

Safety outcomes

Adverse effects of GSATP have been observed in preclinical studies, such as gastrointestinal discomfort, diarrhea, allergies, and skin rash. Any adverse events occurring during the trail will be recorded and assessed. If serious adverse events appear, researchers should terminate the study for the affected participant.

Statistical analysis

Sample size calculation

Reviewing previous study data from our center, after one fresh embryo transfer in women with normal ovarian reserve undergoing IVF-ET, the OPR is approximately 40%. Based on our previous study [7], we assumed that an absolute difference in OPR of 12% would be clinically significant. The aim of this study is to examine a 12% difference in OPRs between GSATP group and the placebo group (52% vs. 40%) at a significance level of 0.05 and a test efficiency of 90%. The minimum sample size calculation for each group is 358. To account for a 10% dropout rate, the sample size of 800 was determined using PASS (Power Analysis and Sample Size) 2021 (NCSS, LLC, Kaysville, Utah), in which 400 participants were in each group. As of November 20, 2023, 452 patients from 20 hospitals who met the inclusion criteria were enrolled, we conducted an interim analysis to preliminarily evaluate the clinical efficacy and safety of GSATP on OPR in NOR women.

Planned data analysis

Study data from all patients will be pooled for intention-to-treat (ITT) analysis, along with per-protocol analysis

after exclusion of loss to follow-up. The ITT analysis will be used for the initial analysis of differences in OPR between the two groups. Multiple imputations will be used to handle missing data for this study. The student's t-test will be used for continuous variables and the χ^2 test will be used for categorical variables. Subgroup analysis will be performed according to pre-set age stratification. If the ITT analysis and the per-protocol analysis are inconsistent, greater weight will be given to the initial analysis when interpreting the trial results. All statistical work will be performed using Jamovi 2.4.8 and R 3.5.1. A p-value of <0.05 will be considered statistically significant.

Results

As of November 20, 2023, approximately half of the expected participants had been recruited for the interim analysis. A total of 452 patients from 20 hospitals who met the inclusion criteria were collected, of whom 371 were included in the efficacy analysis, of these, 186 patients received GSATP treatment and 185 patients received the same dose of placebo (Fig. 1).

Baseline characteristics and clinical index

Table 1 shows the baseline characteristics of the participants on menstrual cycle days 2–3, which indicate no statistically significant differences between the two groups. The clinical index of COS and embryonic laboratory outcomes were similar between the two groups (see Table 2).

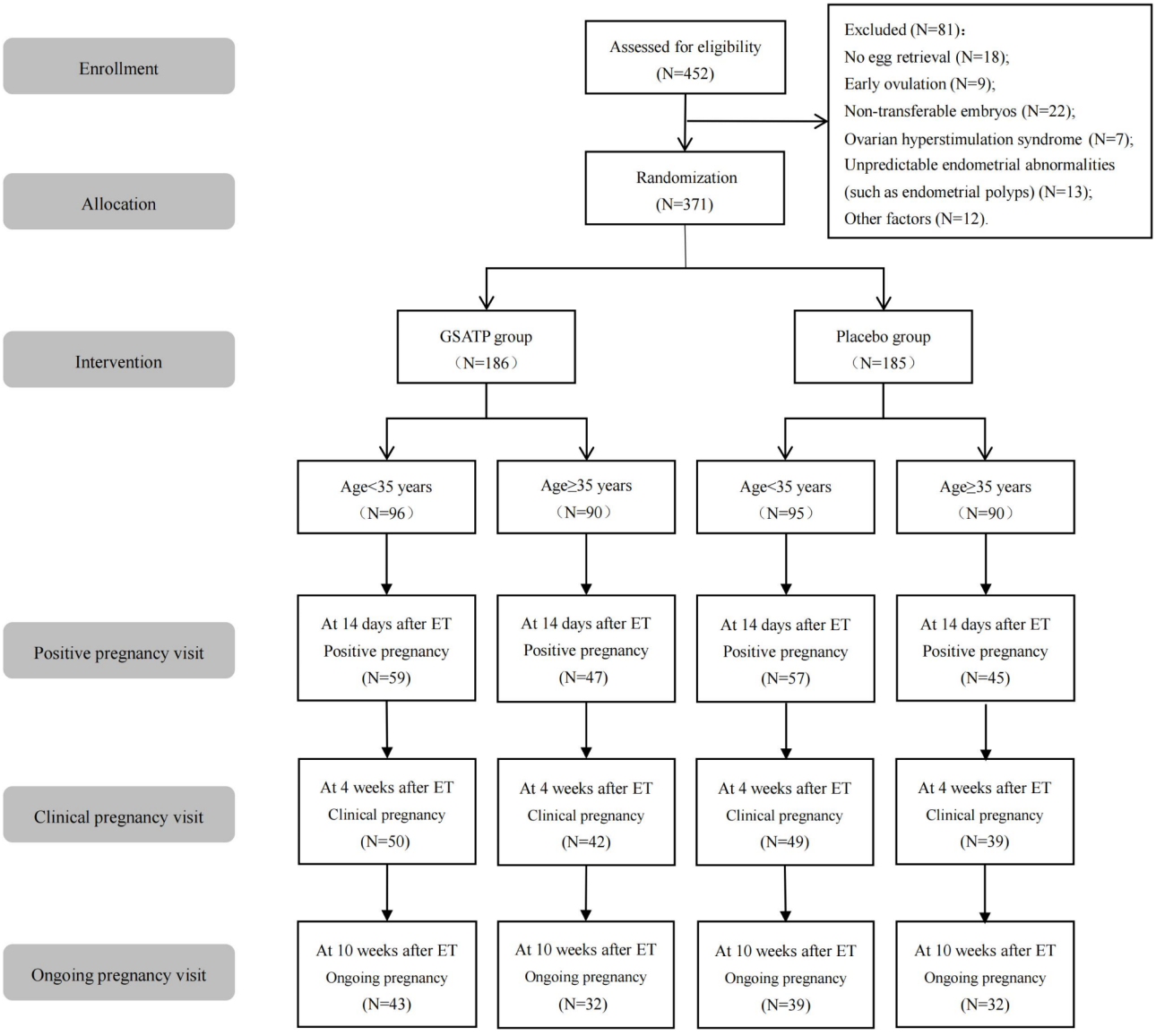

Fig. 1 Flowchart of participant randomization, treatment, and ongoing pregnancy follow-up

Table 1 Participants' baseline characteristics on menstrual cycle days 2–3

Characteristics	Gushen Antai Pill group (*n* = 186)	Placebo group (*n* = 185)	*P* value
Age at inclusion (years; mean (SD))	33.9 (4.5)	34.3 (4.0)	0.581
Age ≥ 37	45 (24.2)	53 (28.6)	0.330
Age ≥ 40	22 (11.8)	18 (9.7)	0.515
Body mass index (kg/m^2; mean (SD)) ⁑	23.1 (3.4)	23.5 (3.2)	0.103
Duration of infertility (years; median (P25, P75))	3 (2, 5)	3 (2, 5)	0.604
Types of infertility			0.882
Primary infertility	73 (39.2)	74 (40.0)	
Secondary infertility	113 (60.8)	111 (60.0)	
Primary cause of infertility:			0.651
Tubal factor	134 (72.0)	122 (65.9)	
Male factor	26 (14.0)	25 (13.5)	
Tubal + Male factor	8 (4.3)	14 (7.6)	
Unexplained infertility	12 (6.5)	16 (8.7)	
Ovulation disturbance	3 (1.6)	2 (1.1)	
Diminished ovarian reserve	2 (1.1)	3 (1.6)	
Endometriosis	1 (0.5)	3 (1.6)	
AMH (ng/ml; median (P25, P75))	2.0 (1.3, 3.0)	2.2 (1.3, 3.0)	0.558
Total AFC (median (P25, P75))	11 (8, 14)	11 (9, 14)	0.913
FSH (mIU/ml; median (P25, P75))	7.1 (6.0, 8.9)	7.1 (5.9, 8.9)	0.734
LH (mIU/ml; median (P25, P75))	4.5 (2.8, 6.1)	4.5 (2.8, 6.5)	0.806
Estradiol (pg/ml; median (P25, P75))	32.4 (25.0, 45.9)	30.7 (24.6, 40.6)	0.122

Data are presented as numbers (%) unless otherwise noted.

In any of the baseline characteristics, no significant differences between groups were observed ($P < 0.05$).

SD = standard deviation; P75 = 75th percentile; P25 = 25th percentile; AMH = anti-müllerian hormone; AFC = antral follicle count; FSH = follicle stimulating hormone; LH = luteinizing hormone.

⁑ Body mass index is weight (kg) divided by height squared (m^2).

Efficacy evaluation

Table 3 presents the efficacy assessment for the primary and secondary outcomes in the per-protocol analysis. The results showed that the OPR was 40.3% in the GSATP group and 38.4% in the placebo group, with the difference not being statistically significant. Vaginal bleeding in early pregnancy was lower in the GSATP group than in the placebo group (11.3% vs. 21.6%, $p = 0.046$), and threatened miscarriage in early pregnancy was lower than in the placebo group compared to the placebo group (16.0% vs. 29.4%, $p = 0.021$). Functional constipation in early pregnancy was significantly lower than that of placebo (9.3% vs. 18.6%, $p = 0.032$), showing a statistically significant difference. The results of the subcomponents showed that there was a statistically significant difference between vaginal bleeding in early pregnancy (13.5% vs. 34.0%, $p = 0.015$) and threatened miscarriage in early pregnancy (19.2% vs. 38.3%, $p = 0.035$) in ≥ 35 yrs. subgroup, but not in < 35 yrs. subgroup.

Additionally, there were no statistically significant differences in the positive pregnancy rates (57.0% vs. 55.1%, $p = 0.7195$), clinical pregnancy rates (49.5% vs. 47.6%, $p = 0.715$), pregnancy loss rates (29.2% vs. 30.4%, $p = 0.857$), ectopic pregnancies (2.8% vs. 2.0%, $p = 0.965$), and embryo implantation rates (34.7% vs. 36.8%, $p = 0.588$) between the two groups.

Safety assessment

Neither the GSATP nor the placebo groups experienced any adverse events. There was no evidence of a difference in the safety indicators of complete blood cell count, liver function, and kidney function before and after treatment in either group.

Discussion

In this study, NOR patients undergoing a fresh ET cycle were focused on to observe the efficacy and safety of GSATP. While GSATP did not result in better OPRs for NOR patients undergoing fresh ETs, it did improve common symptoms observed during the early pregnancy, such as vaginal bleeding, threatened miscarriage, and functional constipation.

The results showed that GSATP reduced the rate of vaginal bleeding in early pregnancy, which was similar to the results of previous studies [7]. Vaginal bleeding in early pregnancy may be related to endometrial implantation [10]. Some medicinal components of GSATP can have estrogenic effects, which can increase serum estrogen concentration and improve conditions for embryo implantation [8], thereby reducing the rate of vaginal bleeding in early pregnancy. The results of this study also showed that the rate of threatened miscarriage in early pregnancy in the GSATP group was lower than

Table 2 Controlled ovarian stimulation and in vitro fertilization-embryo transfer characteristics in study population

Characteristics	Gushen Antai Pill group (*n* = 186)	Placebo group (*n* = 185)	*P* value
No of days of COS (mean (SD))	9.5 (1.7)	9.4 (1.8)	0.647
Total gonadotrophin dose administered (IU; median (P25, P75))	2100 (1615.6, 2700.0)	2112.5 (1687.5, 2775.0)	0.437
Estradiol on hCG trigger day (pg/ml; median (P25, P75))	2222.1 (1686.2, 2544.5)	2073.0 (1858.0, 2746.0)	0.564
Progesterone on hCG trigger day (ng/ml; median (P25, P75))	0.8 (0.5, 1.2)	0.7 (0.5, 1.0)	0.302
Method of fertilization			0.838
IVF	136 (73.1)	137 (74.1)	
ICSI	50 (26.9)	48 (25.9)	
No of oocytes retrieved (median (P25, P75))	9.0 (6.0, 12.0)	9.0 (6.0, 12.0)	0.766
No of mature oocytes (median (P25, P75))	7.0 (5.0, 11.0)	8.0 (5.0, 11.0)	0.550
No of 2PN oocytes (median (P25, P75)) †	6.0 (4.0, 8.0)	6.0 (4.0, 8.0)	0.730
No of Day 3 embryos (median (P25, P75)) *	6.0 (3.3, 8.0)	5.0 (3.0, 8.0)	0.674
No of blastocysts (median (P25, P75)) ‡	3.0 (1.0, 5.0)	3.0 (1.0, 4.0)	0.830
No of embryos transferred			0.273
Single embryo transfer	72 (38.7)	82 (44.3)	
Double embryo transfer	114 (61.3)	103 (55.7)	
Embryo transfer stage			0.856
Cleavage stage	122 (65.6)	123 (66.5)	
Blastocyst stage	64 (34.4)	62 (33.5)	
High-quality embryos transferred			0.424
Yes	159 (85.9)	165 (88.7)	
No	26 (14.1)	21 (11.3)	
Endometrial thickness on hCG trigger day (mm; mean (SD))	10.8 (2.16)	10.9 (2.4)	0.964

Data are number/total number or number (%) unless stated otherwise

COS = controlled ovarian stimulation; SD = standard deviation; P75 = 75th percentile; P25 = 25th percentile; hCG = human chorionic gonadotropin; IVF = in vitro fertilization; ICSI = intracytoplasmic sperm injection

† Two distinct pronuclei defined by four cells, a maximum of 10% fragmentation, and no multinucleation

* Typically, a good, normally growing day 3 embryos will contain between 6 and 10 cells

‡ Defined as Gardner score 3BB or higher

that in the placebo group. Previous research has shown that GSATP combined with dydrogesterone can significantly reduce the rate of threatened miscarriage in early pregnancy. Meta analyses have also shown that GSATP combined with dydrogesterone can effectively improve endocrine levels of serum estradiol, P_4, and β-hCG, making it superior to the use of dydrogesterone alone [11, 12]. Currently, there is a lack of research on whether functional constipation is harmful to pregnancy outcomes or associated with a reduction in pregnancy outcomes, and no clear conclusions have been drawn. However, functional constipation during pregnancy may have a negative impact on maternal health and pregnancy outcomes, affecting the quality of life. For instance, the difficulty in defecation due to constipation may induce or exacerbate hemorrhoids, anal fissures, and anemia during pregnancy [13, 14]. Constipation during pregnancy increases pelvic floor muscle pressure, which may lead to pelvic floor muscle relaxation in the long term, resulting in vaginal wall relaxation and even affecting postpartum sexual quality of life. Additionally, the risk of anemia is already high during the middle and late stages of pregnancy due to increased blood volume and fetal demands, and constipation may further exacerbate this issue. Lastly, excessive strain during defecation may increase the frequency of uterine contractions, theoretically increasing the risk of preterm birth, but more clinical data is needed to support this. However, there was no significant difference in OPR between the GSATP group and the placebo group, which differed from the results of previous studies [7]. This may be due to an insufficient sample size, and we are cautious about the results. Therefore, follow-up and data collection will continue to obtain more accurate evidence-based medicine.

Another interesting finding was that the incidence of functional constipation in early pregnancy was lower in the GSATP group than that in the placebo group, which may be related to the medicinal materials in GSATP that clears heat, cools blood, and lowers blood pressure. Dihuang, Cistanche deserticola, and Polygonum multiflorum enhance the effects of tonifying the kidneys, strengthening the spleen and liver, and nourishing blood. Atractylodes, Uncaria, and Scutellariae would be beneficial for Qi-invigorating and harmonizing, heat-clearing, soothing liver, and detoxifying [12], which can alleviate pregnancy-related constipation and help prevent the occurrence of pregnancy hypertension and eclampsia.

Table 3 Reproductive outcomes for women in Gushen Antai pill and placebo groups

Outcomes	Gushen Antai Pill group ($n=186$)			Placebo group ($n=185$)			*P* value		
	Total	Age < 35 ($n=96$)	Age ≥ 35 ($n=90$)	Total	Age < 35 ($n=95$)	Age ≥ 35 ($n=90$)	Total	Age < 35	Age ≥ 35
Primary outcome									
Ongoing pregnancy rate **	75/186 (40.3)	43/96 (44.8)	32/90 (35.6)	71/185 (38.4)	39/ 95 (41.1)	32/ 90 (35.6)	0.701	0.602	0.999
Secondary outcomes									
Positive pregnancy rate †	106/186 (57.0)	59/96 (61.5)	47/90 (52.2)	102/185 (55.1)	57/95 (60.0)	45/90 (50.0)	0.719	0.837	0.766
Clinical pregnancy rate	92/186 (49.5)	50/96 (52.1)	42/90 (46.7)	88/185 (47.6)	49/95 (51.6)	39/90 (43.3)	0.715	0.944	0.653
Pregnancy loss rate ‡	31/106 (29.2)	16/59 (27.1)	15/47 (31.9)	31/102 (30.4)	18/57 (31.6)	13/45(28.9)	0.857	0.598	0.753
Early spontaneous miscarriage ¶	17/92 (18.5)	7/50 (14.0)	10/42 (23.8)	17/88 (19.3)	10/49 (20.4)	7/39 (17.9)	0.886	0.398	0.517
Ectopic pregnancies ‡	3/106 (2.8)	1/59 (1.7)	2/47 (4.3)	2/102 (2.0)	1/57 (1.8)	1/45(2.2)	0.965	0.491	0.969
Embryo implantation rate *	104/300 (34.7)	59/145 (40.7)	45/155 (29.0)	106/288 (36.8)	62/143 (43.4)	44/145 (30.3)	0.588	0.647	0.804
Vaginal bleeding in early pregnancy	12/106 (11.3)	5/54 (9.3)	7/52 (13.5)	22/102 (21.6)	6/55 (10.9)	16/47 (34.0)	**0.046**	0.775	**0.015**
Threatened miscarriage in early pregnancy	17/106 (16.0)	7/54 (13.0)	10/52 (19.2)	30/102 (29.4)	12/55 (21.8)	18/47 (38.3)	**0.021**	0.223	**0.035**
Functional constipation in early pregnancy	9/106 (9.3)	3/54 (5.6)	6/52 (11.5)	19/102 (18.6)	7/55 (12.7)	12/47 (25.5)	**0.032**	0.334	0.071
Twin pregnancies	11/186 (5.9)	7/96 (7.3)	4/90 (4.4)	13/185 (7.0)	10/95 (10.5)	3/90 (3.3)	0.663	0.432	0.999

Data are number/total number (%) of women unless stated otherwise

** Ongoing pregnancy was defined as a detectable fetal heart beat after 12 weeks of gestation

† Positive pregnancy: serum β-hCG level ≥ 10 mIU/mL

‡ Denominator defined as number of positive β-hCG values (≥ 10 IU/mL) in each group

¶ Early spontaneous miscarriage was defined as a spontaneous abortion that occurs before the 12th week of gestation

* Embryo implantation rate was defined as the number of intrauterine gestational sacs observed divided by the number of embryos transferred

According to TCM, "Kidney Governs Reproduction", and female infertility is closely related to kidney deficiency. The "Pi" (Spleen) transports the water valley subtlety and produces blood, delivering the water valley subtlety to the uterus and providing a material basis for the growth and development of the fetus. The GSATP formula for the treatment of threatened abortion originated from the Yuan Dynasty, from the "Danxi Xin Fa". It is a combination of various medicines primarily aimed at nourishing the kidney. The function of the whole formula is to nourish yin, tonify the kidney and spleen, invigorate qi, nourish the blood, and prevent miscarriage. Modern pharmacological studies have found that GSATP's primary ingredients can improve blood circulation and placental blood supply, regulate immune activity, inhibit uterine contraction, and improve ovarian endocrine function [7, 15]. Therefore, GSATP may play a role in promoting embryo implantation and preventing pregnancy loss.

However, this study has some limitations. Firstly, the good outcomes of pregnancy are much important, however the live birth was not observed in this study due to the short observation period. Secondly, the current sample size was insufficient to determine whether different subgroups (e.g., those of more advanced maternal age) would experience greater benefits from taking the GSATP. The most important point is that the results of primary outcome differed from previous studies, most likely due to insufficient sample size. Follow-up and data collection will continue to obtain more accurate evidence-based medicine.

Women with NOR are the focus of this study, but it will have broader implications for a wide range of individuals. Furthermore, a recent meta-analysis of 11 high-quality RCTs showed that "Freeze-All" strategy is not recommended for patients with NOR compared to fresh embryo transfer due to the increased risk of preeclampsia [16]. Due to the reduction in cleavage-stage embryo transfer, fresh embryo transfer, and double embryo transfer, the generalizability of the conclusions in this study may be somewhat limited. The conclusions, which focus on fresh cleavage-stage transfers, accounting for 55–65% of the cycles at the research center from 2021 to 2023, should be cautiously extrapolated to regions where

blastocyst transfer is dominant. This is because cleavage-stage transfers have not undergone blastocyst culture selection and may include more embryos with unclear developmental potential. The rate of double embryo transfer in this study is 58.5% (reflecting the current situation in this region). As the policy of single embryo transfer progresses, the decrease in multiple pregnancy rates may alter the risk-benefit ratio of preeclampsia. We will continue follow-up and data collection, and as time progresses and the sample size increases, the proportion of single embryo transfers may change. We will also perform sensitivity analyses on single and double embryo transfer subgroups. This conclusion promises to provide Level II evidence according to the Oxford CEBM criteria and is applicable to similar clinical practice environments. The original intention of conducting this clinical trial was that conventional LPS with additional medications (estrogen or hCG) did not have any beneficial effects on pregnancy outcomes after fresh embryo transfer. Therefore, this RCT aims to make a meaningful attempt to optimize the OPR of fresh embryo transfer in this population by adding GSATP to LPS.

Conclusion

In conclusion, the results of this study showed evidence that GSATP may have potential to improve symptoms such as vaginal bleeding, threatened miscarriage in the early stages, as well as functional constipation during the first trimester of pregnancy. Although the primary outcome of OPR is currently similar in the GSATP and placebo groups, the results may vary as more data accumulate. Follow-up and data collection will continue in the future to provide high-quality evidence for the efficacy and safety of GSATP as an adjuvant treatment in IVF-ET for NOR women.

Abbreviations

IVF-ET	in vitro fertilization and embryo transfer
AMH	Anti-Mullerian hormone
AFC	Antral follicle count
RCT	Randomized controlled trial
NOR	Normal ovarian reserve
FET	Frozen-thawed embryo transfer
GSATP	Gushen Antai Pill
OPR	Ongoing pregnancy rate
CRF	Case report form
ITT	Intention-to-treat
LPS	Luteal phase support
hCG	Human chorionic gonadotropin
OHSS	Ovarian hyperstimulation syndrome
TCM	Traditional Chinese medicine
TMC	Trial monitoring committee

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Author contributions

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This study was approved by Ethics Committee of Reproductive Medicine at the affiliated hospital of Shandong university of TCM (SDUTCM-RME2021-0211). Written informed consent will be obtained from each eligible patient before participating in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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