Appendix1 Patient information and consent

Comparison of Acupuncture Pretreatment Followed by Letrozole versus Letrozole alone on Live Birth in Anovulatory Infertile Women with Polycystic Ovary Syndrome: a Randomized Controlled Trial

You are cordially invited to participate in the above named research study. You need to decide whether you want to participate or not. Please take your time to make up your mind. Carefully read the following and feel free to ask the study physician any question which you may have.

Why is this study being done?

Polycystic ovary syndrome (PCOS) is a common endocrine and metabolic disorder affecting 6–10% of women of reproductive age, and it accounts for 70–80% of anovulatory infertility. PCOS is characterized by amenorrhea, hirsutism, acne, obesity, polycystic ovaries, and infertility, and it can have serious effects on general health and quality of life. Insulin resistance (IR), hyperinsulinemia, and dyslipidemia worsen with aging, and the risk of miscarriage is three times greater in women with PCOS compared to healthy women. Women with PCOS are also at an increased risk of pregnancy complications such as impaired glucose tolerance, gestational diabetes mellitus, pregnancy-induced hypertension and pre-eclampsia, and small for gestational age (SGA) children. Thus, the metabolic abnormalities of PCOS can affect not only the woman's health but also herchildren's.

Ovulation induction in women with PCOS suffering from anovulatory infertility includes the use of anti-estrogen, insulin sensitizers, aromatase inhibitors, gonadotropin, and surgical treatment. Clomiphene citrate has been the first-line medication, but a recent large multicenter randomized study demonstrated that letrozole, an aromatase inhibitor, results in a higher ovulation rate and a higher live birth rate than clomiphene citrate.

Despite the fact that pharmacological methods of ovulation induction are often effective, a large number of patients still have difficulty conceiving and they have a high risk of miscarriage that might be related to reduced endometrial receptivity. The high prevalence of IR (up to 60%) in women with PCOS is considered to be one of the major pathophysiological changes of PCOS, and studies indicate that IR and hyperinsulinemia affect the endometrial receptivity in many ways, leading to difficulty in conceiving and an increase in miscarriage.

Acupuncture is becoming popular in research and clinical practice, and recent studies indicate that acupuncture can increase the ovulation rate, improve endocrine profile by decreasing circulating sex steroids, increase menstrual frequency, and decrease weight. It has been shown that acupuncture can regulate endogenous

systems, including the sympathetic nervous system, the endocrine system, and the neuroendocrine system. Acupuncture appears to improve endometrial receptivity in rats by decreasing the impedance of the uterine artery blood flow and improving the blood flow to the uterus, which might enhance implantation and increase the chances of successful pregnancy and live birth.

Furthermore, electroacupuncture has been shown to improve insulin sensitivity in experimental trials. Electroacupuncture can significantly enhance the decrease in blood glucose levels after insulin injection, suggesting an improvement in insulin sensitivity. These results indicate that electroacupuncture improves glucose tolerance in both wild-type and transgenic mice fed a high-fat diet, and that the effect is related to the stimulation parameters used and is probably due to the reduction of free fatty acid. In rat PCOS models, repeated acupuncture has also been demonstrated to normalize insulin sensitivity. Another study showed that electroacupuncture can normalize insulin sensitivity, and ameliorate insulin resistance and hyperinsulinemia in PCOS rats, probably by regulating the function of pancreatic islets β-cells and by reducing oxidative stress and free androgen. During stimulation, electroacupuncture improves insulin sensitivity in a dihydrotestosterone-induced rat PCOS model more than acupuncture with manual stimulation. But post-stimulation both electrical stimulation and manual stimulation show equal effects, and this might be due to the activation of sensory afferents by both methods.

So far, there are still no studies investigating whether low-frequency electroacupuncture pretreatment followed by ovulation induction will increase the live birth rate. Based on previous studies, we hypothesize that acupuncture pretreatment improves insulin sensitivity and leads to a higher ovulation rate and higher live birth rate. This study is intended to test this hypothesis.

Aim of the study

The objective of this multicenter randomized controlled trial (RCT) is to evaluate whether 16 weeks of acupuncture pretreatment followed by letrozole compared to letrozole alone leads to a higher live birth rate in Chinese women with PCOS and anovulatory infertility.

Who should be in this study?

You will be included in this study if you have

1) Women aged between 20 and 40 years.
2) Confirmed diagnosis of PCOS according to the Rotterdam criteria: Oligomenorrhea (an intermenstrual interval>35 days or <8 cycles in the past year) or amenorrhea (an intermenstrual interval>90 days) together with polycystic ovarian morphology, i.e. the presence of ≥12 antral follicles (≤9 mm) and/or ovarian volume>10 ml on transvaginal scanning, and/or clinical or biochemical hyperandrogenism. Clinical hyperandrogenism in China is defined as a

Ferriman–Gallwey (FG) score ≥5, and biochemical hyperandrogenism is defined as total testosterone (T) >2.6 nmol/l and free testosterone ≥6.0 pg/ml.

3) A husband whose sperm concentration meets the World Health Organization standards (2010) of ≥15 × 10^6 /ml and a total motility of ≥40% or a total motile sperm count of ≥9 million.
4) At least one patent tube shown by hysterosalpingogram or diagnostic laparoscopy within 3 years if the patient does not have a history of abortion or pelvic operation. If the patient has a history of pregnancy and no history of pelvic operation within the past 5 years, they are not required to undergo a tubal patency test.

You will not be included in this study if you have

1) Exclusion of other endocrine disorders:
   –Patients with hyperprolactinemia (defined as two prolactin levels of ≥25 ng/ml at least one week apart or as determined by local normative values). The goal of eliminating patients with documented hyperprolactinemia is to decrease the heterogeneity of the PCOS population. These patients might be candidates for ovulation induction with alternate regimens (dopamine agonists). A normal level within the last year or being on treatment is adequate for entry.

   –Patients with FSH levels >15 mIU/ml. A normal level within the last year is adequate for entry.

   –Patients with uncorrected thyroid disease (defined as thyroid stimulating hormone (TSH) <0.2 mIU/ml or >5.5 mIU/ml). A normal level within the last year is adequate for entry.

   –Patients diagnosed with Type I or Type II diabetes who are poorly controlled (defined as a HbA1c level >7.0%) or patients receiving anti-diabetic medications such as insulin, thiazolidinediones, acarbose, or sulfonylureas that are likely to confound the effects of electroacupuncture. Patients currently receiving metformin XR (extended release) for a diagnosis of Type I or Type II diabetes or for PCOS are also specifically excluded.

   –Patients with suspected Cushing's syndrome.

2) Use of hormonal or other medication, including Chinese herbal prescriptions, in the past 2 months that might affect the outcome of the study treatment.
3) Acupuncture in the past 2 months.
4) Pregnancy within the past 6 weeks.
5) Abortion or having given birth in the past 6 weeks.
6) Breastfeeding within the past 6 months.
7) Not willing to give written consent to the study.
8) Patients enrolled simultaneously in other investigative studies that require

medications, prohibit the use of the study medications, limit intercourse, or otherwise prevent compliance with the study protocol.

9) Patients who anticipate taking longer than a one month break from treatments during the study protocol.
10) Additional exclusion criteria

a. Patients with a suspected adrenal or ovarian tumor that is secreting androgens.

b. Couples with previous sterilization procedures (vasectomy, tubal ligation) that have been reversed. The prior procedure might affect the study outcomes, and patients with both a reversed sterilization procedure and PCOS are rare enough that exclusion should not adversely affect recruitment.

c. Subjects who have undergone a bariatric surgery procedure in the recent past (<12 months) and are in a period of acute weight loss or who have been advised against pregnancy by their bariatric surgeon.

d. Patients with untreated or poorly controlled hypertension defined as a systolic blood pressure of 160 mm Hg or a diastolic blood pressure of 100 mm Hg obtained on two occasions at least 60 minutes apart.

e. Patients with known congenital adrenal hyperplasia.

f. Patients on oral contraceptives, depot progestins, or hormonal implants (including Implanon). A 2-month washout period will be required prior to screening for patients on these agents. Longer washouts might be necessary for certain depot contraceptive forms or implants, especially when the implants are still in place. A 2-month washout will be required for patients on oral cyclic progestins.

g. Patients with liver disease defined as aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >2 times normal or total bilirubin >2.5 mg/dl or patients with renal disease defined as blood urea nitrogen (BUN) >30 mg/dl or serum creatinine >1.4 mg/dl.

h. Patients with significant anemia (Hemoglobin <10 g/dl).

i. Patients with a history of deep venous thrombosis, pulmonary embolus, or cerebrovascular accident.

j. Patients with known heart disease that is likely to be exacerbated by pregnancy.

k. Patients with a history of, or suspected, cervical carcinoma, endometrial carcinoma, or breast carcinoma. A normal Pap smear or thinprep cytologic test (TCT) result will be required for women 21 years and older.

l. Patients with a current history of alcohol abuse. Alcohol abuse is defined as >14 drinks/week or binge drinking.

m. Patients taking other medications known to affect reproductive function or metabolism. These medications include oral contraceptives, gonadotropin-releasing hormone (GnRH) agonists and antagonists, anti-androgens, gonadotropins, anti-obesity drugs, Chinese herbal formulas, anti-diabetic drugs such as metformin and thiazolidinediones,somatostatin,

diazoxide, angiotensin converting enzyme (ACE) inhibitors, and calciumchannel blockers. The washout period for all of these medications will be 2 months.

What will I be asked to do?
If you fulfil the inclusion criteria and accept the study design, baseline measurements including measurements of body weight, height, waist circumference, rating of body hair, fasting blood will be drawn for analyses of OGTT, specific hormones and metabolic profile. You will fill in questionnaires regarding health related quality of life and symptoms of anxiety and depression.

If you are randomized into treatment group, acupuncture treatment will start on day 3-5 after a spontaneous period or after a withdrawal bleeding following progestin. And you are requested to use contraception during the 16 week acupuncture pretreatment. You will receive acupuncture treatment three times a week. Each treatment session lasts for 30 minutes and can be separated by an interval of 1-3 days, with a maximum of 48 treatment sessions during 16 weeks. After 16 weeks of acupuncture treatment, LE will start on day 2-3 after a spontaneous period or after a withdrawal bleeding after progestin administration. You will be treated for up to 4 cycles and will be instructed to have intercourse on a regular basis during the cycles.

After the acupuncture treatment, OGTT measurement should be repeated.

If you are randomized into control group, LE will be started on day 3-5 after a spontaneous period or a withdrawal bleeding following progestin. You will be treated for up to 4 cycles and will be instructed to have intercourse on a regular basis during the cycles.

After the last treatment, all baseline measurement should be repeated.

If you become pregnant, the treatments will be stopped, and you will be received follow-up. Your pregnancy outcome data will be collected by us, and we will track the outcomes of you during the course of this study.

How long will I be in the study?
You will be in the study for about 32 or 16 weeks.

How many women will participate in the study?
We plan to recruit 384 subjects.

Will I be paid?
No payment will be made to you for this study, but some of the treatment will be provided for free.

What adverse (bad) effects may happen to me by participating in the study?

Acupuncture is a safe procedure and few side-effects have been reported. Side-effects that may occur during or after the treatment is pricking pain when penetrating the skin, small bleeding when needles are withdrawn, bruising, nausea, dizziness and fainting which all are short-lasting and mild. Symptoms like nausea, dizziness and fainting are almost completely eliminated by treating in supine position. The most common adverse reactions (>20%) of letrozole are hot flashes, arthralgia, flushing, asthenia, edema, arthralgia, headache, dizziness, hypercholesterolemia, sweating increased, bone pain, and musculoskeletal.

What benefit can I expect?

You will get free measurements of OGTT, transvaginal ultrasound, and free acupuncture treatment with the aim to induce ovulation and improve pregnancy rate including live birth rate. OGTT analyses and questionnaires will give you detailed information about your health status.

Can I refuse to be in the study?

Your participation in this study is voluntary. You can choose not to take part in the study, or you can drop out at any time. You will not lose any benefits to which you are otherwise entitled. If you quit the study, you will receive the standard treatment as other patients at our Department.

Confidentiality and privacy

The investigators have always maintained a strict privacy policy. All correspondence to the department is held confidentially; furthermore, at no time will your personal and/or identifying information be shared outside of our organization, for any reason.

Subjects have the rights of access to personal data and known study results, if and when needed, you enjoy or may enjoy rights for the protection of the confidentiality of your personal data, such as those regarding the collection, custody, retention, management, control, use (including analysis or comparison).

By signing and dating this Consent Form, you agree to and do give up, waive, disclaim, adjust and in any way abrogate all or any of the above-mentioned rights. For any query, you should consult the Privacy Commissioner for Privacy Data or his office (020-83062452) as to the proper monitoring or supervision of your personal data protection so that your full awareness and understanding of the significance of compliance with the law governing privacy data is assured.

For questions about the study or reporting of adverse events, please call your physician at telephone.

I *agree / do not agree to save a blood sample for some genetic tests and a feces sample for microbiological detection if it is found to be useful later.

I have read and understood this consent form. All my questions have been answered. I volunteer to take part in this study.

_____	_____	_____
Subject's signature	Patient name	Date

_____	_____	_____
Site Investigator’s signature	Site Investigator’s name	Date

_____	_____	_____
Patient husband’s signature	Patient husband’s name	Date