Protocol

The Application of Zishen Yutai Pill in Advanced Maternal Aged Women Undergoing IVF-ET

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Note: The Steering Committee and the Data and Safety Monitoring Board have discussed and revised this protocol thoroughly, including two major revisions. The final version was written on May 24, 2019, and was approved by the IRB of Sun Yat-Sen Memorial Hospital of Sun-Yat Sen University on May 30, 2019. After its IRB approval, this protocol has been implemented and adhered to without amendment. The Statistical Analysis Plan is contained within the protocol.

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1. Committee composition

1.1 Protocol committee

Table 1. Protocol Committee

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1.2 Steering committee

Table 2. Steering Committee

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1.3 Data coordination committee

Prof. Heping Zhang at Yale University will lead the Data Coordination Committee consisting of the personnel from Sun Yat-Sen Memorial Hospital of Sun Yat-Sen University, including registering the trial at the Chinese Clinical Trial Registry (http://www.chictr.org/cn/) and ClinicalTrials.gov (http://www.ClinicalTrials.gov). The daily operation of the Data Coordination Center is fully executed by the personnel at Sun Yat-Sen Memorial Hospital.

1.4 Third-party data statistical unit

Table 3. Third-party data statistical unit

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1.5 Sponsor and contact information

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1.6 Publication committee

Members of the publication committee include Dongzi Yang, Yu Li.

2. Background

The deferment of childbirth has emerged as a significant concern in recent years. Over the past forty years, the prevalence of primiparity at age of 35 years or older increased by 9 times (1). Advanced maternal age (AMA), defined as pregnancy at 35 years or older, is commonly linked with reduced fecundity (2,3). AMA patients are more inclined to the use of assisted reproductive technology (ART), including in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) (4). Nonetheless, their pregnancy outcomes are typically less favorable compared to their younger counterparts, as aging adversely affects both oocyte quality and ovarian reserve (5).

Traditional Chinese Medicine (TCM) has been employed for over two millennia in the treatment of a myriad of conditions, including infertility (6). A representative formula for the treatment of infertility is the Zishen Yutai Pill (ZYP). ZYP is a patent Chinese medicine invented by Professor Yuankai Luo (7). Since its market debut in 1983, ZYP was renowned for its therapeutic effects on threatened miscarriage and recurrent miscarriage. ZYP is consisted of fifteen Chinese medicine, including Cuscutae Semen, Ginseng Radix et Rhizoma, Dipsaci Radix, Taxilli Herba, Eucommiae Cortex, Morindae Officinalis Radix, Cervi Cornu Degelatinatum, Codonopsis Radix, Atractylodis Macrocephalae Rhizoma, Asini Corii Colla, Lycii Fructus, Rehmanniae Radix Praeparata, Polygoni Multiflori Radix Praeparata, Artemisiae Argyi Folium, and Amomi Fructus (8).

In previous clinical practice, the administration of ZYP was observed to enhance the ovulation and pregnancy rate, while decrease the miscarriage rate among patients with polycystic ovary syndrome (PCOS) (9). Another clinical report had suggested that oral intake of ZYP around the luteal phase could significantly increase the embryo implantation rate (10). Our previous multicenter, placebo-controlled, randomized clinical trial had suggested that the intervention of ZYP among advanced maternal aged women could significantly increase the clinical pregnancy rate during ART.

However, this analysis constituted a post hoc subgroup analysis, lacking the statistical power to detect the between-group difference. Therefore, the design of the current

study aims to provide data regarding the efficacy and safety of ZYP in IVF/ICSI for the AMA patients.

3. Objectives

The present study aims to investigate the efficacy and safety of ZYP on liver birth rates among AMA infertile women during IVF/ICSI-ET. The primary objective is to determine the difference of live birth rate between the ZYP and placebo group. The secondary outcomes will include counts and rates of oocytes/embryos (oocytes retrieved, 2 pro-nuclei zygotes, cleavage zygotes, available embryos, high-quality embryos), pregnancy outcomes (rates of biochemical pregnancy, implantation, clinical pregnancy and miscarriage), incidences of maternal, fetal and neonatal complications, and neonate information (newborn birthweight and length, congenital malformation).

4. Participants

4.1 Inclusion criteria

Participants who meet all of the following criteria will be included in this study.

(1) Infertile women aged between 35 and 42 years old.

(2) Intend to undergo IVF-ET (GnRH-a long protocol or GnRH-ant protocol).

(3) BMI<28 kg/m^2.

(4) Bilateral ovaries exist.

(5) Patients who voluntarily sign the informed consent and agreed to be followed up as required by the study protocol.

4.2. Exclusion criteria

Participants who meet one of the following criteria will be excluded in this study.

(1) Recurrent implantation failure (previous three times or more IVF/ICSI-ET failure).

(2) Adenomyosis, the uterine cavity line constricted by uterine fibroids.

(3) Untreated bilateral hydrosalpinx.

(4) Endometrial diseases that have not been cured.

(5) Known diseases that are not suitable for undergoing ART or at the present not suitable for pregnancy;

(6) Recent therapy (within one month) for infertility with TCM.

4.3 Drop-out

Participants will dropout when:

(1) subjects who have adverse events that cannot be tolerated.

(2) Severe breach of the protocol.

(3) Subjects who exit due to personal or unpredictable reasons (detailed information need to be recorded).

(4) Subjects considered inappropriate to continue to participate in the study for other medical reasons.

5. Study design

5.1 General design

It will be a multicenter, prospective, randomized (1:1 treatment ratio) clinical trial comparing the live birth rates in AMA women assigned to these treatment arms after a fresh ET in 1466 infertile patients undergoing IVF or ICSI. Patients will be randomized into either of two groups, receiving ZYP or the placebo during IVF/ICSI. All of the participants will receive standardized controlled ovarian hyperstimulation (COH) protocols, i.e., GnRH agonist (GnRH-a) long protocol or GnRH antagonist (GnRH-ant) protocol), as appropriate, and then undergo embryo transfer (ET).

5.2 Randomization and blinding

The participants will be randomized 1:1 to receive double-blind single-dummy monotherapy with placebo or ZYP using the permuted block randomization method. The randomization will be stratified by the age (35-37, 38-39 and 40-42) by following the Human Fertilisation and Embryology Authority (11). The web-based Interactive Response Technology system is managed by independent statisticians, guiding drug dispensing.

Patients, investigators and clinical staff performing this trial will be blinded to treatment allocation. The package, appearance and odor are the same in the placebo and ZYP. The allocation and detailed information of randomization will be kept by the independent statisticians managing the Interactive Response Technology system. Blinding will be maintained until the completion of the analysis. Unblinding will be allowed in case of a medical emergency. The cause and time of unblinding will be recorded in detail and signed by the treating physicians.

When all live birth information is retrieved, the computer system will disclose the allocation.

5.3 Intervention of IVF/ICSI and study drug

5.3.1 Controlled Ovarian Hyperstimulation (COH)

Two COH protocols will be applied.

1) GnRH-a long protocol: All subjects in two groups will undergo COH after down-regulation with long-acting or short-acting GnRH-a in midluteal phase. Hormone levels, including follicle-stimulating hormone (FSH), luteinizing hormone (LH) level and estradiol (E_2) will be measured on the gonadotropin (Gn) initiation day. Follicular development (diameter and counts) and endometrial thickness will also be evaluated by means of transvaginal ultrasonography. COH will be initiated when the serum LH < 5 IU/L and E_2 < 50 ng/ml or endometrial thickness < 5 mm. The initiation dose and total Gn dose will be adjusted according to a combined consideration of age, weight, basal hormone levels, antral follicular count (AFC) and

previous cycle response.

2) GnRH-ant protocol: Initiation will be conducted on day 2 to day 4 of menstrual cycle. Hormone levels, including FSH, LH and E_2 levels will be measured on the Gn initiation day. Follicular development (diameter and counts) and endometrial thickness will also be evaluated by means of transvaginal ultrasonography. The initiation dose and total Gn dose will be adjusted according to a combined consideration of age, weight, basal hormone levels, AFC and previous cycle response.

5.3.2 Ovulation monitoring

The mean diameter of all follicles and endometrial thickness will be monitored by means of ultrasonography, along with testing of serum hormonal levels, including FSH, LH, E_2 and progesterone (P_4) on the day 5 to day 6 after Gn initiation.

5.3.3 Oocyte retrieval and embryo transfer

When the diameter and counts of follicles meet the criteria (two leading follicles≥18 mm; or ≥3 follicles ≥ 17 mm; or ≥4 follicles ≥ 16 mm), ovulation will be triggered by injecting human chorionic gonadotropin (HCG). Testing of serum FSH, LH, E_2 and P_4 levels will be conducted on HCG injection day. Oocyte collection will be performed 36 hours later. Luteal support will be started on oocyte retrieval day. Fertilization will be achieved by conventional IVF or ICSI with the husband's semen. On day 3 to day 5 after oocyte retrieval, one to three embryos will be transferred.

High-quality embryos is defined according to Istanbul consensus and Gardner criteria (12, 13).

Day 2	4 cells, cell fragments <10%, no multi-nucleus
Day 3	8 cells, cell fragments <10%, no multi-nucleus
Day 5	stage 4 blastocyst, grade A inner cell mass, trophectoderm at grade A

5.4 Intervention of study drug

Eligible subjects will be allocated to receive ZYP or the placebo 3 times daily, 5 g

each time on down-regulation day (GnRH-a long protocol) or day 19 to day 23 of previous menstrual cycle (GnRH-ant protocol). Study drug will be suspended during the 1st day to the 4th day of menstrual cycle. Continuation of the study intervention will be determined by the serum β-HCG pregnancy test carried out two weeks after embryo transfer. Intervention of study drug will be stopped if the pregnancy test is negative. For patients with positive results on the pregnancy test, the study medicine will be given until a confirmed clinical pregnancy (five weeks after ET).

5.4.1 Zishen Yutai Pill

The manufacture of ZYP complies with the relevant requirements of law of China's Drug Administration and Good Manufacturing Practice (GMP), with approval from the China National Medical Products Administration (Permit No.Z44020008).

Table 5. Herbal drugs used in Zishen Yutai Pill (ZYP) and the origins and medicinal parts

Herbal drugs	Origin of natural medicine
Cuscutae Semen	Ripe dried seed of *Cuscuta Chinensis* Lam.
Ginseng Radix et Rhizoma	Dried root and rhizome of *Panax ginseng* C. A. Mey.
Dipsaci Radix	Dried root of *Dipsacus asper* Wall. ex DC.
Taxilli Herba	Dried leafy stem and branch of *Taxillus chinensis* (DC.) Danser
Eucommiae Cortex	Dried bark of *Eucommia ulmoides* Oilv.
Morindae Officinalis Radix	Dried root of *Marinda officinalis* How
Cervi Cornu Degelatinatum	Residue after water extraction of ossified antler of *Cervus nippon* Temminck
Codonopsis Radix	Dried root of *Codonopsis pilosula* (Franch.) Nannf.
Atractylodis Macrocephalae Rhizoma	Dried rhizome of *Atractylodes macrocephala* Koidz.
Asini Corii Colla	Solid glue prepared by stewing and concentrating from the hide of *Equus asinus* L.
Lycii Fructus	Dried ripe fruit of *Lycium barbarum* L.
Rehmanniae Radix Praeparata	Steamed and dried root of *Rehmannia glutinosa* (Gaertn.) DC.
Polygoni Multiflori Radix Praeparata	Steamed and dried root of *Polygonum multiflorum* Thunb.
Artemisiae Argyi Folium	Dried leaf of *Artemisia argyi* Lévl. et Vant.
Amomi Fructus	Dried fruit of *Amomum villosum* Lour.

5.4.2 Composition and manufacturing process of the placebo

1) Composition: Pregelatinized starch, microcrystalline cellulose, black iron oxide, refined honey, dextrin.

2) Manufacturing process: Pregelatinized starch, microcrystalline cellulose, and black iron oxide are mixed, crushed, and sieved. The refined honey is added to the mixed powder to make the wet pill with the required size. After drying, it is coated with a mixture of black iron oxide and talc powder, 3% dextrin solution, 75% ethanol solution, and refined honey. Finally, eligible pills are polished using Chinese insect wax, selected, and packaged.

5.4.3 Adherence of study drug

Patients' adherence of study drug will be assessed. An actual dosage above 80% of required drug dosage will be considered consistent with protocol requirements.

In order to record and improve the adherence of study drug, a diary card will be used. The diary card includes information of participants, study drug dosage, date of drug administered. In addition, each participant will be assigned a clinical research coordinator to assure the adherence of study drug and carry out follow-up.

5.4.4 Package and label of study drug

Both the ZYP and the placebo will be provided in the same label and appearance (written in Chinese).

Drug No.：

The Application of Zishen Yutai Pill in Aged Women Undergoing IVF-ET

（Clinical Use Only） Dosage of one week

【**Action and Use**】Tonify *Kidney* and *Spleen*, invigorates *Qi* that nourishes *Blood*, placate the fetus and strengthens the body. Used in pregnancy loss due to deficiency of *Kidney* and *Spleen*, debility of *Chong* and *Ren* (treatment and prevention of threatened miscarriage and spontaneous miscarriage)。

【**Package**】5 g/package.

【**Dosage and Administration**】Administered orally with honey water or dilute salt water. 5 g (1 package) each time, tid.

【**Intervention period**】11 weeks.

【**Storage Condition**】Please keep in seal and away from moister.

【**Caution**】Please keep the remaining drug and the package until the end of clinical trial. Stored away from children.

Manufactured by Guangzhou Baiyunshan Zhongyi Pharmaceutical Co. Ltd.

Expired Date: ** Batch No.: **

6. Study procedures and visits

6.1 General visit setting

Briefly, five visit points are scheduled for this study. Visit 1 will be the screening visit. Visit 2 will be performed on the day of Gn initiation. Visit 3 will be carried out on the day of ET. Visit 4 will be carried out two weeks after ET to confirm biochemical pregnancy. Visit 5 will carried out five weeks after ET to confirm clinical pregnancy. Follow-up after delivery will be conducted through web or telephone contact to ascertain the live birth outcomes of participants (**Figure 1**).

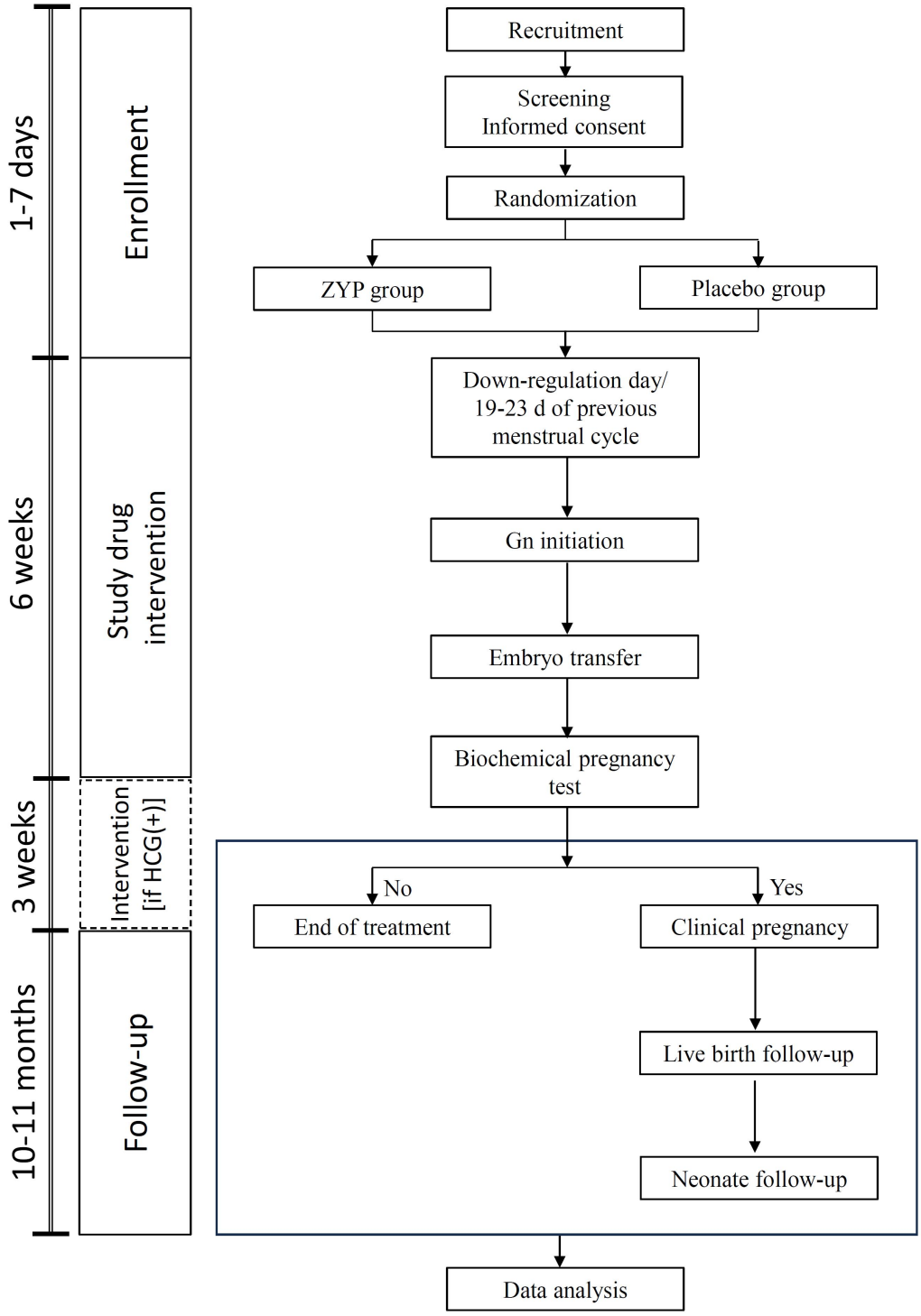

Figure 1. Study flowchart. Gn, gonadotropin; hCG, human chorionic gonadotropin; ZYP, Zishen Yutai Pill.

6.2 Screening visit

If the patient express interest toward the clinical trial, a screening visit will be performed. Patients will be informed about the study thoroughly. During the screening visit, the following procedures will be completed.

(1) Going through all the inclusion and exclusion criteria. Obtaining the signed informed consent form.

(2) Collecting baseline information, including age, medical record number, and birth date.

(3) Reviewing the medical history, including infertility history, childbearing history, gynecological surgery history, disease history and menstrual information.

(4) Carrying out a physical examination and gynecologic examination.

(5) Measuring the baseline sex hormones, including E_2, FSH, LH, prolactin (PRL), and testosterone (T).

(6) Reviewing the previous ET/FET cycle record.

(7) Dispensing information.

6.3 Gn initiation visit

During the Gn initiation visit, the following procedures will be completed.

(1) Adverse events and treatment (if necessary).

(2) Medication on Gn initiation, including type, name, initiation dose.

(3) Serum hormone levels, including E_2, FSH, LH, and P_4.

(4) Dispensing and return information.

6.4 ET visit

During the ET visit, the following procedures will be completed.

(1) Adverse events and treatment (if necessary).

(2) Summary of COH including type, name and total amount.

(3) B-mode ultrasound and basal serum hormonal level.

(4) Information about semen collection, embryo development, embryo transfer information.

(5) Information about the medicines used in luteal phase support, including name, dose, duration, and total amount.

(6) Dispensing and return information.

6.5 Biochemical pregnancy test visit

At 14 days after transplantation, a biochemical pregnancy test will be performed.

(1) Adverse events and treatment (if necessary).

(2) Serum level of β-HCG pregnancy test (β-HCG >50 IU/L is defined as biochemical pregnancy).

(3) Drug dispensing and return information will be recorded.

6.6 Clinical pregnancy test visit

If the pregnancy test is positive, transvaginal ultrasonography will be performed 3 weeks later to confirm clinical pregnancy.

(1) Adverse events and treatment (if necessary).

(2) Clinical pregnancy will be confirmed using B-mode ultrasound. Clinical pregnancy is defined as the presence of intrauterine gestation sac with fetal motion under transvaginal ultrasonography.

(3) Drug return information will be recorded.

(4) If a clinical pregnancy is confirmed, contact information and intention of delivery hospital will be checked.

6.7 Delivery visit

When the participant is preparing to deliver, the investigator will collect the delivery information and infant information according to the form designed for this visit. The delivery information mainly includes the delivery mode and pregnancy complications. The neonate information mainly includes gender, birth weight and length, birth

defects, admission to newborn intense care unit within 1 month and stillbirth.

7. Physical examination

A physical examination will be conducted on all participants by the corresponding investigator. The parameters include height, weight and they will be recorded to the nearest 0.1 cm, 0.1 kg. Height and weight will be measured without shoes, and patients will be dressed in light clothing.

8. Transvaginal ultrasound scan

An ultrasound scan with a transvaginal probe will be carried out. Uterine dimension, endometrial thickness and type, bilateral ovarian dimension, and follicle number will be measured through the ultrasound scan. The uterine size will be determined at the widest diameter. The investigator will determine endometrial types. Endometrial thickness is the largest anterior-posterior measurement of the endometrium in the sagittal plane. Ovarian dimension is measured by analysis of the largest plane of the ovary in two dimensions. In addition, antral follicle count (AFC) will be recorded.

9. Laboratory tests

Hormone and pregnancy tests will be performed at the local laboratories. In addition, blood work will be performed on screening visit, Gn initiation visit, hCG trigger day, and 2 weeks after ET. The blood sample (5 ml) will be collected with an anticoagulant tube and stored at -80℃ for further analysis.

10. Outcome measures

The live birth rate is the primary outcome. The secondary outcomes will include counts and rates of oocytes/embryos (oocytes retrieved, 2 pro-nuclei zygotes, cleavage zygotes, available embryos, high-quality embryos), pregnancy outcomes (rates of biochemical pregnancy, implantation, clinical pregnancy and miscarriage),

incidences of maternal, fetal and neonatal complications, neonate information (newborn birthweight and length, congenital malformation) (14).

Live birth is defined as delivery of any viable infants after 28 weeks of gestation.

The number of oocytes retrieved will be defined as the total number of oocytes retrieved through ultrasound-guided transvaginal aspiration. The rate of oocytes retrieved will be defined as the number of retrieved oocytes divided by the number of follicles with a diameter ≥10mm on the HCG injection day. Matured oocytes will be defined as those with pronuclei or polar bodies observed on Day 1 after oocyte retrieval in IVF patients, or as the number of MII oocytes on the day of oocyte retrieval in ICSI patients. Cleavage oocytes will be defined as the number of fertilized oocytes that undergo cleavage on Day 2 after oocyte retrieval. The rate of cleavage will be defined as the number of cleaved embryos divided by the number of fertilized oocytes (2PN + 1PN + multiple PN). Available embryos will be defined as the sum of the number of embryos transferred and the number of embryos frozen. The rate of available embryos will be defined as the number of available embryos divided by the number of cleaved oocytes. High-quality embryo will be defined according to the day of embryo transfer, following the Istanbul consensus and Gardner criteria, Day 2: 4 cells, cell fragments <10% and no multi-nucleus; Day 3: 8 cells, cell fragments <10%, no multi-nucleus; Day 5: stage 4 blastocyst, grade A inner cell mass, grade A trophectoderm (12,13). The rate of high-quality embryos will be defined as the number of high-quality embryos divided by the number of available embryos.

Biochemical pregnancy will be defined as positive when β-HCG >50 IU/L. Implantation rate will be defined as the number of gestational sacs per the number of embryos transferred. Clinical pregnancy will be defined as the presence of intrauterine gestation sac with fetal motion under transvaginal ultrasonography. Miscarriage rate will be calculated among patients with biochemical pregnancies and patients with clinical pregnancies.

Incidences of maternal, fetal and neonatal adverse events were assessed, including moderate or severe OHSS, gestational diabetes mellitus (GDM), gestational

hypertension, postpartum hemorrhage, preterm delivery, congenital anomalies, puerperal infection, stillbirth, neonatal jaundice, neonatal infection, neonatal death, ectopic pregnancy, and low birth weight infant. The definitions of these safety indexes were shown in **Table 6**.

Table 6. Definitions of safety outcomes

Safety outcome	Definitions
Ovarian hyperstimulation syndrome (OHSS)	OHSS is defined according to the Golan criteria. Mild OHSS is diagnosed by the presence of abdominal distension and discomfort with or without nausea, vomiting, and/or diarrhea. Moderate OHSS is diagnosed when ultrasonographic ascites were present in addition to the above features. Severe OHSS is diagnosed when there is clinical evidence of ascites and/or hydrothorax or breathing difficulties with or without hemoconcentration, coagulation abnormalities, and diminished renal function.
Pregnancy loss	Pregnancy loss is defined as pregnancies that eventuate in spontaneous abortion or therapeutic abortion that occurred throughout pregnancy.
Gestational diabetes mellitus	GDM is defined as carbohydrate intolerance of variable severity with onset or first recognition during pregnancy as determined from the diagnosis in the obstetrical medical record.
Gestational hypertension	Gestational hypertension is defined as blood pressure ≥140/90 mmHg with negative proteinuria, which appeared during pregnancy and returned to normal 12 weeks after delivery. It may have upper abdominal discomfort or thrombocytopenia.
Postpartum hemorrhage	Postpartum hemorrhage is defined as the loss of 500 ml of blood or more after completion of the third stage of labor.
Preterm delivery	Delivery of a fetus at less than 37 and more than 28 weeks gestational age.
Congenital anomalies	Congenital anomalies are defined as structural or functional anomalies that occur during intrauterine life, including minor and major anomalies.
Puerperal infection	Any bacterial infection of the genital tract after delivery and during puerperium.

Stillbirth	The absence of signs of life at or after birth.
Neonatal jaundice	Neonatal jaundice is yellowing of the skin and other tissues of a newborn infant.
Neonatal infection	Neonatal infection is defined as a variety of infections in neonates caused by bacteria, fungi, viruses, etc., as determined from the diagnosis in the neonatal medical record.
Neonatal death	The death of a live-born neonate within 28 days after delivery.
Low birth weight infant	Neonatal birth weight ≤ 2500 g.
Ectopic pregnancy	Ectopic pregnancy is one in which the blastocyst implants at any site other than the endometrial lining of the uterus cavity.

11. Timeline and recruitment plan

The planned duration of recruitment will be 24 months with 12 centers. The enrollment target of our clinical trials is 1466 randomized participants. The number of participants taken in each center will be allocated according to the actual situation. The treatment period may need about 3 months and another 9 months to trace the pregnancy outcome. In short, a total of 36 months will be required to complete this trial, from initial recruitment to pregnancy outcome period.

12. Statistical analysis plan

12.1 General statistical consideration

For continuous variables, the Kolmogorov-Smirnov test will be applied to evaluate the distribution. Variables with normal distribution will be presented as means and standard deviation (SD), and intergroup comparisons will be performed using the 2-tailed, student's t test. Variables with non-normal distribution will be presented as medians and interquartile ranges (IQR), and intergroup comparisons will be performed using the Mann-Whitney U test. Categorical variables will be summarized with frequencies and percentages and compared using chi-square test or Fisher exact test, as appropriate. All statistical analysis will be conducted with the use of the statistical package SPSS, version 19.0 (SPSS Inc). A two-sided P value of <0.05 will be considered as significantly different.

12.2 Sample size estimation

In our previous clinical research, the live birth rate was 0.42 per embryo transfer in ZYP treated group and 0.33 per embryo transfer in placebo control group among AMA women. By assuming the same live birth rates, a sample size of 454 women in each group can provide a power of 80% at a significance level of 0.05. According to previous study, the dropout rate before ET was 15% and the cycle cancellation rate was 23% in AMA women. For this trial, a total rate of 38% (dropout and cancellation) is considered in the sample size calculation. Therefore, a total of 1466 participants

will be recruited in this study, 733 participants in each group.

12.3 Type of analysis

We will utilize an intent-to-treat approach to examine differences in the live birth rates for the first embryo transfer cycle in the two treatment arms in the primary analysis by the Pearson $\chi 2$ test. Safety parameters and secondary efficacy parameters, such as pregnancy rate, miscarriage rate and other rates, will be analyzed using the Pearson $\chi 2$ test or Fisher exact test as appropriate. Secondary efficacy parameters, including the counts and rates of oocytes/embryos, will be analyzed using the Mann-Whitney U test.

For the primary and secondary outcomes, we will also perform analysis in the per-protocol set (PPS), excluding those who have major protocol deviation(s), cancel cycle or do not complete the pre-set minimum exposure dosage of the assigned study drug (at least 80% compliance), from the ITT population. Subgroup analysis will be conducted as stratified by age (35-37, 38-39, 40-42).

Prior to unblinding, missing value in the primary outcome (live birth) and secondary outcomes (biochemical pregnancy, clinical pregnancy) in the ITT analysis will be imputed as not having an event.

12.4 Interim analysis

No interim analysis is planned.

13. Adverse event reporting

13.1 Risks and discomforts

Compared with the usual IVF patients, participating in this study will not increase additional risks. The possible risks and discomforts in common IVF technology are detailed in the informed consent, including in vitro fertilization ET, embryo freezing, and so on. The table below lists all procedures, including related risks and discomforts.

Table 7. Potential risks and discomfort of intervention during study process

Procedures and events	Risks and discomfort
Controlled ovarian hyperstimulation (COH)	Frequent subcutaneous injection, frequent venipuncture, frequent transvaginal ultrasound scan. Supra-physiologic E_2 may increase the risk of cancer ovary torsion or ovary rupture.
Ovarian hyperstimulation syndrome (OHSS)	Massive enlargement of the ovaries, ascites, bloating, nausea, and vomiting. Severe cases may have thoracic edema, breathing difficulties, oliguria, even anuria, and may require hospitalization, medication, or puncture drainage. A very severe case may suffer from thrombosis, damage to the liver or renal function, and even death.
Oocyte retrieval	Anesthesia accident, pelvic organ injury, intra-abdominal hemorrhage, puncture site hemorrhage, in serious case surgery or transfusion may be needed, infection.
ICSI	Microinjection may injure an oocyte, pass an unknown disease gene to the next generation.
Embryo transfer	Infection.
Embryo frozen and thaw	Embryotic development arrest. The survival rate of thawed embryos is 95%.
Standard venipuncture for blood work	Slight pain, ecchymosis at the site of puncture, infection, or bleeding at the site.
Transvaginal ultrasound	Abdominal or pelvic discomfort.
Ectopic pregnancy	May require medical or surgical treatment. In severe cases, pregnancy site rupture can result in intra-abdominal hemorrhage, even shock, or death if treatment is delayed.
Multiple pregnancies	May require embryo reduction, increase risk of pregnancy

	complication, fetus abnormalities, and preterm delivery.
Infertility treatment	Anxiety or emotional distress to various degrees.
Zishen Yutai Pills	It has been reported that some patients who took the Zishen Yutai pill suffered from nausea, dry mouth, and constipation that disappeared after drug withdrawal.

The participants are not expected to have all of these complications, and they will be allocated to a treatment group at random. The treatment may be less effective or have more complications than the other research treatment.

In this study, the GnRH-a long protocol and GnRH-ant protocol will be performed according to each patient's actual situation. The GnRH-ant protocol will be used to minimize the risk of OHSS. The initial dose will be determined according to the age, basal FSH level, basal AFC, weight, and previous situation promoting ovulation. The initiation dose and total Gn dose will be adjusted according to a combined consideration of age, weight, basal hormone levels, AFC and previous cycle response. In addition, in case of high ovarian response, the cycle will be canceled to avoid OHSS. If there are three or more fetuses, then a reduction will be performed to minimize the risks of multiple pregnancies. A responsible investigator or a resident doctor on 24 h call can be contacted at each site if any adverse event occurs during this study.

Every effort will be taken to avoid injury as a result of participation. If adverse events occur, active treatment will be provided. Furthermore, if a medical dispute is involved, it will be disposed of as a routine medical event.

13.2 Adverse event definitions

13.2.1 Definition of adverse event

Adverse event means any untoward or unfavorable medical occurrence associated with the subject's participation in the research, whether or not considered related to the study intervention.

Adverse events can be any of the following:

• Physical signs or symptoms, including medication side effects.

• Abnormal laboratory values.

• Changes in vital signs, physical exam findings, or test results.

• An increase in the frequency or intensity (worsening) of a condition or illness presents before study enrollment.

Note: In this trial, adverse events will not include:

• Pre-existing conditions or illnesses that do not worsen during the study period (record these in the medical history).

• Normal conditions associated with pregnancy.

13.2.2 Definition of serious adverse event

Serious adverse event: Any event temporally associated with the subject's participation in research that meets any of the following criteria:

• Death.

• Life-threatening (at immediate risk of death).

• Severely or permanently disabling.

• Requires in-patient hospitalization or prolongation of existing hospitalization.

• Pregnancy loss after 20 weeks gestation.

• Results in a congenital anomaly/birth defect.

• Or any event so deemed as serious by the PI at the site.

Note: A "severe" adverse event is not the same as a "serious adverse event" or SAE. Severity is based on the event's intensity, whereas seriousness is based upon the event outcome as it poses a threat to the patient's life or functioning.

13.2.3 Definition of Serious Unexpected Suspected Adverse Reaction (SUSAR)

SUSAR is defined as an serious adverse reaction that is both unexpected and meets the definition of an serious adverse reaction.

An adverse event is considered "unexpected" if its characteristic or severity exceed

current available product information, including the instruction of product or Investigator's Brochure.

13.3 Recording of adverse events

All adverse events will be observed during the clinical trial. The investigators will require the participants to reflect the change in patients' condition truthfully after using the drug and avoid suggestive questions. Adverse events and unexpected side effects (including symptoms, signs, and laboratory tests) will be observed while observing the curative effect. In order to determine whether adverse events are associated with the experimental drug, they will be recorded in the eCRF in detail, including the occurrence time, symptoms, signs, degrees, duration, laboratory examination indicators, treatment methods, procedures, results, follow-up time, and so on. Concomitant medication will be recorded in detail to analyze the correlation between adverse events and experimental drugs. In addition, the record will be signed and dated.

When adverse reactions occur, the investigator will take necessary measures, such as adjusting the dose, temporarily discontinuing the medication, and decide whether to terminate the trial or not. If a serious adverse event occurs, the unit undertaking the study must immediately take necessary treatment measures to ensure the subject's safety.

13.4 Causality and severity assessment

13.4.1 Causality assessment

According to the documented adverse events and abnormal test findings, the investigator will need to determine that if the abnormal test finding should be classified as an adverse event and if the adverse events are related to the study intervention or meet the criteria for a serious adverse event. The relationship between the experimental drug and adverse events are divided as "related", "probably related", "possibly related", "possibly unrelated", and "unrelated". AEs with the former three

kinds of relations are defined as adverse drug reactions (ADRs). The considerations of causality analysis include five aspects:

(1) There is a reasonable chronological relation between drug administration and the occurrence of suspected ADRs (occurrence after drug administration).

(2) Suspected ADRs are in accordance with known ADRs of the drug (literature compliance).

(3) Suspected ADRs cannot be explained by concomitant medication, previous medication, existing clinical conditions of patients, or the effects of other therapies (other explanation).

(4) After drug discontinuation or dose reduction, suspected ADRs disappear or relieve (disappearance after drug discontinuation).

(5) Suspected ADRs recur after re-exposure to the same drug (Recurrence after re-administration).

The investigators should assess the possible relation between AEs and the research drug or the concomitant medication.

Consideration	Occurrence after drug administration	Literature compliance	Other explanation	Disappearance after drug discontinuation	Recurrence after re-administration
Related	+	+	-	+	+
Probably related	+	+	-	+	?
Possibly related	+	+	±	±	?
Possibly unrelated	+	-	±	±	?
Unrelated	-	-	+	-	-

13.4.2 Severity assessment

The level of adverse event response will be evaluated and reported as follows:

Mild: The participant can tolerate the event, and it does not affect the treatment. It need not take special actions and is not harmful to the participant.

Moderate: The participant is intolerant and requires withdrawal or special treatment, which has a direct impact on their health.

Severe: It is a life-threatening, fatal, or disabling event and requires withdrawal or emergency treatment immediately.

13.5 Reporting of serious adverse events and unanticipated problems

Whether or not related to the study drugs, serious adverse events during the trial should be treated and reported to the primary investigator in the trial center promptly. Moreover, it should be reported to the Ethics Committee of Sun Yat-Sen Memorial Hospital of Sun Yat-Sen University within 24 h. The investigator will have to document this serious adverse event and take necessary measures to ensure the participants' safety and interests. It should also be timely reported to the drug administration according to the legislation. At the same time, it should be notified to the investigator involved in the same clinical trials. If it is confirmed as a serious adverse event related to the experimental drug, the investigator will bear the rescue and treatment cost and the corresponding economic compensation.

The investigator must fill in the "serious adverse events report form". The time, the treatment and to whom the SAEs are reported should be recorded in the original data.

14. Concomitant medication

To avoid possible interference from other traditional Chinese medicine, recent therapy (one month prior to and during the IVF/ICSI process) for infertility with TCM will be considered as protocol violation.

The following medications will be allowed during this study, and concomitant medication should be recorded.

(1) Anti-diabetic agents and anti-hypertension agents.

(2) Folic acid supplement aimed at preventing neural tubal defect.

(3) For patients with abnormal bleeding/prolonged amenorrhea, progestin, micronized progesterone, or dydrogesterone.

(4) During controlled ovarian stimulation, human menopausal gonadotropin will be allowed to use in patients with slow E_2 increase or follicles development.

(5) For patients with moderate or severe OHSS, routine clinical treatment, such as fluid infusion, albumin infusion, aspirin, or preventive antibiotics, will be used.

(6) For patients with threatening abortion, an extra dose of progesterone will be allowed to use. Concomitant medication will be recorded.

(7) For patients with pregnancy complications, clinical standard care will be performed. Concomitant medication will be recorded.

15. Monitoring

15.1 Data and Safety Monitoring Board

The clinical trial management office will review and interpret data generated from the study and review the protocol's revisions before their implementation. Its primary objectives are to ensure the safety of study subjects and the integrity of the research data. The office will advise on research design issues, data quality and analysis, and research participant protections for the study. The office will hold regular conference calls to review the protocol for ethical and safety standards, monitor the trials' safety, monitor the data's integrity for original study design, and provide advice on study conduct. The office will review the trial's progress, adjudicate adverse events, and decide on any premature closure of the study. The board will coordinate the call and provide study updates before the call via email.

Table 8. Data and Safety Monitoring Board

Name	Affiliation	Email
Zhaosi Xu	Guilin University of Electronic Technology	zhaosi.xu@jeeyor.com
Xiaoli Chen	Sun Yat-Sen Memorial Hospital of Sun Yat-Sen University	gzxiaolichen@163.com
Jiewen Zhou	Guangzhou University of Chinese Medicine	zhoujiewen0808@163.com

15.2 Ethics

Ethics approval has been sought from the Ethics Committee at Sun Yat-Sen Memorial Hospital, with an ethic approval file entitled: "2017 Reproduction Ethnic Approval No.2" (in Chinese, 2017 生殖伦审字第(02)号). Ethics approval will be obtained from each participating center.

16. Data handling and record-keeping

Prof. Heping Zhang at Yale University will oversee the data collection and management (including quality assurance/compliance measures) team consisting of investigators from Sun Yat-Sen Memorial Hospital.

16.1 Data entry and electronic case report form (eCRF)

The trial investigators must go through Good Clinical Practice (GCP) training and understand the protocol and relevant information in advance adequately. The protocol will be executed strictly, and clinical trial drugs will be provided to enrolled subjects after screening.

Clinical research coordinator (CRC) is responsible for entering the data of CRF into the database. All data required on the eCRF must be recorded. Each blank of the eCRF must be completed, and all items should be filled in. If the item is “not done”, then select “ND”. Double data entry is performed by two independent CRCs separately to make two databases, which will be compared by the data manager to generate a list of inconsistent data. Any inconsistencies between the two databases are identified by the data manager, and queried via the eCRF system until final settlement.

16.2 Data quality control and query management

Data will be verified by independent statisticians to generate the data query list according to the data verification plan (DVP). Then clinical research associate (CRA)

asks the corresponding investigator to answer the queries in the form, after which the form with answers will be given back to the data manager. The database will be revised based on these forms.

16.3 Data security

The database managers, Dr. Yu Li, biostatistician Prof. Heping Zhang, and project leader Prof. Dongzi Yang, will be in charge of the eCRF records, and are responsible for the assignment of the jurisdiction to the users. The database managers have the highest jurisdiction to manage and monitor the data and actions. The users of each sub-center will be allowed to enter their patients' information and study results in their center. The database managers take the responsibility to decide which data could be disclosed to the public. Patients' information that will or may lead to identity recognition, includes but not limited to name, address, medical card number, telephone numbers, will be critically protected and will never be allowed to be disclosed.

In addition to the internal safeguards built into the computerized system, external safeguards will be implemented. Data will be stored at the servers housed at Sun Yat-Sen Memorial Hospital with access overseen by Prof. Heping Zhang. Records will be regularly backed up, and record logs are maintained to prevent a catastrophic loss and ensure the data's quality and integrity.

16.4 Audit

Audit will be performed during the whole trial process to ensure the data quality. The investigator team will compare data in the database against medical record in the hospital information system (HIS). Identified errors will be resolved between the DCC and clinical sites. The visits will assure data quality and patient protection.

16.5 Medical Coding

Adverse events are coded using MedDRA 21.0 (or higher version).

17. Publication policy

It is anticipated, there will be up to 14 authors in the final manuscript. Yu Li will be the first author. Dongzi Yang and Heping Zhang will be the last two author. The other authors' order for the participating sites will be based upon subject recruitment, data accuracy, and promptness of data report and will start at the position 2 and go to position n-2. Each site's PI will be responsible for documenting the contributions to the study of that site's authors. We encourage the site investigators to establish the second hypothesis and have publications by sharing these data under the publication committee's supervision.

18. Acknowledgment section

The acknowledgment section will include other investigators and study personnel who contributed substantially to the study by site and members of the advisory board and Data Safety Monitoring Board. The designation will list the initials of the individual, followed by their highest degree. Significant contributions include but are not limited to protocol review, initiation and participation at each site, subject recruitment and enrollment, study conduct, data analysis, and manuscript preparation.

19. Protocol revision history

The Application of Zishen Yutai Pill in Aged Women Undergoing IVF-ET. After the initial version was prepared by the protocol committee, this protocol underwent two major revisions by the investigators.

Version	**1**
Date written	**December 26, 2016**
Date approved	**January 12, 2017**
Version	**2**
Date written	**August 22, 2018**

Date approved	**September 20, 2018**
Version	**3**
Date written	**May 24, 2019**
Date approved	**May 30, 2019**

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