Integrative Medicine Research 11 (2022) 100775

Contents lists available at ScienceDirect

Integrative Medicine Research

journal homepage: www.elsevier.com/locate/imr

Original Article

Chinese herbal medicine (Bu-Shen-Tian-Jing Formula) for outcomes of IVF in Chinese patients with polycystic ovary syndrome: a retrospective cohort study

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ARTICLE INFO

Article history:
Received 26 February 2021
Revised 19 August 2021
Accepted 20 August 2021
Available online 8 September 2021

Keywords:
Anti-Müllerian hormone
Chinese herbal medicine
growth differentiation factor-8
in vitro fertilization
polycystic ovary syndrome

ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is one of the most common causes of anovulatory infertility. Chinese herbal medicine (CHM) has many advantages in treating PCOS. We conducted a retrospective cohort study to investigate the effects of CHM (Bu-Shen-Tian-Jing Formula, BSTJF) on the outcomes of IVF in Chinese patients with PCOS and the potential underlying mechanism.

Methods: A total of 111 patients with PCOS who undergone IVF between November 2009 and July 2018 were included. Fifty-four patients received a three-month BSTJF therapy before controlled ovarian hyperstimulation, while the other 57 patients didn't. The data of the PCOS patients was collected. Anti-Müllerian hormone (AMH), growth differentiation factor-8 (GDF-8) levels in the follicular fluid were evaluated.

Results: BSTJF helped patients with PCOS to get more retrieved oocytes ($P<0.05$) and fertilized oocytes ($P<0.05$). The clinical cumulative pregnancy rate, live birth rate, and term delivery rate were significantly higher in the same stimulated cycle of the PCOS patients with BSTJF treatment ($P<0.05$). No significant differences existed between the two groups in the rate of fertilization, hospitalization rate of ovarian hyper stimulation syndrome and obstetrical or neonatal complications. BSTJF significantly decreased the AMH levels in the follicular fluids ($P<0.05$).

Conclusion: BSTJF significantly may improve the outcomes of IVF in Chinese patients with PCOS through decreasing AMH levels in follicular fluids. However, the evidence is limited due to the small sample size and the several potential bias.

1. Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine and metabolic disorder in women, affecting approximately 8-13% women of reproductive age.[1] The manifestations are heterogeneous, usually characterized by oligomenorrhea and ovulatory dysfunction, clinical or biochemical hyperandrogenism and polycystic ovary morphology.[2] It is accompanied by abnormal metabolic and psychological features.[3] PCOS accounts for 80% of cases of anovulatory infertility.[4] In vitro fertilization (IVF) is required in cases where women with PCOS exhibit poor response to first- or second-line ovulation induction treatment or for other indications. However, PCOS patients undergone IVF had lower implantation rate, clinical pregnancy rate and live birth rate,[5] but higher risks of ovarian hyper stimulation syndrome (OHSS), obstetrical complications and neonatal complications.[6]

As the majority of conventional western drugs contain single active ingredients, they have limited capacity to address the various symptoms of PCOS.[2] Chinese herbal medicine (CHM) has a long history of treating gynecological problems and infertility of patients with PCOS.[7] As a holistic medicine, the advantage of CHM is that multiple herbs are combined in a formula and can therefore target reproductive and metabolic defects simultaneously.[8-11] Multiply researches proved that, as a co-treatment with lifestyle intervention or traditional medication, CHM can improve different aspects of PCOS, such as altering serum hormones to normal levels,[12, 13] promoting menstrual regularity and ovulation,[9, 10] increasing pregnancy rate,[14] improving insulin sensitivity,[15] regulating glucose and lipid metabolism,[8, 9] reducing inflammatory reac-

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https://doi.org/10.1016/j.imr.2021.100775

tion and oxidative stress.[16] So, CHM becomes more and more popular among women with PCOS nowadays.[17]

However, little is known whether CHM can improve pregnancy outcomes of IVF in patients with PCOS and the potential underlying mechanism. Our research is designed to investigate the effects of Bu-Shen-Tian-Jing Formula (BSTJF), a prescription of CHM on the outcomes of IVF in Chinese patients with PCOS and the potential underlying mechanism.

2. Methods

2.1. Study design

This study was conducted as a retrospective cohort study. Patients with PCOS undergone IVF were selected for inclusion in the CHM group (n=54) and non-CHM group (n=57), separately. The pregnancy outcomes of IVF, the levels of anti-Müllerian hormone (AMH) and growth differentiation factor-8 (GDF-8) in the follicular fluids were compared.

2.2. Ethical statement

Procedures of study were performed under the approval of institutional review board of Ethics Committee of Women's Hospital, Zhejiang University School of Medicine, China (registration number IRB-20200029-R).

2.3. Participants

Participants were diagnosed as PCOS if they met two out of three of the Rotterdam criteria[18]: oligo or anovulation, clinical or biochemical signs of hyperandrogenism and polycystic ovaries by ultrasonography. The patients with PCOS undergone IVF in our reproductive center between November 2009 and July 2018 were recruited. Informed consent was obtained from them. To minimize the bias, inclusion criteria were as follows: age above 20, diagnosed with PCOS based on Rotterdam criteria, underwent IVF treatment by the standard long agonist controlled ovarian hyperstimulation (COH) protocol and conventional fertilization. Exclusion criteria were as follows: underwent oocyte or sperm donation, in vitro maturation, preimplantation genetic diagnosis, intracytoplasmic sperm injection, testicular sperm aspiration, blastocyst transfer, or had severe endometriosis (III and IV stage) diagnosed by laparoscopic surgery. Data was collected from the clinician records and telephone follow-ups.

2.4. Interventions

2.4.1. The treatment of CHM

The therapeutic principle of BSTJF used in the present study is to tonify *Kidney* (in Chinese: Bu-Shen) and fill *Essence* (in Chinese: Tian-Jing). It consists of nine herbs as shown in Table 1. These were obtained from Huadong Medicine Co., Ltd. (Hangzhou, China). One dose of BSTJF were boiled at 100°C for 1.5 hours with pure water, and the extracted aqueous solution was taken in the morning and evening every day. Fifty-four patients received a three-month BSTJF therapy before controlled ovarian hyperstimulation (COH) on their own will, while the other 57 patients didn't receive any CHM therapy before COH. Table 2

2.4.2. The treatment of IVF

All the included patients underwent the standard long agonist COH protocol, administrating recombinant follicle-stimulating hormone (FSH) (Gonal-F,Serono, Switzerland) with or without highly purified HMG (HMG, Lizhu, China). Ovulation was induced with 10,000 IU human chorionic gonadotropin (HCG) or 250μg recombinant HCG (Ovitrelle, Merck KCaA, Germany) when at least two follicles had reached 18 mm in diameter. Oocytes were retrieved 34–36 hours after HCG administration by guidance of vaginal ultrasound. Conventional fertilization was performed in all the patients. Fresh embryos were transferred on day 3, except in cases with few embryos ($\leq$2), which were transferred on day 2. Although one or two embryos were usually transferred, in women over 35-year-old, after counselling the risk of multiple gestation, transfer of three embryos was also allowed. For frozen-thawed embryo transfer, endometrial preparation was conducted using a hormone replacement therapy or a mild stimulation cycle. Embryos were transferred into the patients at a time point when the endometrial thickness came to a minimum of 8 mm with exhibiting evidence of a triple line pattern upon ultrasonic assessment, which resulted in appropriate endometrial synchronization.

2.5. Outcome measures

2.5.1. Primary outcome

The primary outcome was cumulative clinical pregnancy rate. Cumulative clinical pregnancy rate was defined as the rate of clinical pregnancy following the transfer of all (fresh or frozen-thawed) embryos available from the same stimulated cycle. Clinical pregnancy was confirmed by the presence of a gestational sac and fetal cardiac activity on vaginal ultrasound examination six weeks after embryo transplantation (ET). Ectopic pregnancy was confirmed by the absence of intrauterine gestational sac with positive HCG. Miscarriage was defined as a pregnancy loss before 28 full weeks of gestation.

2.5.2. Secondary outcome

The secondary outcome was live birth rate. Live birth rate meant the rate of live birth (>28W) following the transfer of all (fresh or frozen-thawed) embryos available from the same stimulated cycle. Live birth (>28W) was defined as deliveries in which

Table 1
The basic components of Bu-Shen-Tian-Jing Formula

Chinese Pinyin name	English name	Part used	Process	Dried herbal daily dosage (g)
Xian Ling Pi	Short-horned Epimedium Herb	Leaf	Dry in the sun or in the shade	9
Rou Cong Rong	Desertliving Cistanche Herb	Rhizome	Dry in the sun	9
Bai Zhu	Largehead Atractylodes Rh	Rhizome	Dry in the sun	20
Nv Zhen Zi	Glossy Privet Fruit	Mature fruit	Dry after slightly steamed	20
Fu Pen Zi	Palmleaf Raspberry Fruit	Fruit	Dry after slightly steamed	10
Tu Si Zi	Semen Cuscutae	Seed	Dry in the sun	15
Bu Gu Zhi	Malaytea Scurfpea Fruit	Fruit	Dry in the sun	15
Huang Qi	Milkvetch Root	Rhizome	Dry in the sun	10
Dan Shen	Dan-Shen Root	Rhizome	Dry in the sun	10
Total prescription weight (g)				118

Table 2
Descriptive characteristics of PCOS patients undergone IVF

Variable	CHM Group (n=54)	Non-CHM Group (n=57)
Age (years)	29.33±2.91	30.07±3.99
Height (cm)	160.28±4.32	160.13±4.88
Weight (kg)	57.22±7.84	59.97±9.08
BMI (kg/m^2)	22.27±2.90	23.37±3.27
Type of infertility		
Primary (%)	31/54 (57.4%)	28/57 (49.1%)
Secondary (%)	23/54 (42.6%)	29/57 (50.9%)
Duration of infertility (years)	3.98±2.12	3.91±2.77
Comorbidity	2/54 (3.7%)	6/57 (10.5%)
Diagnostic criteria†		
Oligo or anovulation	54/54 (100%)	57/57 (100%)
Clinical or biochemical signs of hyperandrogenism	6/54 (11.1%)	3/57 (5.3%)
Polycystic ovaries by ultrasonography	51/54 (94.4%)	55/57 (96.5%)
Basal hormone levels††		
FSH (U/L)	5.59±2.40	6.33±2.34
LH (U/L)	8.01±4.15	7.62±5.14
LH /FSH	1.49±0.72	1.20±0.76
E_2 (pmol/L)	122.73±80.04	119.13±57.88
PRL (ng/mL)	17.31±7.23	13.66±7.10
T (nmol/L)	1.33±0.98	1.10±0.79

BMI, Body mass index, defined as weight in kilograms divided by the square of height in meters; E_2, estradiol; FSH, follicle-stimulating hormone; IVF, in vitro fertilization; LH, luteinizing hormone; PCOS, polycystic ovary syndrome; PRL, prolactin; T, testosterone.
Continuous data were analyzed using t-test. Categorical variables were compared using Chi-square test. There were no statistical significances between two groups.
†According to Rotterdam criteria.
††The basal hormone levels are exhibited by mean ± standard deviation.

at least one baby was born alive after 28 completed weeks of gestation. Premature delivery was defined as delivery between 28 to 37 completed weeks of gestation. Term delivery was defined as delivery after 37 completed weeks of gestation.

2.6. Biochemical analysis of follicular fluid

Follicular fluid samples were obtained from mature follicles during oocyte retrieval. They were centrifuged at 3000 r/min at 15°C for 5 min to eliminate cells and debris. The supernatant was collected in sterile polypropylene tubes and stored in -80°C fridge.

AMH levels in the follicular fluids were measured by the Human AMH enzyme-linked immunosorbent assay (ELISA) Kit (CSB-E12756h; Cusabio Biotech Co., Wuhan, China). GDF-8 levels in the follicular fluids were quantified using the Human GDF-8 ELISA Kit (KE00120; Proteintech-China, Wuhan, China). The test kit was placed under room temperature for 30 min. The assay was performed in a 96 well plate according to the manufacturer's instructions. The absorbance was read at 450 nm (reference wavelength). Linear regression function was plotted for deducing sample concentrations. The concentrations of AMH and GDF-8 in the follicular fluids were presented as picogram per milliliter (pg/ml).

2.7. Statistical analysis

All analyses were executed using SPSS version 22.0 (SPSS, Chicago, IL, USA). The continuous variables were tested by t-tests. Categorical variables were compared using Chi-square test. Results were presented as mean ± standard deviation or frequency (%). P values less than 0.05 were considered to be significant.

3. Results

3.1. Selection process of participants

The selection process of participants was shown in Figure 1. Fifty-four PCOS patients who received a three-month BSTJF therapy before COH were selected for inclusion in the CHM group. Fifty-seven PCOS patients who didn't receive any CHM therapy before COH were selected for inclusion in the non-CHM group. Following transfer of fresh or frozen-thawed embryos available from the same stimulated cycle, 47 patients got clinical pregnant and 43 of them achieved live birth, among which, 32 patients achieved term delivery in the CHM group. In the non-CHM group, 27 patients got clinical pregnant and 22 of them achieved live birth, among which, 14 patients achieved term delivery.

3.2. The characteristics and treatments of PCOS patients

There were no significant differences between the two groups in the demographic characteristics, the diagnostic criteria (Rotterdam criteria) indicators, and the levels of hormones on the 3rd day of menstruation between the two groups .

3.3. The effects of CHM on the outcomes of IVF in patients with PCOS

3.3.1. The treatment regimens

There were no significant differences in the treatment regimens between two group except the number of retrieved oocytes, and fertilized oocytes (Table 3).

3.3.2. Outcomes

The CHM group achieved a higher cumulative clinical pregnant rate (87.0% vs. 47.4%, $P<0.05$), live birth rate (>28W) (79.6% vs. 38.6%, $P<0.05$) and term delivery rate (59.3% vs. 24.6%, $P<0.05$) than the non-CHM group (All pregnancies following fresh or frozen-thawed embryo transfer in the same stimulated cycle were included) (Table 4). While the miscarriage rate, preterm delivery rate didn't increase in the CHM group ($P>0.05$).

Forty-three patients in the CHM group and 22 patients in the non-CHM group had babies alive for more than 28 gestational age (GA) (Table 5). There were no differences in GA, weight gain during pregnancy, blood loss during labor, caesarean section rate, obstetrical and neonatal complication rate between the two groups ($P>0.05$).

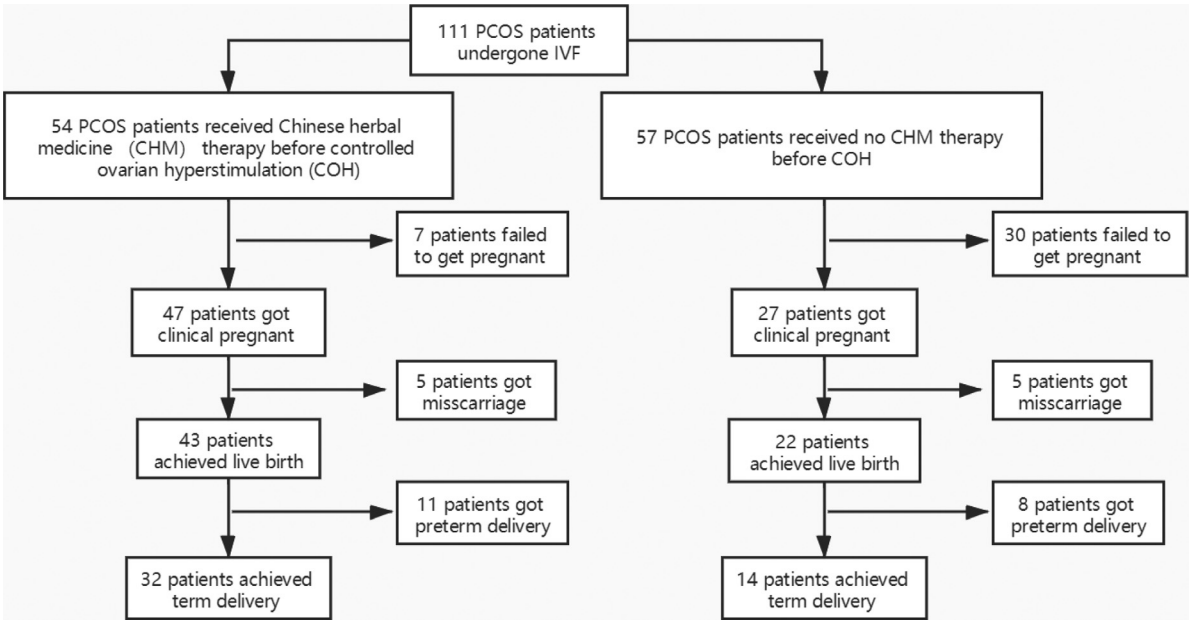

Figure 1. Flowchart of the study.

Table 3
The effects of CHM on the characteristics of IVF treatment of PCOS patients

Variable	CHM Group (n=54)	Non-CHM Group (n=57)
Dose of FSH (IU)	1462.73±676.43	1559.96±541.52
Dose of HMG (IU)	307.78±620.31	375.09±629.01
Total dose of Gn (IU)	1770.51±675.31	1934.17±705.03
Duration of COH (d)	10.48±3.34	10.81±2.97
Numbers of retrieved oocytes	18.33±7.94*	13.58±7.33
Numbers of fertilized oocytes	9.80±5.75*	6.39±4.54
Fertilization rate (%)	55.37±20.51	47.79±23.91
Numbers of transferred embryos	1.85±0.45	1.70±0.78
Hospital admission rate for OHSS (%)	4/54 (7.4%)	1/57 (1.8%)

CHM, Chinese herbal medicine; COH, controlled ovarian hyperstimulation; Fertilization rate, was calculated as the ratio of number of embryos obtained over the number of oocytes retrieved; FSH, follicle-stimulating hormone; Gn, gonadotropin; HMG, human menopausal gonadotropin; IVF, in vitro fertilization; OHSS, ovarian hyper stimulation syndrome; PCOS, polycystic ovary syndrome.
Continuous data were analyzed using t-test. Categorical variables were compared using Chi-square test. *P< 0.05, compared with the non-CHM group.

Table 4
The effects of CHM on the pregnancy outcomes of PCOS patients undergone IVF

Variable	CHM Group (n=54)	Non-CHM Group (n=57)
Cumulative clinical pregnant rate (%)	47/54 (87.0%)*	27/57 (47.4%)
Singleton pregnant rate (%)	31/53 (58.5%)*	21/57 (36.8%)
Multiple pregnant rate (%)	15/53 (28.3%)*	6/57 (10.5%)
Miscarriage rate (%)	5/54 (9.3%)	5/57 (8.8%)
Live birth rate (%) (>28W)	43/54 (79.6%)*	22/57 (38.6%)
Preterm delivery rate (%)	11/54 (20.4%)	8/57 (14.0%)
Term delivery rate (%)	32/54 (59.3%)*	14 /57(24.6%)

CHM, Chinese herbal medicine; IVF, in vitro fertilization; PCOS, polycystic ovary syndrome.
In the CHM group, one patient who was diagnosed as heterotopic pregnancy was excluded when calculating singleton and multiple pregnant rate.
Categorical variables were compared using Chi-square test. *P< 0.05 compared with the non-CHM group.

No significant differences were found in one minute or five minute Apgar score and neonatal birth weight in both singleton and multiplets between the two groups (P>0.05) (Supplement 1).

The AMH levels in the follicular fluids of CHM group (n=40) were significantly lower than those of the non-CHM group (n=30) (798.65±619.71 vs. 1153.43±785.60, P<0.05), while the GDF-8 levels in the follicular fluids didn't differ from each other (368.42±149.40 vs. 343.59±163.98, P>0.05) (Supplement 2).

4. Discussion

The results of our retrospective study implied that CHM have positive effects on the outcomes of IVF in Chinese PCOS patients. The possible reasons might lie in that CHM can help PCOS patients to retrieve more oocytes and get more fertilized oocytes. With more embryos available, they had more chances of embryo transplantation, which had more opportunities of getting pregnant.

Table 5
The influences of CHM on the PCOS patients undergone IVF with GA>28 weeks during perinatal period

Variable	CHM Group (n=43)	Non-CHM Group (n=22)
GA (weeks)	37.23±2.28	36.68±2.61
Weight gain during pregnancy (Kg)	15.09±3.53	15.59±2.60
Blood loss during labor (mL)	358.14±251.41	342.86±92.58
CS rate (%)	26/43 (60.5%)	14/22 (63.6%)
Obstetrical complication rate (%)	20/43 (46.5%)	8/22 (36.4%)
Neonatal complication rate (%)	13/43 (30.2%)	4/22 (18.2%)

CHM, Chinese herbal medicine; CS, caesarean section; GA, gestational age; IVF, in vitro fertilization; PCOS, polycystic ovary syndrome.
Continuous data were analyzed using t-test. Categorical variables were compared using Chi-square test.

The evidences above implied that CHM is a good and safe intervention for PCOS patients undergoing IVF, which was in accordance with previous researches.[19, 20]

Serum AMH levels were significantly higher in PCOS patients than the healthy women,[21] even in their offspring, they were statistically higher.[22] They were associated with gonadotrophin dosages and risks of severe OHSS in PCOS patients undergone IVF.[23] So people proposed AMH as one of the factors related to the degree of severity of PCOS.[24] Follicular fluid is the site for oocyte growth and development. It contains important regulatory factors to induce oocyte maturation, which provides unique conditions for oocyte development and promoting the maturation of dominant follicles.[25] Yet, it has not been sufficiently explored. AMH levels in follicular fluids were also elevated in PCOS patients compared with healthy women, and they might contribute to failure of follicle growth and ovulation in PCOS.[26] Whether AMH levels in follicular fluids have any effects in IVF outcomes, it was controversial. The study of Mehta showed that the AMH in follicular fluids share an inverse correlation with clinical pregnancy,[27] while the study of Sacha showed the opposite.[28] The study of Bastu revealed that there were no significant correlations between pregnancy rates and AMH.[29] However, Ciepiela believed that there was a positive correlation between oocyte matched AMH levels in follicular fluids and the live birth rate after fresh single embryo transfer.[30] We hypothesized that the positive effect of CHM on the outcomes of PCOS patients undergone IVF may have relationship to the decrease of AMH levels in follicular fluids. However, the downstream molecular mechanism needs further study.

The conclusion might have a tendency to bias due to the following limitations: (1) the small sample size makes it hard to represent the whole population characteristics of PCOS; (2) the inherent bias existed in our retrospective study makes it possible to have recall bias, (3) the lack of a placebo group prevents identification placebo effect of the CHM, (4) we only obtained part of the patients' follicular fluid samples, which makes it impossible to carry out more detailed study on the multiple biological samples to discover the potential mechanism.

To get a more reliable conclusion, a prospective, multi-center, randomized controlled clinical trial with large samples should be conducted and multiple biological samples (such as serum, granulosa cells, placenta) should be applied to research. Besides, we should pay more attention to the long term effects of CHM on the offspring of patients with PCOS undergone IVF.

In conclusion, Bu-Shen-Tian-Jing Formula may improve the pregnancy outcomes of IVF in Chinese patients with PCOS through decreasing AMH levels in follicular fluids. However, the result is limited due to the small sample size and the several potential bias.

Author contributions

Conceptualization: FQ, XP. Methodology: FQ, XP. Software: XP. Validation: FQ, XP. Formal analysis: XP. Investigation: XP, YG, XZ, FW. Resources: XP, YG, XZ, BS, LC, FW. Data curation: FQ, XP. Writing-Original Draft: FQ, XP. Writing-Review & Editing: FQ, XP. Visualization: FQ, XP. Supervision: FQ. Project administration: FQ, XP. Funding acquisition: FQ, FW.

Conflict of interest

The authors declare that they have no conflicts of interest.

Funding

This research was supported by National Natural Science Foundation of China (No. 81874480, 82074476, and 81873837).

Ethical statement

This research was reviewed and approved by the institutional review board of Ethics Committee of Women's Hospital, Zhejiang University School of Medicine, China (registration number IRB-20200029-R). Informed consent was obtained from all participants.

Data availability

The data that support the findings of this study are available from the corresponding author on reasonable request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.imr.2021.100775.

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