Effect of Traditional Chinese Medicine Formula Guilu Xian on in vitro Fertilization and Embryo Transfer Outcome in Older Women with Low Prognosis: Study Protocol for a Prospective, Multicenter, Randomized Double-Blind Study

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Study protocol

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Abstract

Introduction

In recent years, the prevalence of infertility has significantly increased and has become a global reproductive health problem. The female ovarian reserves have been shown to decrease progressively with an increase in age. Besides, the rate of embryo implantation and clinical pregnancy also decreases. Traditional Chinese medicine has been widely applied in assisted reproductive technology. It is reported to have a significant influence on improving the quality of oocytes, improving endometrial receptivity, increasing clinical pregnancy rate, reducing pregnancy-related complications, etc. Therefore, this study will investigate the effect of Guilu Xian, a traditional Chinese medicine formula on IVF-ET outcome in older women with low prognosis.

Methods and analysis

This trial is a prospective, multicenter, randomized double-blind clinical trial. A total of 120 infertile patients with low prognosis and receiving IVF or ICSI in 3 public hospitals in China will be randomly divided into two parallel groups: Guilu Xian group (n = 60) and placebo group (n = 60). Patients in both groups will be treated with antagonist regimens to promote ovulation, and all the patients will be required to take the medication from the 2nd to 4th day of the menstrual cycle to the day of egg retrieval. A comparison of the total number of oocytes obtained, the fertilization rate, clinical pregnancy rate, embryo quality, embryo implantation rate and early spontaneous abortion rate between the experimental group and the placebo group will be performed.

This study was registered in the Chinese Clinical Trials Registry Platform (ChiCTR1900028255).

Introduction

Infertility affects about 8 to 12% of couples of childbearing age globally, and this accounts for more than 186 million people, and the majority of them being residents of developing countries. Increasing age in women is one of the most powerful negative predictors of fertility [3]. Rapid social and economic development has led to a gradual increase in the number of people with late marriages and childbearing, especially with the liberalization of the policy of "comprehensive second child". This has increased in the number of older women seeking assisted reproductive technology (ART) treatment. The reproductive ability of women gradually decreases with increasing age [4]. Irreversible aging of the ovaries may lead to diminished ovarian reserves (DOR), however, in vitro fertilization-embryo transfer (IVF-ET) technology has become an effective treatment for DOR in older women. Although IVF-ET is increasingly being used to deal with infertility, the clinical pregnancy and live birth rate among older women with a poor ovarian response to exogenous gonadotropin stimulation (DOR) remains unsatisfactory.

Low prognosis is considered one of the most common and intractable problems of IVF-ET treatment occurring in about 47% of women. Out of these, 55% are women above the age of 35 years and with insufficient ovarian reserves [6–8]. The main cause of diminished ovarian response declines in the number

of follicles responsive to FSH. This phenomenon is most common in older women with severely decreased ovarian reserves due to accelerated follicle loss [9]. However, in some cases, patients with good ovarian reserves may experience poor ovarian response owing to the gonadotropin dosage used [10] or due to the impact of genetic polymorphisms of endogenous gonadotropins and their receptors [11–13]. These factors change the response of recruitable follicles to exogenous gonadotropins, and this is associated with several problems such as a small number of eggs, poor embryo quality, poor pregnancy outcomes, etc. Therefore, patients with low prognosis is in need of high gonadotropin (Gn) dose and cycles for ovarian stimulation during the IVF cycle. This causes emotional and physical pain and financial burden to couples [9]. Currently, there are no effective ways of improving clinical pregnancy and live birth rates for patients with increased age and poor ovarian reserve [17]. The follicular fluid provides an important microenvironment in the growth and development of oocytes. Several metabolites in the follicular fluid are associated with the oocyte ability to undergo fertilization, embryonic development, and pregnancy outcome [18].

Traditional Chinese medicine has a significant effect on improving the quality of oocytes, improving the endometrial receptivity, increasing clinical pregnancy rate, and reducing pregnancy-related complications. Tortoise deer erxian ointment, also known as tortoise deer erxian gum, was first recorded by Wang Sancai, a Chinese physician in the Ming Dynasty(1569). Guilu Xian reported the addition of turtle shell glue to the original "Guilu Erxian Ointment" and adjusted the dosage to strengthen the effect of nourishing yin and latent yang. The prescription is based on the tortoise shell glue and turtle shell glue as the main medicine, which functions in tonifying the kidney and as a nourishing essence and yin and latent yang. The application of deer horn glue is based on the fact that "those who are good at tonifying yin must seek yin in yang, then yin is born in yang, and the source of spring is not thirsty". This maximizes the tonifying effect in the balance of yin and yang. Therefore, Guilu Xian has the effect of tonifying the kidney essence, nourishing yin and latent yang, replenishing qi and nourishing spirit, and delaying aging. According to TCM theory, the pathogenesis of older women with low prognosis is deficient kidney essence, spleen qi deficiency, liver depression, and blood deficiency. "tonifying the kidney, regulating the liver and invigorating the spleen" is the main treatment method, which corresponds to the tortoise deer fairy syndrome previously described. Recent studies have shown that TCM formula Guilu Erxian ointment has a good effect on the treatment of the reproductive system, and has an estrogenic effect [19–22]. However, there is no clear case on how to improve prognosis among older women. Therefore, there is necessary that a well-designed randomized controlled trial to confirm the effectiveness and safety of Guilu Xian in improving IVF-ET outcome in older women with low prognosis.

Methods And Analysis

Aims of the study

To explore the effect of Guilu Xian, a traditional Chinese medicine formula on the IVF-ET outcome in older women with low prognosis.

Study participants

The study institutions were the Genetic and Reproductive Center of Traditional Chinese and Western Medicine, Affiliated Hospital of Shandong University of Traditional Chinese Medicine, the Second Affiliated Hospital of Shandong University of Traditional Chinese Medicine and Jinan Central Hospital. The project leader regularly coordinates and communicates with each unit to understand the progress of the project and ensure the smooth progress of the project and the guarantee of quality.

The inclusion criteria for this study will be patients aged between 35 and 42 years old, with insufficient ovarian reserve parameters (The POSEIDON criteria AFC < 5, AMH < 1.2 ng/mL)[23], and poor ovarian response (that is, the number of oocytes obtained after standard ovarian stimulation is less than 3). Patients should meet the diagnostic criteria of western medicine infertility, that is, have regular sex life for more than one year, no contraception, and not pregnant women in their reproductive period[24].

The exclusion criteria are women with BMI ≥ 25 Kg/m2; women who suffer from genetic diseases that are not suitable for childbearing as stipulated in the Maternal and Child Health Care Law; women with severe endometriosis, adenomyosis, and immune infertility; with untreated hydrosalpinx; with congenital or acquired abnormal development of the uterus and severe deformities of other reproductive organs; women with a history of endocrine dysfunction such as reproductive system tumor, thyroid dysfunction, and hyperprolactinemia; women receiving ovarian stimulation therapy or OCP in recent three months; women with a previous history of gynecological surgery such as the ovary; and all other contraindications of assisted reproductive technology.

The discontinuation criteria will be women who do not take their medication on time and stop the use without permission, or fail to return to see the doctor; women with adverse reactions such as nausea and obesity occurring during medication, women with no dominant follicular growth, early follicular ovulation and no egg acquisition during ovarian stimulation. Three days after egg harvesting, the embryo was not formed.

Technical route

Schedule of the study process

	STUDY PERIOD							
	Enrolment	Allocation	Post-allocation					Close-out
TIMEPOINT**		0 weeks	1–5 weeks	6–10 weeks	11–15 weeks	16–20 weeks	21–25 weeks	
ENROLMENT:								
Eligibility screen	V							
Informed consent	V							
Basic information login	V							
Double-blind assignment		V						
INTERVENTIONS:								
Guilu Xian treatment								
Placebo treanment								
ASSESSMENTS:								
Basic information (age,BMI,AFC,AMH, FSH,etc)	V							
Main indicators (number of follicles)				V				
Secondary endpoints (2PN number, implantation rate, persistent pregnancy rate,etc)								
Fetal Development Assessment								V
Maternal assessment of hepatic and renal function								V

Case registration and allocation

The clinicians being involved in the study will record the basic AFC and AMH values of the patients after obtaining informed consent from the participants. Upon meeting the inclusion criteria, the patients will be randomly distributed in groups, and the clinicians will use the case questionnaire to record their identity, age, and other basic information.

The clinicians will give medication to participants within 3 days after screening. Data will be recorded on drug distribution and use, and timely reports provided to the clinicians on the drug dose and patients' discomfort during the process.

This study will include 120 patients who will be recruited and randomly divided into two groups using the R language version 3.5.1: the elderly test group and the elderly control group. Sixty, patients will be treated with Guilu Xian and 60 with the placebo. The elderly and the experimental group will be treated with an antagonist, and the tortoise deer fairy and placebo will be administered from the third day of menstruation before IVF.

Ovarian stimulation and egg extraction and transplantation.

The flexible GnRH antagonist regimen will be utilized in the COS regimen in both groups. On day 3 of the cycle, gonadotropin (Serono) or urinary gonadotropin (Lubao biochemical Pharmaceutical Company, Zhuhai Lizhu Group) 300 IU will be administered, and regular monitoring of follicular development performed. When ≥ 1 dominant follicle is at least ≥ 14 mm in diameter or LH 10 U/L, GnRH-ant will be administered. However, when ≥ 2 dominant follicles are ≥ 18 mm in diameter, urogenic hCG 8000 ~ 10000U or r-hCG 250 μg will be administered. Transvaginal ultrasound-guided paracentesis will be performed 36 hours after the injection of HCG. Fresh or frozen embryo transfer will be based on the patient's characteristics such as intima growth value, P-value, and embryo score. Following transplantation, progesterone will be injected into the supporting luteal muscle at 40 mg/ day or application of progesterone vaginal sustained-release gel at 90 mg/day. Drug administration will be stopped from the day of transplantation to the 10th week of gestation.

Estimation of the sample size (see attachment for specific calculation).

In a recent study in China, it was reported that in conventional IVF or ICSI, the total number of retrieved eggs in the treatment group (259.77 ± 25.45) was significantly higher than that in the placebo group (235.59 ± 26.44). In this study, the number of eggs obtained in the Guiluxian group was 11 under the original hypothesis and 9 under the alternative hypothesis (9 in the placebo group). The bilateral Z test of the combined variance will be used to estimate the sample size at 0.05 level of significance. The ratio between groups will be1:1. The minimum sample size found for each group is 49 people, hence a total of 98 people. Besides, 90% of the detection ability will be obtained, and the difference in ratio between groups is 0.15. Considering a dropout rate of 15%, we will be expected to enroll 120 participants, with 60 participants in each group(see supplementary file 1 for details).

Randomization and blinding

Stratified block randomization will be used to randomly assign the participating volunteers to one of the two study groups at a ratio of 1:1. The R software version 3.5.1 will be used to generate a random number. The random number list which is strictly confidential will be maintained by the drug administration center staff. The active drug (Guilu Xian) and placebo will be manufactured with similar appearance and smell. The clinicians and patients will be blinded to the drug allocation until the end of the study.

Intervention

The Guilu Xian/placebo will be given to the patients at 5 g three times daily from the third day of their menstruation period before IVF for 40 days (provided by the preparation room of Affiliated Hospital of Shandong University of Traditional Chinese Medicine, and the extraction procedure will be based on the standard of preparation room SOP). Drug composition: tortoise glue, turtle glue, antler glue, Chinese wolfberry, American ginseng, Cornus officinalis, Hawthorn seed, jujube.

Patient compliance

Three months before the start of the study, all participants will be allowed to take any other Chinese medicine supplements or nutritional supplements that may increase ovarian responsiveness. Such drugs or nutritional supplements may affect the therapeutic effect of modified Guilu Erxian ointment. Assessment of patient's compliance, including the bottle count of the drugs and weekly telephone follow-up, will be performed to find out if the participants are taking the medicine as agreed and any reasons for non-compliance. All unused drugs will be counted and registered.

Patient and public involvement

Patients and public were not involved in developing the research questions nor the study design. Moreover, they will not participate in the recruitment exercise or the conduct of the study. Results of the study will be disseminated to participants and their families via telephone and patient organization platforms.

Outcome measurement

The primary outcome will be the number of eggs obtained, and the secondary outcome will include the 2PN number, cleavage number, grade I embryo number, implantation rate, abortion rate, persistent pregnancy rate, trigger day $\geq$ 14 mm follicles, Gn days and Gn. The data will be expressed by the average value ($x \pm s$). The t-test will be used to compare the differences in the sample mean between the two groups when the variance is uniform, and the correction t-test will be used for the uneven variance. Fisher's exact rate method will be used to compare the rates between the two groups and a significance level of 0.05 will be considered.

Data management

All data collected will be saved for five years after publication. Electronic data will be stored in password-protected computers and access will only be allowed to the principal investigator. Data obtained will only be restricted for use in this study.

Adverse events

The safety analysis will be performed on all participants who will have received at least one Guilu Xian treatment.

Regular quality assurance monitoring will be carried out for any adverse effects and to ensure that the study is safe and in line with the implementation plan. This will also ensure that the data are accurately recorded and stored. Any serious adverse effects will be reported to the principal investigator with immediate effect.

Ethical approval and consent

This study has been approved by the Reproductive Medicine Ethics Committees of the Affiliated Hospital of Shandong University.

Clinicians will receive written informed consent from each patient participating in the study before the study commences. This study was approved by the Ethics Committee of the Reproductive and genetic Center of Integrated traditional Chinese and Western Medicine in Jinan City, Shandong Province, China.

Discussion

Previous studies have confirmed that traditional Chinese medicine formula Guilu Erxian ointment is effective in the treatment of male impotence, semen abnormalities, female dysfunctional uterine bleeding, peri-menopausal osteoporosis, and other reproductive system diseases. A recent study found that Jiawei Guilu Erxian ointment effectively increased the number of elementary ovarian follicles in mice with primary ovarian insufficiency[25]. However, Guilu Xian treatment has not been reported in an older population of infertile women. Therefore, this prospective, multi-center, randomized double-blind clinical trial will investigate the effect of Guilu Xian, a traditional Chinese medicine formula on IVF-ET outcome in older women with low prognosis.

This study is a multi-center prospective clinical trial with certain limitations. The trial will not completely rule out clinical treatment bias. It will be difficult to guarantee that patients have similar backgrounds when comparing the two groups.

There is no current study to predict the impact of the Chinese Medicine Guiluxian on elderly infertile patients. Therefore, a prospective study is required to determine the feasibility and effectiveness of the turtle deer fairy.

Declarations

DATA DISSEMINATION

The findings of this study will be widely disseminated through conference papers, research reports, and academic publications.

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Contributors MM and ZS designed this study. XC, LL, and YZ modified the article. DG and XW made significant contributions to sample size estimation and performed the statistical analysis.

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