

NATIONAL CLINICAL TRIAL BASE OF CHINESE MEDICINE

《国家中医临床研究基地》妇科慢病项目



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Longjiang Gynecology

**Protocol
Final Version**

**Effect of Acupuncture and Clomiphene Citrate on Live Birth in Anovulatory Women
with Polycystic Ovary Syndrome: a Randomized Controlled Trial
(NCT01573858, ChiCTR-TRC-12002081)**

Funded by State Administration of Traditional Chinese Medicine

January 15, 2013

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1. Committee composition

1.1 Protocol committee

Table 1. Protocol committee

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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 with the help of personnel from Heilongjiang University of Chinese Medicine, including registering the trial at clinicaltrials.gov (**NCT01573858**) as well as the Chinese Clinical Trial Registry (<http://www.chictr.org/cn/>), prior to randomization of any subjects (**ChiCTR-TRC-12002081**).

1.4 Publication Committee

Members of the publication committee include Richard S. Legro, Xiaoke Wu, Heping Zhang, Elisabet Stener-Victorin, Ernest HY Ng, Lihui Hou, Jianping Liu and Taixiang Wu.

2. Background

Polycystic ovary syndrome (PCOS) is the commonest endocrine disorder in women of reproductive age and affects 5-10% of premenopausal women and is characterized by hyperandrogenism, ovulatory dysfunction and polycystic ovaries (PCO)¹. In addition to hirsutism, irregular menses, and infertility, women with PCOS display a number of metabolic abnormalities including hyperinsulinemia, insulin resistance, dyslipidemia, and obesity². Obese PCOS women will benefit from weight loss, as this might lead to resumption of spontaneous periods and ovulation and will also improve their response to ovulation induction. Clomiphene citrate (CC) is considered as the first line drug to induce ovulation in women with PCOS. A compilation of published results from 5,268 patients revealed an ovulation rate of 73% per patient, pregnancy rate of 36% per patient and live birth rate of 29% per patient³. Women who do not ovulate while receiving the 150 mg dose are considered to be CC resistant. Inability of CC to induce ovulation is more likely in patients who are obese, insulin resistant and hyperandrogenic compared with those who do respond⁴. Only about 50% of women who ovulate with CC will conceive. This may be partly explained by the peripheral anti-estrogenic effect of CC at the level of endometrium and cervical mucus or by hypersecretion of luteinizing hormone (LH). Side effects of CC are related to its combined estrogenic and antiestrogenic properties, which include hot flushes, breast discomfort, abdominal distension, nausea, vomiting, nervousness, sleeplessness, headache, mood swings, dizziness, hair loss and disturbed vision. Approximately 7% of pregnancies resulting from CC-induced ovulation are twin pregnancies and 0.5% are triplet pregnancies⁵. Gonadotrophin treatment can be offered as the second line drug when anovulatory PCOS women fail to respond to CC. The use of gonadotrophin is more expensive and associated with a much higher risk of multiple pregnancies and development of ovarian hyperstimulation syndrome.

Acupuncture is an integral part of Traditional Chinese Medicine (TCM), which dates back more than 3,000 years. In recent years, the use of acupuncture within the area of reproductive endocrinology and infertility has gained increased popularity worldwide⁶⁻⁸. According to the principles of TCM, there are patterns of energy flow (Qi) through the body that are essential for health. Disruption of this flow in terms of energy disturbance or imbalances or organ deficiencies and excesses is believed to be responsible for anovulatory subfertility. Acupuncture may correct any imbalances in the flow of life force along meridians and thus cure specific conditions.

From a physiological perspective, insertion of acupuncture needles in areas with sensory innervations corresponding to the affected organs, e.g. ovaries and uterus may modulate autonomic and endocrine function^{9,10}. The underlying mechanisms of the effect of acupuncture in the treatment of reproductive endocrinology and fertility may be via modulation of endogenous regulatory systems, including the sympathetic nervous system, the endocrine and the neuroendocrine system^{9,10}.

In PCOS women with ovulatory dysfunction, uncontrolled trials revealed that repeated acupuncture treatments decreased total testosterone and other sex steroid levels, reduced LH/follicle-stimulating hormone (FSH) ratio, and improved menstrual frequency without negative side-effects¹¹⁻¹³. In a randomized controlled trial (RCT), fourteen low-

frequency electroacupuncture (EA) treatments (combination of electrical and manual stimulation) during 16 weeks and 16 weeks of physical exercise compared with no intervention, improved menstrual bleeding pattern and decreased high levels of sex steroid precursors, estrogens, androgens, and glucuronidate androgen metabolites in women with PCOS¹⁴. Acupuncture was superior to physical exercise when compared directly after the treatment. In a quasi-randomized study, daily abdominal acupuncture during 6 months improved menstrual frequency and decreased circulating testosterone more effectively than metformin during 6 months¹⁵. Recently in another RCT, twelve treatments of true acupuncture was compared with sham acupuncture (so called placebo needles) during eight weeks and they found similar ovulation frequency and improvement in LH/FSH ratio in both groups of women with PCOS¹⁶. Thus, they were unable to demonstrate differences between true and sham acupuncture and they did not include a non-intervention group. These results are in line with previous studies on different pain conditions and nausea caused by chemotherapy, demonstrating that true acupuncture is not more effective than sham acupuncture, although all these trials found a significant effect when compared with a non-intervention group¹⁷⁻¹⁹. These results indicate that sham acupuncture is not an inert method and it highlights methodological difficulties in the design of acupuncture trials.

From a physiological perspective, the effect of acupuncture is most likely mediated via activation of sensory nerve afferents which in turn modulate the sympathetic outflow from the ovaries and the central nervous system. Support for the theory that acupuncture, at least in part, acts via the autonomic nervous system arises from the findings that acupuncture normalizes the ovarian expression of sympathetic markers and modulates ovarian blood flow as a reflex response via ovarian sympathetic nerves²⁰⁻²². Recently, direct intra-neural recordings of sympathetic nerve activity in women with PCOS revealed increased sympathetic nerve activity that correlated with the high testosterone levels in these patients²³. It has also been demonstrated that low-frequency EA and physical exercise reduce high sympathetic nerve activity in women with PCOS²⁴.

The frequency of electrical stimulation seems to be of importance in the effect of acupuncture and the most optimal frequency to modulate ovarian sympathetic nerve activity in female rats were either 2Hz EA delivered as 0.1-s bursts at a frequency of 80 Hz (pulse duration: 0.18 ms), or 10Hz EA delivered as single pulses (pulse duration: 0.5 ms)²¹. In addition, needle placement in abdominal muscle, corresponding to ovarian innervations, resulted in stronger effect compared with placement only in the legs, whereas the combination seems to induce the strongest effect^{21,22}. Previous studies in a rat PCOS model demonstrates that low-frequency EA improved ovarian morphology, restored estrus cyclicity and decreased an increased expression of hypothalamic androgen receptor and gonadotropin releasing hormone (GnRH)-immunoreactive cells^{25,26}. More frequent treatments resulted in stronger effects indicating a dose response effect^{25,26}.

In summary, findings from previous clinical and experimental studies indicate that acupuncture may induce ovulation and improve pregnancy in women with PCOS. Experimental studies indicates that needle location, stimulation frequency and treatment frequency play an important role, and it may explain possible mechanisms of action of

the effects observed in women with PCOS. However, these specific variables have not been tested in detail in women with PCOS. In the design of an acupuncture trial it is of great importance to define an adequate dose of acupuncture for the specific condition to be studied by taking all existing clinical and experimental findings into account²⁷, and to follow the Standards of Reporting Interventions in Clinical Trials of Acupuncture (STRICTA)²⁸.

CC is the first-line therapy for ovulation induction in PCOS but is associated with significant adverse effects, while acupuncture can be employed as an alternative therapy to induce ovulation in women with PCOS with fewer side-effects. Importantly, there is no previous study comparing the first-line therapy CC with acupuncture.

3. Objectives

The objectives of the present trial are to test the following three hypotheses in anovulatory women with PCOS:

- 1) Acupuncture protocol 1 plus CC (Arm A) is more likely to result in live birth than acupuncture protocol 2 combined with CC (Arm B),
- 2) Acupuncture protocol 2 plus CC (Arm B) is more likely to result in live birth than acupuncture protocol 1 plus placebo (Arm C),
- 3) Acupuncture protocol 1 plus placebo (Arm C) is more likely to result in live birth than acupuncture protocol 2 plus placebo (Arm D).

4. Study procedures

4.1 Pre-screening

Questionnaire related to symptoms and signs of PCOS, family planning, partner situations and previous examination sheets for couples (see Appendix 12) .

4.2 Screening Visit (Visit 1: physician)

1. Obtain signed informed consent.
2. Ensure the patients agreeing to frequent blood tests and not having frequent business visits.
3. Take a comprehensive history and perform complete physical examination including vital signs, height, weight, hip and waist measurements, BMI, pelvic exam with Pap smear or thinprep cytological test (TCT) or Human Papillomavirus (HPV) DNA test.
4. Assessment of hirsutism by Ferriman Gallwey score and acne standard acne lesion counts
5. Perform transvaginal ultrasound of the uterus and ovaries.
6. Perform urine pregnancy test.
7. Perform the following tests at the local lab: Total testosterone (T), estradiol (E2), Progesterone (P4), follicle stimulating hormone (FSH), luteinizing hormone (LH), sex hormone binding globulin (SHBG), thyroid stimulating hormone (TSH), Prolactin, Hemoglobin A1C (HgA1C), fasting blood glucose and CBC, Renal, and Liver Profile.
8. Complete 4 questionnaires: For health related quality of life (HRQoL) the short form 36 (SF-36)²⁹, the Polycystic Ovary Syndrome Questionnaire (PCOS-QOL)³⁰ and the Chinese Quality of Life (ChQOL)³¹ and questionnaire for symptoms of anxiety and depression: Zung Self-Rating Anxiety Scale and Zung Self-reported Depression Scale (SAS and SDS).
9. Check Inclusion Criteria of the couple (no other infertility factors) with assessment of tubal potency, uterine cavity and semen analysis.
10. Provide pre-conception counseling (Appendix 3), TORCH screening (Toxoplasmosis, Rubella, Cytomegalovirus and Herpesvirus) and HIV screening, and folic acid prescription (dispense folic acid after randomization).
11. Provide progestin prescription to induce withdrawal bleed, with instructions to begin medication once eligibility is determined.

4.3 Baseline Visit (Visit 2: Day 1-5 of the subsequent menstrual cycle with Day 1= first day of vaginal bleeding)

1. Randomization.
2. Dispense home pregnancy test.
3. Dispense study medication and folate.
4. Distribute intercourse and menstrual journal logs.
5. Take blood sample for repository.
6. Take blood sample for DNA which is used for the pharmacogenomics study.
7. Assess TCM syndromes of patients. Syndrome differentiation (Bian Zheng) in traditional Chinese medicine (TCM) is the comprehensive analysis of clinical information gained by the four main diagnostic TCM procedures: observation, listening, questioning, and pulse analysis, and it is used to guide the choice of treatment either by acupuncture and/or TCM herbal formulae³². In PCOS, patients are empirically differentiated to be four

categories: 1) phlegm-dampness syndrome, 2) blood stasis syndrome, 3) phlegm and blood stasis, 4) liver stagnation, (5) others.

4.4 Weekly acupuncture Visit:

1. CC 50mg or one placebo tablet will be started daily from day 3 to day 7 after a menstrual period.
2. Acupuncture treatments twice a week at interval of 2-4 days given by TCM physicians at the convenience of subjects during the whole study, totally 32 sessions. Acupuncture treatment will start on day 3 after a spontaneous period or a withdrawal bleeding following progestin, when starting with CC/placebo tablet.
3. Complete the credibility check questionnaire after the third acupuncture treatment.
4. Record adverse events and concomitant medications.
5. Collect menstrual and intercourse journal logs at the end of every cycle.

4.5 Weekly Serum Progesterone Level and Pregnancy Test

Progesterone level and qualitative pregnancy test will be checked in the local lab every week after initiation of study interventions (CC/placebo drug tablets and acupuncture). Timing would be at day of the second acupuncture day each weekly acupuncture visit, and coordinated to be at the same time as one acupuncture session for patient's convenience. Further details of determining response or non-response to treatment based on the weekly visit can be found in Section 23 below.

4.6 Pregnancy Visits (only with conception)

1. Complete adverse event query
2. Take blood for serum quantitative hCG levels until the level reaches a point where a pregnancy can be visualized by transvaginal ultrasound (1500-2000 IU/mL).
3. Perform transvaginal ultrasound when the hCG level or clinical presentation dictates. Determine the number of gestational sacs, location, dimensions, presence and size of fetal parts, and documentation of visualization of fetal heart motion, documentation of any pregnancy related abnormalities.

4.7 End of Treatment Visit (all subjects):

1. To take place after pregnancy or after completion of the 4th cycle without pregnancy.
2. Complete the credibility check questionnaire after the last acupuncture treatment.
3. Whether or not to perform transvaginal ultrasound for subjects is determined by the local PI on the case-by-case basis.
4. Perform physical examination, including vital signs, height, weight, hip and waist measurements as well as repeating hirsutism and acne assessments.
5. Take blood sample for progesterone level, hCG assay, and send the blood sample to the central core lab.
6. Repeat Safety Labs. These samples will be collected and shipped to the core laboratory for final analysis.
7. Repeat questionnaires including: 1) Risk Factors for Genetic Disorders; 2) Questionnaire for PCOS Quality of Life; 3) Questionnaire for Chinese Quality of Life; 4) SF-36 Health Survey; 5) Zung Self-Rating Anxiety/Depression Scale.
8. Collect menstrual and intercourse journal logs.
9. Record adverse events and concomitant medications.

10. Arrange follow up for subjects who have conceived and obtain release of records for pregnancy and neonatal records.

5. Study design

This is a multicenter, randomized, controlled, 2 by 2 factorial clinical trial for Chinese women with PCOS.

6. Subject selection and exclusion

Chinese women suffering from anovulation related to PCOS and requesting ovulation induction. PCOS is defined by the modified Rotterdam criteria: oligomenorrhea or amenorrhea, together with presence of ≥ 12 antral follicles (≤ 9 mm) or ovarian volume >10 ml on transvaginal scanning, and/or clinical/biochemical hyperandrogenism.

Oligomenorrhea is defined as an intermenstrual interval >35 days and <8 menstrual bleedings in the past year. Amenorrhea is defined as an intermenstrual interval >90 days. Clinical hyperandrogenism in China Mainland is defined as a Ferriman-Gallwey (FG) score ≥ 5 ³³.

7. Inclusion criteria

1. Age of women between 20 and 40 years.
2. Confirmed diagnosis of PCOS according to the modified Rotterdam criteria and all subjects must have anovulation plus either polycystic ovaries and/or hyperandrogenism.
3. Patency of at least one tube and a normal uterine cavity shown by hysterosalpingogram, HyCosy or diagnostic laparoscopy.
4. Semen analysis:
 - (1) a. sperm concentration $\geq 15 \times 10^6$ /ml and b. total motility (a+b+c) $\geq 40\%$ or forward motility (a+b) $\geq 32\%$ in the semen analysis of the husband (based on World Health Organization, 2010).
 - (2) Total motile sperm count ≥ 9 million [based on WHO (2010) criteria, volume 1.5 ml; conc. 15 million; motility 40%. $1.5 \times 15 \times 0.4 = 9$ million].

8. Exclusion criteria

1. Exclusion of other endocrine disorders: ①Patients with hyperprolactinemia (defined as two prolactin levels at least one week apart 25 ng/mL or greater 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 may be candidates for ovulation induction with alternate regimens (dopamine agonists). A normal level within the last year or on treatment is adequate for entry. ②Patients with FSH levels > 15 mIU/mL. A normal level within the last year is adequate for entry. ③Patients with uncorrected thyroid disease (defined as TSH < 0.2 mIU/mL or > 5.5 mIU/mL). A normal level within the last year is adequate for entry. ④Patients diagnosed with Type I or Type II diabetes who are poorly controlled (defined as a HbA1c level $> 7.0\%$), or patients receiving antidiabetic medications such as insulin, thiazolidinediones,

acarbose, or sulfonylureas likely to confound the effects of study medication; patients currently receiving metformin XR (extended release) for a diagnosis of Type I or Type II diabetes or for PCOS are also specifically excluded. ⑤Patients with suspected Cushing's syndrome.

2. Use of hormonal or other medication including Chinese Herbal prescriptions which may affect the outcome at least in the past 2 months.
3. Pregnancy within the past 6 weeks.
4. Within 6 weeks post-abortion or postpartum.
5. Breastfeeding within the last 6 months.
6. Not willing to give written consent to the study.
7. Additional exclusion criteria
 - a) Patients on oral contraceptives, depot progestins, or hormonal implants (including Implanon). A two month washout period will be required prior to screening for patients on these agents. Longer washouts may be necessary for certain depot contraceptive forms or implants, especially where the implants are still in place. A one-month washout will be required for patients on oral cyclic progestins.
 - b) Patients with liver disease defined as AST or ALT > 2 times normal or total bilirubin >2.5 mg/dL. Patients with renal disease defined as BUN > 30 mg/dL or serum creatinine > 1.4 mg/dL.
 - c) Patients with significant anemia (Hemoglobin < 10 g/dL).
 - d) Patients with a history of deep venous thrombosis, pulmonary embolus, or cerebrovascular accident.
 - e) Patients with known heart disease that is likely to be exacerbated by pregnancy.
 - f) Patients with a history of, or suspected cervical carcinoma, endometrial carcinoma, or breast carcinoma. A normal Pap smear result will be required for women 21 and over.
 - g) Patients with a current history of alcohol abuse. Alcohol abuse is defined as > 14 drinks/week or binge drinking.
 - h) Patients enrolled simultaneously into other investigative studies that require medications, proscribe the study medications, limit intercourse, or otherwise prevent compliance with the protocol.
 - i) Patients who anticipate taking longer than a one month break during the protocol should not be enrolled.
 - j) Patients taking other medications known to affect reproductive function or metabolism. These medications include oral contraceptives, GnRH agonists and antagonists, antiandrogens, gonadotropins, anti-obesity drugs, Chinese herbal formula, anti-diabetic drugs such as metformin and thiazolidinediones, somatostatin, diazoxide, ACE inhibitors, and calcium channel blockers. The washout period on all these medications will be two months.
 - k) Patients with a suspected adrenal or ovarian tumor secreting androgens.
 - l) Couples with previous sterilization procedures (vasectomy, tubal ligation) which have been reversed. The prior procedure may affect study outcomes, and patients with both a reversed sterilization procedure and

PCOS are rare enough that exclusion should not adversely affect recruitment.

- m) Subjects who have undergone a bariatric surgery procedure in the recent past (<12 months) and are in a period of acute weight loss or have been advised against pregnancy by their bariatric surgeon.
- n) Patients with untreated poorly controlled hypertension defined as a systolic blood pressure 160 mm Hg or a diastolic 100 mm Hg obtained on two measures obtained at least 60 minutes apart.
- o) Patients with known congenital adrenal hyperplasia.

9. Recruitment and Strategy

Every site is expected to randomize at least 1 case per week. Posters/flyers within university/hospital facilities and direct mailings were previously proved to be the most successful recruitment strategies for an acupuncture randomized clinical trial of reproductive age women³⁴. Communications of experience among site investigation/coordinators by telephone conference are effective to promote recruitment. To encourage competition among site investigators, the SC will award a quarterly certificate of merit to the site with the most subjects recruited.

9.1 Hospital/Local Health Care Referrals

Each PI and other investigator will recruit subjects from her/his individual practice as well as faculty/resident continuity clinic.

9.2 Local Publicity Office

PIs should meet with their local Public Relations officer and plan a news release about the study. They should be available for any newspaper, radio, or TV stories that result from this. The full gamut of local media sources should be utilized. Often there is greater yield with more extensive coverage in smaller local outlets, than brief mentions in outlets with larger circulation. News releases should mention the uniqueness of a study with pregnancy as an outcome and the benefits of improving care of women, as well as the development of new methods for fertility treatment.

9.3 Local Advertisements

Local advertisements include unpaid flyers and paid advertisements. Flyers should be posted in the medical center and local clinics or other public places where allowed. Advertisements will be placed on local radio stations, local newspapers and hospital/university website on a regular basis and money for this has been budgeted. Suggested copy should advertise for “Are you infertile with 8 or fewer menstrual periods a year with a wish to get pregnant...” and not PCOS per se. All responses to paid advertisements (# of contacts, # screened, # randomized) should be tallied. This will allow us to determine the cost effectiveness of these advertisements and their role in enhancing diversity in our study. These strategies should be reviewed and data reviewed from individual sites to guide the budgeting of future funds towards the most cost effective strategy, which may vary from site to site.

10. Prescreening

Subjects who are interested will first be prescreened and recorded by an intake to verify that they meet basic inclusion criteria (i.e. age, oligomenorrhea, partner availability, etc.). The study will be explained in detail and all questions, as well as the written informed consent to help patients understand. The male partner will also be required to read and understand the written informed consent together with participants at the time of the prescreening. It is important to obtain his consent for participation in the study due to the requirements of intercourse, semen analysis for the inclusion criteria and the information collected.

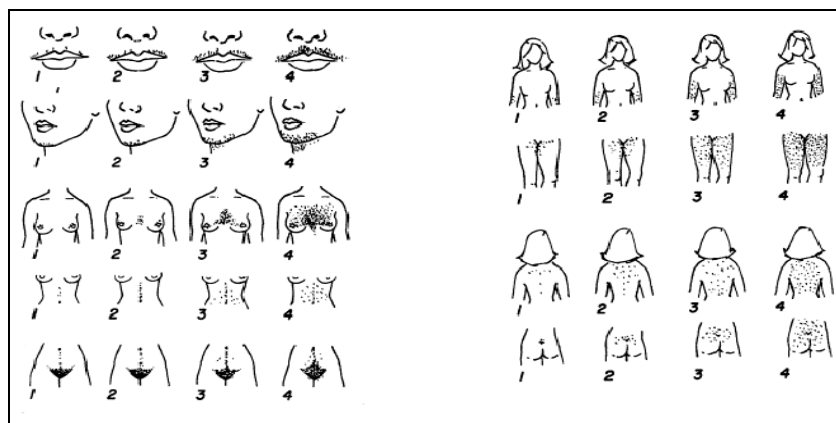
11. Screening Visit

The goal of screening will be to establish a diagnosis of PCOS, to exclude major medical illnesses, and to verify that there are no other significant infertility factors in the couple. These tests may all be performed in one day or in the case of the couple inclusion criteria over several days. All baseline and follow-up measures (i.e. physical exam, serum for the core lab, ultrasound, and questionnaires) should be obtained on a single day. However, questionnaires may be spread between the Screening and Baseline visit. Local inclusion labs or safety labs may be obtained at an earlier (for instance T, SHBG, FSH, Prolactin) or at a later visit (i.e. safety labs).

12. Physical Examination

A physical examination with a standard pelvic and breast exam will be performed on all patients by a study physician. Height, weight and waist and hip circumferences will be recorded to the nearest 0.1 cm, 0.1 kg and 1 cm, respectively. Waist will be measured at the level of the umbilicus and hip circumference will be measured at the widest diameter. Participants will be weighed while dressed in light clothing, without shoes. Weight will be collected at each individual visit, however height, waist and hip circumferences are only collected at the screening and termination visits. Blood pressure will be determined in the right arm in the sitting position. Large cuffs will be used when necessary. Blood pressure will be assessed at each visit. Elevated blood pressures ($\geq 160/100$ mmHg) will be repeated following a short period of rest. A hirsutism assessment will be made via the modified Ferriman-Gallwey hirsutism score by trained study personnel³³.

Figure 1. Ferriman-Gallwey hirsutism Scale



An acne assessment will be made by trained personnel using a standard acne lesion assessment (count) diagram and definitions.

Table 3. Acne lesion assessment

Name	Description
Open Comedone (Blackhead)	Results when resident skin oil, makeup, dirt, dead skin, and small hairs impact a sebaceous follicle and prevent the pore from functioning correctly. If the pore is open, the comedone will be dark in appearance, and is called a blackhead.
Closed Comedone (Whitehead)	Non-inflammatory comedone that resembles a small red bump on the skin.
Pustule	An inflammatory comedone that resembles a small red bump on the skin.
Pustule	An active infection of the skin that consists of dead skin cells and bacteria. These lesions are spherical in appearance and are filled with pus. Often reddish in color, pustules may be painful and will break open easily if scratched or bumped.
Nodule	A natural progression of a papule. They appear very similar to papule, but are inflamed and penetrate deep into the skin. They are often very painful.

Table 4. Acne lesion scoring

Scale	Type	Clinical manifestation
0	None	None
1	Slight	Less than 10 bumps (≥ 2 mm) on face or trunk
2	Mild	10-20 bumps
3	Moderate	Over 20 bumps or less than 20 pustules
4	Severe	Over 20 pustules
5	Cystic acne	Inflammatory skin lesions ≥ 5 mm

When counting facial acne lesions, it is important that all lesions be counted, both noninflammatory and inflammatory, examining areas of the forehead, cheeks, and chin and avoiding the nose.

13. Transvaginal Ultrasound Examination and menstrual cyclicity

In PCOS patients with oligomenorrhea/amenorrhea, a pelvic ultrasound examination will be performed with a transvaginal probe on any cycle day or in the early follicular phase such as day 2-5 of the induced cycle. The following information will be obtained at baseline: uterine dimensions, presence and size of leiomyoma, other uterine abnormalities, endometrial thickness in the sagittal plane, any endometrial pathologies, ovarian size in three dimensions, the size of the largest ovarian follicle/cyst and size of

every follicle with a mean diameter greater than 10 mm, and total antral follicle count of each ovary (small follicles < 10 mm mean diameter). Thereafter only the endometrial thickness, ovarian size, measure of cysts or follicles > 10 mm, and antral follicle count should be performed. Ovarian size is determined by measuring the largest plane of the ovary in two dimensions and then turning the vaginal probe 90 degrees and obtaining a third measurement. Ovarian volume is determined by the formula for a prolate ellipsoid (length x width x height x $\pi/6$)³⁵. Polycystic ovary volume is determined by the revised Rotterdam criteria of Balen et al³⁶, which is defined as at least one ovary having a volume greater than 10 cm³ with no cysts or follicles greater than 9 mm mean diameter. If the patient has had no prior test of tubal potency, this may be the desired time to perform a sonohysterogram to determine tubal potency.

Oligomenorrhea is defined as an intermenstrual interval >35 days and <8 menstrual bleedings in the past year. Amenorrhea is defined as an intermenstrual interval >90 days.

14. Laboratory Examination

Fasting blood will be taken as described above in the inclusion and exclusion criteria to identify appropriate study subjects. This is found in Table below and the tests will be performed in the local lab.

Table 5. Screening labs of PCOS women (run in local labs)

PCOS Diagnostic Labs (if no hirsutism, F-G <5)	PCOS Inclusion/Exclusion labs	PCOS Safety Screen
1. T OR 2. SHBG and calculated Free androgen index	1. Prolactin 2. TSH 3. FSH 4. Progesterone 5. Fasting blood glucose 6. Pregnancy 7. Hemoglobin <10g/dL 8. Glycosylated Hemoglobin A1c level	1. Liver Profile ALT/AST Total Bilirubin 2. CBC 3. Renal Profile BUN or Creatinine 4. Pap smear or Thinprep cytological test (TCT) or HPV DNA test (if no recent results available, per ACOG guidelines).

Every patient should have T and SHBG assay in local labs. Free androgen index will be calculated. Women who deviate from the recommended cutoffs for laboratory screens will proceed with further evaluation as necessary (e.g. repeat prolactin) or be excluded from the study. Costs of these blood tests that determine if the anovulation is consistent with PCOS will be covered by the study.

The Central Laboratory: Heilongjiang University of Chinese Medicine Laboratory

Given the variability of assays between labs, secondary analyses of baseline predictive

factors and response variables will be performed in Heilongjiang University of Chinese Medicine as the central core laboratory. Glucose, insulin, serum androgens (total T, androstenedione, DHEAS, as well as estradiol, progesterone SHBG, etc.), will be measured in singlet at the central laboratory of Heilongjiang University of Chinese Medicine issued by ISO 15189.

15. Quality of Life Measures (QOL)

Mood, quality of life, and sexual function will be assessed at baseline and at the end of the study visit. HRQoL will be assessed by the SF-36, ChiQOL and PCOSQ questionnaires (Appendix 4-6). We will also assess symptoms of anxiety and depression by the Zung SAS and Zung SDS questionnaires (Appendix 7).

16. Exclusion of Other Infertility Factors

Other infertility factors that would require additional evaluation or alternate therapies will be excluded prior to randomization in the study. These factors include male factor and tubal factor. It is estimated that, if necessary in the case of a couple with no prior testing, the screening evaluation could be completed in one visit, if there has been no prior evaluation. This would consist of the patient bringing in a semen specimen from the partner, obtaining baseline history, physical, and blood tests, and performing an ultrasound with sonohysterogram or hysterosalpingogram on the subject to determine tubal patency and uterine cavity. An uncomplicated intrauterine non-IVF pregnancy and uncomplicated delivery and postpartum course resulting in live birth within the last one year will also serve as sufficient evidence of a patent tube and normal uterine cavity as long as the subject did not have, during the pregnancy or subsequently, risk factors for Asherman's syndrome or tubal disease or other disorder leading to an increased suspicion for intrauterine abnormality or tubal occlusion.

Sonohysterogram is a procedure where fluid (usually sterile saline) can be infused through an intrauterine catheter that contains a balloon to occlude the cervical canal. This can examine the contour of the uterine cavity to confirm the uterine cavity is normal and the patency of at least one fallopian tube by accumulating free fluid in the posterior cul de sac. (Chinese Obstetrics and Gynecology (Second Edition))

Hysterosalpingogram is a similar procedure however, this procedure is performed under radiography using a radioactive contrast dye that is injected and visualized flowing through the fallopian tube. The uterine cavity has to be normal and at least one fallopian tube is patent. (Chinese Obstetrics and Gynecology (Second Edition)).

An HSG test is generally acceptable if it is done within 3 years unless a patient had a history of pelvic surgery or pelvic inflammatory disease after the HSG. In that case, the patient should have an updated HSG test within 1 year. In addition, if the updated HSG report indicates that the tubes are patent but not fluent, the doctor in charge should read the HSG films and make the final determination whether the tubes are patent or not; that is, there is no need to report the fluency of the tubes. For example, the doctor can document the tubes as patent if he or she sees that the dye fills the tubes and spills out

from the tubes. In the event that there are discrepancies between the radiologist and the site PI concerning the patency of the tubes, they should discuss the test and reach a consensus for the diagnosis and sign off on the final report.

Sonohysterogram has been found to have a sensitivity approaching hysterosalpingography for detecting uterine anomalies and tubal patency (around 70-80%)³⁷. It was used successfully in Legro's CC/metformin study³⁸. Sonohysterography is chosen for this study because of its lower cost compared with hysterosalpingography. However, if it is judged by the principal investigator to be clinically preferable based on the standard clinical practice at each site, the principal investigator may deem it necessary to perform the hysterosalpingogram or other test of tubal patency such as a laparoscopy, if needed, and these results will be accepted. These studies will be performed by staff at each site experienced in these techniques.

If a patient (or her partner in case of the semen analysis) has had any test in the last year that is normal or within normal range for the reference lab, this may be accepted in lieu of repeating the test for screening. This applies to all inclusion/exclusion criteria except labs that qualify as safety labs (See Safety Screens). In the case of a test for tubal patency, the results will be accepted if performed within the previous one year. Consent forms should explicitly state what tests will be covered by the study, and what other tests will be the responsibility of the patient.

17. Preconception Counseling

During the baseline evaluation all patients will be provided with preconception counseling. This will usually consist of offering TORCH (Toxoplasmosis, Rubella, Cytomegalovirus, Herpes Virus) if there is no history of previous episodes, and offering HIV screening and referral for treatment as necessary. In addition, all patients will be given a prescription for prenatal vitamins containing a minimum of 400 micrograms (0.4 mg) of folic acid after randomization. Giving a prescription for folic acid alone is also acceptable. Subjects are to take this pill daily during the study and once pregnancy takes places.

Previous studies have demonstrated that increasing baseline BMI is a significant negative predictor for live birth as well as associated with a variety of adverse pregnancy outcomes and complications. Therefore, all subjects whose BMI is above 25 will be counseled about the potential benefits of weight loss prior to conception and recommended to pursue this as a first line therapy (although there are no adequate studies to document that this improves outcomes in this population or any obese population). However, because anti-obesity therapy, whether it be lifestyle or pharmacologic is frequently unsuccessful, obesity per se will not be an exclusion criteria. Bariatric surgery for morbidly obese women with PCOS remains a treatment option, but because the role of bariatric surgery in improving reproductive outcomes is uncertain and because these procedures involve substantial risk and cost to study subjects, this will not be incorporated into the study protocol.

Similarly smokers will also be counseled about adverse effects and advised to stop

smoking, though continued smoking will not be an exclusionary item.

18. Screen Failures

Many screen failures may qualify for rescreening after correction of modifiable conditions (such as wash out of exclusionary medications) or re-testing of borderline cutoffs (i.e. prolactin levels). It is anticipated that patients who may fail screening due to a medically treatable condition, such as hypothyroidism, hypertension, or mild iron deficiency anemia, may eventually become eligible for re-screening, assuming that other inclusion/exclusion criteria have not changed (e.g. oligo-amenorrhea as an inclusion criteria, or elevated prolactin levels as an exclusion criteria). These cases will be handled on an individual basis after consultation with the Protocol Leader and the DCC.

19. Counseling about Menstrual and Intercourse Diaries

All subjects will receive counseling and journal logs to track menstrual bleeding and intercourse during the study.

Timing and Tracking of Intercourse Instructions: All subjects will be instructed to have intercourse on a regular basis during the study period. The optimal frequency will be once every 2-3 days. Subjects will be counseled that multiple episodes of intercourse within a short period (i.e. <24 h) can lead to diminishing sperm count, and therefore they should not “batch” their intercourse. The visit of every cycle will include a query about dates of intercourse and review of the intercourse diary. The importance of regular intercourse should be mentioned if the frequency on any week is < 2 episodes/week. An inquiry regarding possible causes should be done when intercourse has occurred <2 times per week for two consecutive visits. Menstrual diaries will be collected every month.

20. Adjuvant Therapy

No adjuvant therapy or sonography monitoring is allowed including other ovulation induction agents (metformin, letrozole and gonadotropins), or triggering ovulation with hCG, or performing intrauterine or intracervical inseminations for study subjects in this protocol. This extends to extra acupuncture, herbal therapies, etc.

21. Progestin Withdrawal

All patients will undergo withdrawal bleeding with progestin when started but not during the study. The patient will be given a progestin prescription at the discretion of the local PI during the baseline visit (such as medroxyprogesterone acetate, MPA, 5mg x 10 days), and progestin availability can be instituted immediately after the screening visit, once eligibility criteria have been verified.

22. Study Specific Procedures/Visits

An overview of the study visits is found in Figure below.

Flow chart of the study: Effect of acupuncture and clomiphene citrate for ovulation induction and live birth in infertile women with polycystic ovary syndrome: Randomized controlled trial. Please note that a serum progesterone level and hCG will be sent only once per week, whereas there will be two acupuncture sessions per week.

Table 6. Overview of the study visits

Visit #	Screening Visit	Baseline Visit	Week 1		Week 2		Week N+ 1		Final Treatment Visit Week 16-20		End of Treatment Visit
	1	2	3	4	5	6	N	N+1	33	34	35
Sign Consent	x										
History	x										x
Urine pregnancy test	x		x	x	x	x	x	x	x	x	
Physical Exam	x										x
Transvaginal ultrasound	x										x
Semen Analysis	x										
Hysterosalpingogram or sonohysterogram (SHG)	x										
Safety Eligibility Lads	x										x
Fasting Phlebotomy For Study Parameters		x									x
QOL Measure	x										x
Progesterone assay	x			x		x		x		x	x
HCG assay	x			x		x		x		x	x
Acupuncture Visits (twice weekly)			x	x	x	x	x	x	x	x	
Assess adverse events and concomitant meds				x		x		x		X	x

22.1 Screening Visit: Assessment for Eligibility. The results of the inclusion and exclusion labs which are performed locally should be available within a few days. If the subject is eligible to participate, she will be instructed by telephone or email to take the progestin if needed (i.e. anovulatory serum progesterone level). If not eligible, the subject will discard the progestin and will not participate in the study. The screening visit will have no fixed timing in relation to last menses or day of the week, but will be scheduled at the

discretion of the study subject and investigative team. Randomization will take place as soon as the eligibility requirements are met, although study drug will not be dispensed until the next visit (baseline visit).

22.2 Baseline Visit: The baseline visit will occur on Day 1-5 of the subject's cycle that occurs either spontaneously (because the patient ovulated at the screening visit), or after a progestin challenge. Day 1 is defined as the first day of vaginal bleeding (spotting does not count). The subject will call in to the study coordinator with onset of her menses to schedule the baseline visit, which will take place within 5 days of the onset of normal menstrual flow.

If the subject does not have a menstrual bleed, either after progestin or spontaneous ovulation, she will be managed on a case-by-case basis by the site PI depending on if they are pregnant and the results of the baseline ultrasound.

At the baseline visit, the subject will undergo the brief examination and the fasting blood sampling as described above in the eligibility visit and in the table of study visits. The subject will be instructed not to start the treatment until she has received a call from the study coordinator regarding the result of the serum pregnancy test taken. If the test is negative, she will be instructed to begin her study treatment the same day. The subjects will be dispensed home pregnancy tests to be used throughout the cycle if pregnancy is suspected, and their journal logs to record menstrual bleeding, drug administration, intercourse frequency and adverse events throughout the study.

22.3 Weekly acupuncture Visit

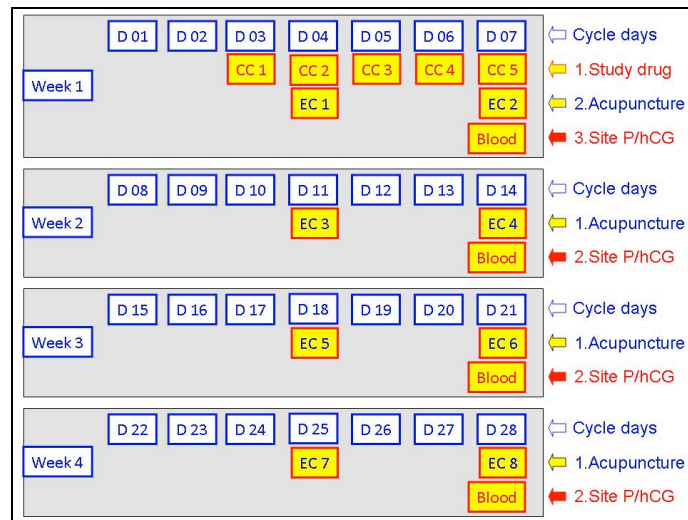
Treatment starts after one spontaneous period and or induced period by progestin on day 3.

1. Dispense one CC 50 mg or placebo tablet at cycle days 3-7.
2. On day 3, acupuncture treatments will start with an interval of 2-4 days every week by TCM physicians at the convenience of subjects during the whole study, totally 32 sessions.
3. Patients treated with acupuncture protocol 1 and 2 should be separated on different dates or time of day.
4. Check urine pregnancy test before acupuncture treatment (treatments 1 and 2). If positive, no acupuncture treatment should be performed.
5. Weekly serum progesterone level test is needed.
6. Query for adverse events and concomitant medications.
7. Collect menstrual and intercourse journal logs at the end of every cycle.

23. Cycle treatment program

We may find three situations on base of weekly serum P/HCG assay and two acupuncture sessions deviated from normal treatment program. It is generally accepted to deviate 1-3 days from fixed date of blood draw and acupuncture treatment to facilitate patient's schedule.

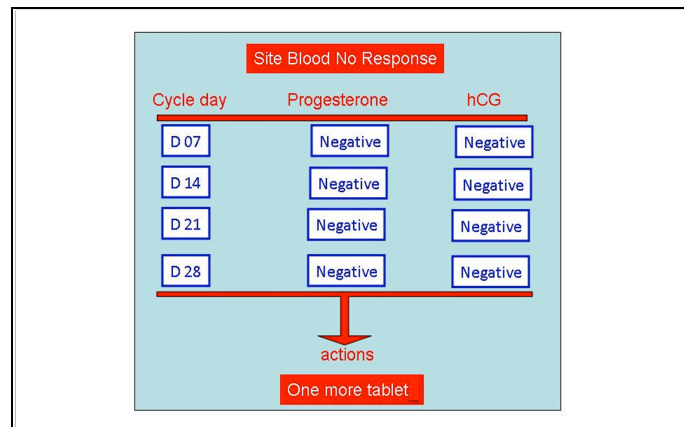
Figure 2. Normal Treatment Program



23.1 Non-Responders

Non-response will be defined after 4 weeks of CC/Placebo administration with 3 consecutive weekly negative P4 and hCG levels. Then during the fifth week a higher dose consisting of one more tablet per day (up to 3 tablets per day at third or fourth cycles, if they remain non-responders) will be given 5 days, together with 2 weekly sessions of acupuncture and one weekly blood draw for progesterone analyses. Generally, CC 50mg or one placebo tablet will be started daily from day 3 to day 7 after a menstrual period. If there is no period after 3 weeks and the patient is not pregnant, CC 100mg or 2 placebo tablets will be given daily from day 3 to day 7. If there is again no period after 3 weeks and the patient is found to be not pregnant and not ovulating by P4 levels, CC 150mg or 3 placebo tablets will be given daily from day 3-7. The same CC dose will be continued in the next cycle if the patient is shown to be ovulating. A maximum of CC 150mg or 3 placebo tablets daily are to be given even if the patients do not ovulate during the study period. Thus a patient who is anovulatory after three consecutive cycles can receive an additional cycle of 150 mg x 5 days. Non-responders will not receive progesterone for withdrawal bleeding before a higher dose of CC unless at discretion of the site investigator.

Figure 3. Non-Responder’s Treatment Program

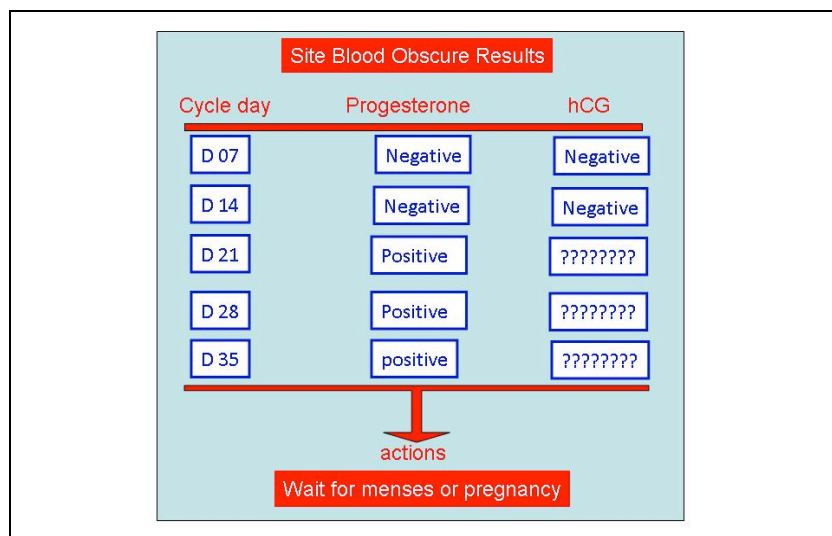


23.2 Responders

Responders will be defined as having at least one weekly-elevated serum P4 level indicative of ovulation. A patient who has ovulated will be followed weekly until she has a period or a positive pregnancy test. If she has menses, the same amount tablet will be given at days 3-7 of spontaneous menses together with 2 sessions of acupuncture and one blood draw. One special situation is when there are extra weeks beyond the 4-week cycle with continued positive P4 levels BUT negative HCG levels and no menses. Then waiting another week is appropriate to anticipate menses and as well as pregnancy by positive HCG. During these 1-2 waiting weeks, acupuncture sessions should continue even without application of CC/placebo drugs.

Responders will be identified on the basis of an elevated progesterone level consistent with ovulation (generally this is a Progesterone > 3 ng/mL, however cutoffs may vary from lab to lab). An elevated level > 3 ng/mL is evidence of response³⁹. After the menses with a documented negative serum beta hCG assay, the patient will again take the study treatment. Responders will continue at the same dose until pregnancy, or a total of four cycles is reached.

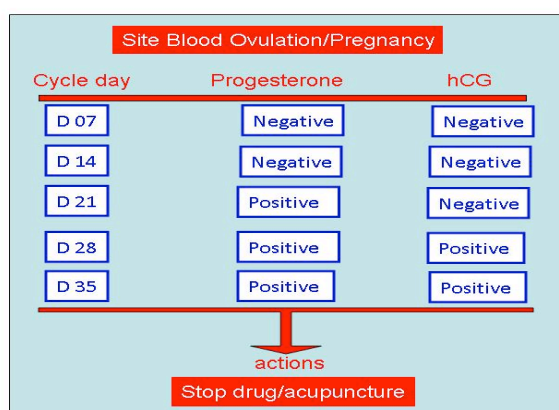
Figure 4. Responder’s Treatment Program



23.3 Pregnancy

In the case of a positive pregnancy test, medication and acupuncture will be stopped with subsequent focus on determining if there is a viable intrauterine pregnancy by ultrasound. Once a patient conceives she is out of the study, even if she has a biochemical pregnancy or early miscarriage. Pregnant women who presents with threatened miscarriage or are considered to have a significant risk for miscarriage can be given oral Duphaston. No other medications including hCG, herbal medications and acupuncture should be given by the investigators. Should patients choose to take any other medications on their own, the medicines should be clearly documented in the CRFs.

Figure 5. Pregnancy Treatment Program



23.4 Dispensing of Study Drug before Every Cycle

Before the beginning of each cycle, each participant will be dispensed the study drug for that cycle on the same day they are supposed to take medications, and the woman should sign off after receiving the medication. The dose of study drug should be determined by the study coordinator based on the prior dose and if the patient is ovulatory or not and appropriate amount administered. The study coordinator will instruct the subject not to take any medication until serum progesterone and beta hCG results are available for review with the subject. If anovulation is confirmed, the study coordinator will instruct the subject to begin study drug for that cycle. No subject will receive more than the maximum dose for one cycle (maximum of 15 pills). It is anticipated that each study site will store and dispense the medication in this trial.

23.5 Breaks in Study Protocol

There may be breaks in the protocol for both anticipated and unanticipated reasons, but the breaks should not exceed 4 weeks. If personal or professional commitments will result in more than a four-week break from the protocol, subjects should not be enrolled. A break or breaks, up to four weeks, is allowable to facilitate timing of intercourse if there are other commitments or to allow recovery from a persistent ovarian cyst. Urinary

pregnancy tests can be dispensed to check weekly for pregnancy during these breaks. Longer break(s) may be an indication for dismissing the patient from the protocol as non-compliant and should be cleared with the Protocol Leader and DCC.

23.6 Pregnancy Visits

Serum progesterone and hCG are determined locally at sites to document ovulation and pregnancy during weekly visit for acupuncture treatment. Subjects should be encouraged to come in on the same day every week of treatment so that there are ~7 days between each visit. In case of appropriate serum beta hCG results, pelvic ultrasound will be arranged for confirmation of the pregnancy site, the number of gestational sacs length of a fetal pole, and presence of fetal heart motion. Patients who conceive will be followed through the study until the pregnancy has advanced to the point of determining the number of gestational sacs, their location, and fetal viability as determined by visualization of fetal heart motion by ultrasonography. At this point, at approximately 9 weeks gestational age, women will be referred to their prior or referring practitioner or to an appropriate health care provider for antenatal care by China law for family planning.

23.7 End of Treatment Visit

At the end of study, a visit will be arranged at the end of the ovulation induction phase for women who do not conceive in the trial or with pregnancy if women conceive. The end of treatment visit should be performed as early in pregnancy as possible. A brief examination with the addition of a repeat of acne and hirsutism will be collected. Subjects will return remaining study drug, their journal logs, and a final assessment of adverse events and concomitant medications will be done. Baseline measures will be repeated in all subjects, including safety labs and 5 QOL surveys including 1) Risk Factors for Genetic Disorders; 2) Questionnaire for PCOS Quality of Life; 3) Questionnaire for Chinese Quality of Life; 4) SF-36 Health Survey; 5) Zung Self-Rating Anxiety/Depression Scale. A final collection of blood for the core lab will also be done.

For those women who have an ongoing pregnancy, arrangements will be made to follow the outcome of the pregnancy at the end of first trimester and also after delivery or termination of gestation. All pregnancies (including multiples) will be followed to determine the abortion rate, complication rates, and to determine pregnancy outcomes. Patients will be informed to notify study personnel of the outcome of the pregnancy and we will obtain release of record forms from treating physicians to obtain copies of relevant medical records. Delivery records of both mother and newborn will be requested to determine the birth weight, length of gestation, and any prenatal complication of mother or neonatal complication of the infant. All reported congenital anomalies will be collected as part of the study. Phone contacts will be initiated if the patient has not contacted study personnel by six weeks beyond the original estimated date of confinement.

23.8 Final Pregnancy Outcome data

We will collect pregnancy outcome data. We will track the outcomes of all randomized subjects who have a positive serum pregnancy screen during the course of this study. We will record biochemical pregnancies (defined as positive serum pregnancy screens

without ultrasonically detected pregnancies), ectopic pregnancies, and all intrauterine pregnancy losses both before and after 20 weeks including missed abortions, spontaneous abortions, elective abortions, fetal demises, and stillbirths. We will review pregnancy and birth records of the mother and of the fetus to establish neonatal morbidity and mortality and the presence of fetal anomalies. We will extract from these records concomitant medical and obstetrical conditions, exposure information on all other medical products used, including prescription products, over-the-counter (OTC) products, dietary supplements, vaccines, and insertable or implantable medical devices. We will file individual case reports for all congenital anomalies, which will be considered a serious adverse event.

24. Randomization

Subjects will be randomized into one of the four treatment arms: A) Acupuncture treatment 1 and CC, B) Acupuncture treatment 2 and CC, C) Acupuncture treatment 1 and placebo CC, and D) Acupuncture treatment 2 and placebo CC by an interactive online computer program in a central office. Using a 1:1:1:1 treatment ratio, there will be 250 women assigned to each treatment group including 20% dropout. The DCC statisticians will generate the randomization scheme for the study. The CC and placebo assignments will be double-blind and the randomization scheme will be disclosed to the DCC data manager, and not to any site investigators or staff, including the Protocol Lead Investigator. The acupuncture treatments 1 and 2 will be known only to the TCM doctor beside the DCC data manager. Unless otherwise specified, treatment group data will be presented in a blinded fashion within DSMB reports. The randomization will be stratified within each of the 25 sites by a random block algorithm.

The CC and placebo will be organized in a kit consisting of 4 bottles for each patient with 5, 10, 15, and 15 tablets, respectively. The kit will be labeled with an ID number mapped to the CC and placebo known only to the DCC personnel. After the patient has signed an informed consent and all required baseline evaluation procedures have been completed, an Investigator or designee will take a medication kit and scan (or enter) the ID number into the web-based database. The TCM doctor logs a password protected secured website designed by the DCC to find the true or sham acupuncture corresponding to the ID number. It is anticipated this will occur in the ~14 days between the screening visit and the onset of the withdrawal bleed after the subject has taken progestin. The Study Coordinator usually being the investigator's master/PhD student at each site will be responsible for storing, dispensing, and performing pill counts on the study medication. The medication bottles should have all necessarily warning and instruction labels produced by the manufacturer.

25. Treatment Arms

25.1 Acupuncture protocols 1 and 2

We base the rationale of acupuncture protocols on Western Medical theories and the study protocol follows the CONSORT⁴⁰ and STRICTA²⁸ recommendations with detailed descriptions of the treatment including number of needle used, how needles will be stimulated (manual, electrical), frequency of sessions and length of treatment period.

In both the acupuncture protocols 1 and 2 groups, we will use fixed acupuncture protocols. All participants will receive treatment twice a week; each treatment session can be separated by an interval of 2-4 days, with a maximum of 32 treatment sessions during 16 weeks and up to 20 weeks if a patient should have longer cycles after ovulation (i.e. 5 week cycles instead of 4). A total of 32 acupuncture treatments should be maintained. If the participant misses two to four scheduled acupuncture treatments (1-2 weeks), she will take additional acupuncture sessions during the following treatments and make up to 32 times in the end. If the participant misses 6-8 acupuncture treatments (3-4 weeks), she should be scheduled not to take study medication CC either and have this treatment cycle “rested” until she can get acupuncture. Each treatment session lasts for 30 minutes. Acupuncture treatment will start on day 3 after a spontaneous period or a withdrawal bleeding following progesterin, when starting with CC/placebo tablet. The design of acupuncture protocol 1 is based on existing data from experimental studies, on a previous RCT in PCOS women¹⁴, and on an unpublished RCT registered at ClinTrial.gov (NCT00921492). The acupuncture protocol 2 is chosen in contrast to acupuncture protocol 1 and designed in such way that we believe it is less effective. The rationale for having two acupuncture protocols despite knowledge that there is no inert sham procedure for acupuncture is that we don’t know the effectiveness of acupuncture and we want to evaluate the effect of acupuncture in the most unbiased manner.

Both acupuncture protocols will be given by a TCM doctor with science qualification consisting of master training and 1 year clinical experience in acupuncture. Each TCM doctor at each site will receive training in PCOS, theoretical and practical acupuncture with protocol designed by reviewing previous clinical and experimental studies, after discussions with clinicians and in the steering committee. Before the start of the study, we will conduct a one-day study-specific education, including theoretical sessions with a Western Medical approach to acupuncture physiology, practical sessions with demonstrations of acupuncture protocols 1 and 2, and lectures on research methodology with a focus on RCT.

Acupuncture protocol 1—Disposable, single-use, sterilized needles made of stainless steel, 0.25 x 30 mm and 0.30 x 40/50 mm (Hwoto, Suzhou Medical Appliance Fact. 215005 Suzhou, China) will be inserted to a depth of 15–35 mm in segmental acupuncture points located in abdominal and leg muscles with innervations corresponding to the ovaries⁴¹. Two sets of acupuncture points will be alternated every second treatment. The first set consists of conception vessel (CV) 3, CV 6, and stomach (ST) 29 bilaterally and in the muscles below the knee, spleen (SP) 6, and SP 9 bilaterally. Needles will also be placed in extrasegmental acupuncture points that do not innervate the ovaries large intestine (LI) 4 bilaterally and governor vessel (GV) 20. In total 11 needles will be placed and all will be stimulated manually by rotating the needle to evoke needle sensation (de qi) once when inserted. CV 3, CV 6, ST 29, SP 6, and SP 9 will thereafter be connected to an electrical stimulator (Export Abteilung, Schwa-Medico GmbH, Wetzlarer Str. 41-43;35630 Ehringshausen) and stimulated with low-frequency EA of 2Hz, 0.3 ms pulse length and the intensity will be adjusted to produce local muscle contractions without pain or discomfort. Needles not connected to the electrical stimulator will be manually stimulated to evoke needle sensation every 10 min, in total 4 times. The second set

consists of 13 needles placed in segmental abdominal points; stomach (ST) 25 and 29 bilaterally (electrical stimulation) and CV3 and CV6 (manual stimulation), leg points; SP6 and liver (LR) 3 bilaterally (electrical stimulation). Extrasegmental points are pericardium (PC) 6 bilaterally and GV20 (both manual stimulation).

Acupuncture protocol 2 —Disposable, single-use, sterilized needles made of stainless steel, 0.20 x 20 mm (Hwoto, Suzhou Medical Appliance Fact. 215005 Suzhou, China) will be inserted to a depth of <5 mm, one in each shoulder and one in each upper arm at non-acupuncture points. Placement of needles is unlikely to affect ovulation in women with PCOS. Electrodes will be attached to the needles and the stimulator will be turned on at an intensity of zero (no active current) in order to mimic EA in the Acupuncture protocol 1 group. No manual stimulation of the needles will be performed.

25.2 Clomiphene citrate (CC) and Placebo CC

CC (Fertilan, Codalysnto.Ltd, Limas sol, Cyprus) and placebo pills will be packed and tested by a commercial pharmacy supply company (BaoquanPharma, Hegang, Heilongjiang, China) specifically for this study. Each participant will receive a monthly medication bottle consisting of CC (CC in 50-mg pill or matching placebo). Participants are treated for up to four cycles, or 20 weeks.

One pill (CC 50 mg or placebo) per day from the bottle for 5 days, beginning on day 3 of menses (spontaneous or induced). If there is an adequate response with ovulation, this dose will be maintained. In patients with no response, the dose will be increased to two tablets (CC 100mg or placebo) a day, either after 5 weeks of anovulation or after menses until a maximum dose of three tablets (CC 150 mg or placebo) per day will be reached. The same CC dose will be continued in the next cycle if the patients are shown to be ovulating. A maximum of three tablets daily are given even if the subject does not ovulate during the study period.

25.3 Study Drugs packaged and dispensed

Study drug will be supplied to the sites in packaged, labeled and numbered kits. Each kit will contain study drug for one or more cycles as follows. The kit will contain either:

- 1 bottle containing 5 pills of 50mg of Clomiphene Citrate or CC placebo
- 1 bottle containing 10 pills of 50mg of Clomiphene Citrate or CC placebo
- 2 bottles containing 15 pills of 50mg of Clomiphene Citrate or CC placebo

The kit will be labeled with an ID number mapped to the CC and placebo known only to the DCC personnel. After the patient has signed an informed consent and all required baseline evaluation procedures have been completed, an Investigator or designee will take a medication kit and scan (or enter) the ID number into the web-based database. The TCM doctor logs in a password protected secured website designed by the DCC to find the acupuncture protocol 1 or acupuncture protocol 2 corresponding to the ID number. Instruct patient to wait to begin medication until called by the coordinator.

Study drug will be shipped via Express Company. If a shipment does not arrive as scheduled or there are questions about the study medication, please notify Rong Wang

(the head nurse at Heilongjiang University of Chinese Medicine) immediately. She will track the shipment and answer queries.

Study drug should be stored at controlled room temperature (20° - 25° C or 68° - 77° F). All study drug boxes must be stored together in a secured area with limited access. All personnel involved in dispensing study drug should be aware of the location and any change in location.

26. Monitoring

Serum P and HCG concentrations will be measured once a week during the study period. If serum HCG concentration is elevated above 10 IU/L, pelvic ultrasound will be arranged 2 weeks later for the number of the gestational sacs and the viability of the pregnancy. Another pelvic scanning will be performed 2 weeks later to confirm the continued viability of the fetus. Patients will be referred out for the antenatal care at 9 weeks. The antenatal outcomes of all pregnant patients will be tracked.

Upon pregnancy or at the end of the 16 week treatment, fasting blood samples for measurement of FSH, LH, total T, SHBG, DHEAS, glucose and insulin concentrations, and lipid profile in those patients who are not pregnant at that time. Patients will complete the HRQoL and symptoms of anxiety and depression questionnaires.

27. Outcome measures

27.1 Primary outcomes: Live birth rate

27.2 Secondary outcomes:

1. Ovulation rate
2. Ongoing pregnancy rate (around gestation 8-10 weeks)
3. Multiple pregnancy rate
4. Miscarriage rate: loss of an intrauterine pregnancy before 20 completed weeks of gestation.
5. Change in hormonal profile: FSH, LH, total T, SHBG and DHEAS
6. Change in metabolic profile: glucose and insulin concentrations, cholesterol, triglycerides, high density lipoprotein (HDL-C) and low density lipoprotein (LDL-C)
7. Side effect profile

28. Timeline and Recruitment Plan

The targeted duration of recruitment will be 18 months with 21 sites (selected from 35 potential sites). In order to achieve our enrollment goal of 1,000 randomized subjects, each site must contribute 40-50 subjects, which equals about 1 randomized subject per site per week. There will be a four month treatment period and another nine month period to follow up the pregnancy outcome. Therefore from initial recruitment to complete follow up, a total of 31 months (2.5 years) will be required to complete the study (June 2012 to November 2014).

29. Statistics

29.1 Statistical tests

The primary analysis will use an intent-to-treat approach to examine differences in the live birth rate among the four treatment arms. Primary efficacy analysis will be done by comparing the treatment groups with respect to the primary outcome of live birth using the Pearson chi-square test. For the secondary, supportive analysis, we will fit a logistic regression model to compare the treatment arms with respect to the primary outcome of live birth, adjusting for other factors such as randomization stratification of study site and prior exposure to study medications. The analysis of other secondary (supplemental) outcomes measured over time will entail the application of statistical methods that have been developed for correlated data since repeated observations will be made over time on each individual. For secondary outcomes such as hormone levels, a linear mixed-effects model will be fit where the main independent variables will be treatment group, time, and their interaction as well as the designed randomization stratification factors as covariates. Logistic regression models will be used in secondary analyses to evaluate the predictive value of treatment arm, clinical site, prior exposure to CC, body mass index and other explanatory variables on binary outcomes (e.g., singleton live birth, abortion). Cox proportional hazards models and a Kaplan-Meier method will be applied to compare time to pregnancy in the treatment groups.

29.2 Sample size estimation

The overall live birth proportion for subjects to receive CC is estimated to be 0.20 for 4 months according to Chinese Obstetrics and Gynecology (Second Edition), with ovulation rate 76%, pregnancy rate 38% and live birth rate 30% for 6 months treatment. Due to lack of existing data on the effectiveness of acupuncture, we cannot rely on the literature for the sample size determination. Instead we hypothesize that the order of effectiveness of the treatments is as follows:

- Acupuncture protocol 1 + CC (A) > Acupuncture protocol 2 + CC (B) and all other treatment arms
- Acupuncture protocol 2 + CC (B) > Acupuncture protocol 1 + placebo CC (C)
- Acupuncture protocol 1 + placebo CC (C) > Acupuncture protocol 2 + placebo CC (D)

The respective live birth rates are projected with 10% difference as follows and the average rate among four arms is 15%:

Intervention s	Acupuncture protocol 1	Acupuncture protocol 2
CC	A=25%	B=15%
Placebo CC	C=15%	D=5%

Using a two-sided trend test, our study is designed to have >80% power at the significance level of 0.05. The sample size has been inflated from 220 to 250/arm to allow for a dropout rate of slightly over 10%, totally 1000 cases for four arms.

30. References

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31. Assessment of safety, quality control and quality assurance

31.1 Safety analysis

Adverse events will be categorized and percentage of patients experiencing adverse events and serious adverse events during the treatment period will be documented. Chi-square tests will be performed to examine differences in the proportion of total and categories of adverse events within each treatment arm. For each Data and Safety Monitoring Board (DSMB) report, a list and summary of the reported adverse events will be presented in a blinded fashion, unless otherwise formally requested.

31.2 Interim Analysis

We propose to do an interim analysis at stage of 1/3 enrollment, to avoid the serious deviation of clinical habits of local PIs from our standard PCOSact protocol. The final data analysis will be completed after all live births in the trial. Unblinding of individual study subjects will not take place until all subjects have delivered and reported outcomes to the DCC.

32. Data and Safety Monitoring Board (DSMB) and Ethics

32.1 DSMB

We will establish an independent Data and Safety Monitoring Board (DSMB) to review and interpret data generated from the study and to review revisions of the protocol prior to their implementation. Its primary objectives are to ensure the safety of study subjects and the integrity of the research data. The DSMB advises on research design issues, data quality and analysis, and research participant protections for the study.

The DSMB will hold regular conference calls in English to review the protocol with respect to ethical and safety standards, monitor the safety of the trials, monitor the integrity of the data with respect to original study design, and provide advice on study conduct. The DSMB will review the progress of the trial, adjudicate adverse events, and decide on any premature closure of the study. The call will be coordinated by the DCC who will provide study updates prior to the call via email.

32.2 DSMB Members

Table 7. DSMB Members

Name	Role on DSMB	Affiliation	High Level Responsibilities
Robert Rebar, MD	Chair of DSMB voting member	American Society for Reproductive Medicine	<ul style="list-style-type: none"> ▪ Chair the DSMB discussion and prepare written recommendations to China Government. ▪ Ensure the safety of study subjects, the integrity of the research data. ▪ Provide China Government with advice on the ethical and safe progression of studies conducted in the current project. ▪ Advises on research design issues, data quality and analysis, and research participant protection for each prospective and on-going study.
Esther Eisenberg MD, MPH	Voting member	Vanderbilt University Medical Center	<ul style="list-style-type: none"> ▪ Ensure the safety of study subjects, the integrity of the research data. ▪ Provide China Government with advice on the ethical and safety progression of current study. ▪ Advises on research design issues, data quality and analysis, and research participant protection for this prospective and on-going study.
Weiliang Weng, MD 翁维良	Voting member	China Academy of Traditional Chinese Medicine	
Sulun Sun, MD 孙塑伦	Voting member	Beijing University of Traditional Chinese Medicine	
Wei Zou, MD 邹伟	Voting member	Heilongjiang University of Traditional Chinese Medicine	
Zidan Chen 陈子丹	Voting member	China University of Mining and Technology (Retired)	

32.3 Ethics

Ethics approval has been sought from Ethics Committee at First Affiliated Hospital in Heilongjiang University of Chinese Medicine on behalf of the State Administration of Traditional Chinese Medicine (2010HZYLL-010). Ethics approval will be obtained from each participating centre; or a permission of using ethics approval of First Affiliated Hospital of Heilongjiang University of Chinese Medicine will be obtained.

33. 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 Heilongjiang University of Chinese Medicine.

33.1 Data Entry and Forms

Case Report Forms (CRFs) will be developed in WORD form and will be implemented in a Web-based data management system at <http://www.clinicaltrialecrf.org>. The Web data entry forms will be implemented to be similar to the WORD (paper) forms with the same questions. However, the Web forms usually have more flexibility than the paper forms, such as pull down menus.

33.2 Data Security

For the purpose of managing the data, monitoring the process and promoting the transparency of the study, a web based database, Clinical Trial Electronic Case Record Form (eCRF system) (<http://www.clinicaltrialecrf.org>), will be used to record and deposit the patients' data and results.

The database managers, Dr. Yan Li, biostatistician Prof. Heping Zhang, and project leader Prof. Xiaoke Wu, will take the responsibility to establish the project space in the eCRF system and manage the user's accounts of each sub-center, establish individual folders for each sub-center and assign the jurisdiction to the users.

The database managers have the highest jurisdiction to manage and monitor the data and actions of putting the data; and the users of each sub-center will be allowed to entry their patients' information and study results in their individual folders. The database managers take the responsibility to make decision of which data could be disclosed to public. The private information of patients includes name, age, telephone number, will be critically protected and will never be allowed to disclose.

The eCRF system service provider, Chinese Clinical Trial Registry, will takes responsibility to ensure the safety of the database and the study data, provides technique support of using the database, and maintains the database keep working, but do not allowed to change anything in the study data. The contact person of the eCRF system is Prof. Taixiang Wu.

33.3 Data Quality Control

Competency to Perform Procedures / Tests in the Protocol.

The site PI will be responsible for ensuring that study related tests are performed by competent personnel. The criteria for determination of competency may vary between sites in the study.

33.4 Quality Control Steps

Quality control of data will be handled at three different levels. The first level is the real-time logical and range checking built into the web-based data entry system. The research coordinators and data entry clerks at the participating sites are required to ensure the data accuracy as the first defense. The second is the remote data monitoring and validation that is the primary responsibility of the data manager and programmer at the DCC. The data manager will conduct monthly comprehensive data checks, as well as regular manual checks (within the database system). Manual checks will identify more complicated and less common errors. The data manager will query sites until each irregularity is resolved. The third level of quality control will be the site visits, where data

in our database will be compared against source documents. Identified errors will be resolved between the DCC and clinical sites. The visits will assure data quality and patient protection.

33.5 Audit Trails

An audit trail will be added as another security measure. This will ensure that only authorized additions, deletions, or alterations of information in the electronic record have occurred and allows a means to reconstruct significant details about study conduct and source data collection necessary to verify the quality and integrity of data. Computer generated, time-stamped audit trails will be implemented for tracking changes to electronic source documentation.

Controls will be established to ensure that the system's date and time are correct. This is a multicenter clinical trial and will be located in within China mainland. System documentation will explain time zone references as well as zone acronyms. Dates and times will include the year, month, day, hour, and minute to the date provided by international standard-setting agencies. The ability to change the date or time will be limited to authorized personnel, and such personnel will be notified if a system date or time discrepancy is detected.

In addition to internal safeguards built into the computerized system, external safeguards will be implemented. Data will be stored at the servers housed at Heilongjiang University of Chinese Medicine with the access overseen by Prof. Heping Zhang. Records will be regularly backed up, and record logs maintained to prevent a catastrophic loss and ensure the quality and integrity of the data.

34. Financing

The study is funded to Xiaoke Wu/Lihui Hou by

1. National Public Welfare Projects for Chinese Medicine (201107005) and National Trial Base for Chinese Medicine in Gynecology from State Administration of Traditional Chinese Medicine of People's Republic of China.
2. Key discipline construction fund on Gynecology of Traditional Chinese Medicine from Ministry of Education of People's Republic of China.
3. Key discipline construction fund on Gynecology of Traditional Chinese Medicine from State Administration of Traditional Chinese Medicine of People's Republic of China.

35. Publication policy

35.1 Major Publications Authorship Order:

Table 8. Authorship Order Category

1	Xiaoke Wu
2	Lihui Hou
3	Hongying Kuang
4	Yan Li
5, n+1, etc.	21 site investigators

n-5	Taixiang Wu
n-4	Jianping Liu
n-3	Ernest HY Ng
n-2	Elisabet Stener-Victorin,
n-1	Richard S. Legro
n	Heping Zhang

It is anticipated there will be up to 30-40 authors per major manuscript. The authorship order for the participating sites will be based upon subject recruitment, data accuracy and promptness of data report and will start at position 7 and go to position 28. Data accuracy will be ranked according to the rate of missing or false data entries/randomized subject at each site. Inquires that show data was accurately entered will not count against this rate of data inaccuracy. Each site's PI will be responsible to document the contributions to the study of that site's authors. We encourage the site investigators to establish the second hypotheses and have publications by sharing these data under the supervision of publication committee.

35.2 Acknowledgement Section:

The acknowledgement section will include other investigators and study personnel who contributed substantially to the study by site, as well as 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 preparation of the manuscript.

36. Protection of Human Subjects Section

36.1 Risk to subjects

The major risks to the subject are side effects from clomiphene and acupuncture and the risks of pregnancy. Clomiphene is improved as an ovulation induction method by the U.S. FDA and is a first line drug for this indication. The major risk of clomiphene is multiple pregnancies. Other major risks which are extremely rare are pituitary apoplexy and deep venous thrombosis. Common side effects include hot flashes, mood changes, bloating, formation of ovarian cysts, and pain. The major risks of acupuncture are local skin irritation, discomfort, and vasovagal reactions during the procedure.

36.2 Adequacy of protection against risks

We will be excluding subjects with a history of major medical morbidity including a history of deep venous thrombosis. Subjects will be seen on a weekly basis and we will collect at this time any unusual side effects. We will be using both interventions according to their tradition use, in the case of acupuncture this has been thousands of years. We will use single use acupuncture needles to reduce any local skin reactions. In regards to the pregnancy complications, all subjects will undergo preconception counseling and we will seek to identify any potential risk factors for a high risk pregnancy. Clomiphene is associated with a 5-7% multiple pregnancy rate. Clomiphene has no known fetal teratogenicity. We will be obtaining regular pregnancy tests both urine and serum and will stop both Clomiphene and acupuncture treatments as soon as we have a

positive pregnancy test. We will follow subjects and treat for any early pregnancy losses including the treatment of ectopic pregnancy.

We will be collecting systematically adverse events and SAEs. These will be reviewed on a quarterly basis by the DSMB, and SAEs will be immediately adjudicated (See section 32.1). There is a risk of loss of personal identifying information, but this will be minimized by giving each patient a unique identifier and eliminating personal identifying information from any documents and accessible databases. Additionally we will be protecting patient confidentiality.

36.3 Potential benefits to the subjects and others and importance of the knowledge to be gained

The subjects, all of whom belong to couples with infertility, will benefit from receiving treatment, all of which alone and in combination may offer an increased chance for pregnancy. This study will help guide therapy for future couples with the same issues. Intensive infertility therapies such as superovulation and in vitro fertilization are invasive and expensive and result in excessive rates of multiple pregnancies. Simpler therapies, including holistic treatments such as acupuncture are now widely utilized by infertile couples in the West and East. Establishing the evidence based nature of these practices is an important public health aim.

Appendix 1: Agreement for site investigators

Effect of acupuncture and clomiphene citrate on live birth in anovulatory women with polycystic ovary syndrome: A Randomized controlled trial

We have read the research protocol and related documents and agree to participate in this project.

It is understood and agreed to that the below identified discloser of confidential information may provide certain information that is and must be kept confidential. To ensure the protection of such information and to preserve any confidentiality necessary, it is agreed that:

1. The Confidential Information to be disclosed can be described as and includes: Clinical protocols of the study, also including all study related materials, such as Manuals of Operations.
2. The Recipient shall limit disclosure of Confidential Information within its own organization to its institutional officials, research staff, employees and/or Institutional Review Board (collectively referred to as “affiliates”) for the need to execute the protocol as part of collaboration with the Heilongjiang University of Traditional Chinese Medicine. The recipient and affiliates will not disclose the confidential information obtained from the discloser unless required to do so by law.
3. This Agreement imposes no obligation upon Recipient with respect to any Confidential Information (a) that was in Recipient’s possession before receipt from Discloser; (b) is or becomes a matter of public knowledge through no fault of Recipient; (c) is rightfully received by Recipient from a third party not owing a duty of confidentiality to the Discloser; (d) is disclosed without a duty of confidentiality to a third party by, or with the authorization of, Discloser; or (e) is independently derived by Recipient.
4. The site investigators will receive stipend by 500 RMB for one case. All medical expenses of patients will be covered by the project.
5. This Agreement states the entire agreement between the parties concerning the disclosure of Confidential Information. Any addition or modification to this Agreement must be made in writing and signed by all parties.
6. If any of the provisions of this Agreement are found to be unenforceable, the remainder shall be enforced as fully as possible and the unenforceable provision(s) shall be deemed modified to the limited extent required to permit enforcement of the Agreement as a whole.

WHEREFORE, the parties acknowledge that they have read and understand this Agreement and voluntarily accept the duties and obligations set forth herein.

Recipient of Confidential Information:

Name (Print or Type): _____

Signature: _____

Date: _____

Discloser of Confidential Information:

Name (Print of Type): _____

Signature: _____

Date: _____

Appendix 2: Patient information and consent

Effect of acupuncture and clomiphene citrate on live birth in anovulatory 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 doctor any question which you may have.

Why is this study being done?

Acupuncture is an integral part of traditional Chinese medicine (TCM), which dates back more than 3,000 years. In recent years, the use of acupuncture as an adjuvant treatment to the management of various obstetrical and gynecological conditions has gained increasing popularity worldwide. The underlying mechanisms of the effect of acupuncture in the treatment of gynecological problems may be via modulation of endogenous regulatory systems, including the sympathetic nervous system, the endocrine system and the neuroendocrine system.

Clomiphene citrate (CC) is the first line pharmacological therapy for ovulation induction in women with polycystic ovary syndrome (PCOS), the most common endocrine disorder during reproductive age. CC is associated with significant adverse effects, while acupuncture has been employed as an alternative therapy to induce ovulation in women with PCOS with few side-effects. Importantly, there is no previous study regarding CC combined with acupuncture in treating PCOS. We still don't know whether acupuncture might increase the live birth rate in women with PCOS and whether any treatment protocol involving acupuncture is better or not. Therefore, in the present study, one acupuncture protocol is based on previous studies on animals and women with PCOS and the other acupuncture protocol is chosen as a contrast in order to elucidate the importance of the site of acupuncture points. There is no evidence that either acupuncture protocol being utilized is more effective than the other, which is why we are conducting this research.

Aim of the study

The aim of the present study is to evaluate the efficacy of two different acupuncture protocols with CC or placebo for live birth rate in anovulatory women with PCOS.

You will randomly be allocated to one of four groups:

- A) Acupuncture protocol 1 + CC
- B) Acupuncture protocol 2 + CC
- C) Acupuncture protocol 1 + placebo
- D) Acupuncture protocol 2 + placebo

Who should be in this study?

You will be included in this study if you have the following:

1. Age between 20 and 40 years.

2. Confirmed diagnosis of PCOS according to the modified Rotterdam criteria: Oligo-, amenorrhea (less than 8 cycles per year) and one of the following two criteria; clinical or biochemical hyperandrogenism and/or polycystic ovarian morphology.
3. Not using hormonal medications at least in the past 3 months.
4. Not pregnant within the past 6 weeks.
5. The husband's sperm concentration $\geq 15 \times 10^6$ /ml and total motility $\geq 40\%$, or Total motile sperm count ≥ 9 million.
6. At least one tube is patent and the uterine cavity is normal in a tubal patent test such as hysterosalpingogram, sonohysterography or diagnostic laparoscopy.
7. Promise of weekly blood test/acupuncture, and no business travels during a four-month intervention

You will not be included in this study if you have the following:

1. Received hormonal medication within the past 3 months.
2. Had abortion or delivery within 6 weeks.
3. Unwillingness to give written consent to the study.

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 specific hormones. You will fill in questionnaires regarding health related quality of life and symptoms of anxiety and depression. Also, TORCH (Toxoplasmosis, Rubella, Cytomegalovirus, and Herpes Virus) and HIV screening should be performed before you are recruited and your HIV status should be negative.

On day 3 to day 7 after a spontaneous menstrual bleeding or a withdrawal bleeding following progestin, you will receive a call from the study coordinator regarding the result of the serum pregnancy test taken. Do not start the medication before you hear from the study coordinator. If the test is negative, the study coordinator will tell you to start with the tablet: CC or placebo (once daily for five days). Then, you will start with acupuncture treatment (30 minutes/treatment) twice a week during 16 weeks, up to a total of 32 treatments.

During the entire study period, you are asked to have regular intercourse, every 2-3 day. Intercourse frequency should be reported each month.

Once a week, blood samples will be drawn for analyses of progesterone which gives information if you have ovulated and hCG which indicates if you are pregnant or not. If you get a bleeding, this should be registered in a diary. The tablets will be increased to twice and three times daily for five days if you do not have evidence of ovulation.

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

There is no need to use drugs for withdrawal bleeding if you do not have any period after taking the tablets and acupuncture treatment. And there is no need to use any further treatment, luteal phase support or herbal medication when you get pregnant.

How long will I be in the study?

The treatments will last for 16 weeks/4 month cycles. You will be in the study for about 20 weeks including screening and waiting for withdraw bleeding.

How often will I be expected to attend the clinic?

During the entire study period, you will be expected to attend the clinic at most 35 times, including: 1 screening visit, 1 baseline visit, 32 acupuncture treatments and 1 end of treatment visit. On an average, you will attend clinic about twice a week. The procedures you will be taken during every visit are demonstrated in the table below.

Overview of the study visits

Visit #	Screening Visit	Baseline Visit	Week 1		Week 2		Week N+ 1		Final Treatment Visit Week 16-20		End of Treatment Visit
	1	2	3	4	5	6	N	N+1	33	34	35
Sign Consent	x										
History	x										x
Urine pregnancy test	x		x	x	x	x	x	x	x	x	
Physical Exam	x										x
Transvaginal ultrasound	x										x
Semen Analysis	x										
Hysterosalpingogram or sonohysterogram (SHG)	x										
Safety Eligibility Lads	x										x
Fasting Phlebotomy For Study Parameters		x									x
QOL Measure	x										x
Progesterone assay	x			x		x		x		x	x
HCG assay	x			x		x		x		x	x
Acupuncture Visits (twice weekly)			x	x	x	x	x	x	x	x	
Assess adverse events and concomitant meds				x		x		x		X	x

How many women will participate in the study?

We plan to recruit 1000 subjects.

For what will I need to pay?

We supply study drugs and acupuncture treatments freely. You are expected to have standard tests results before you can be recruited in the study. Those standard tests are uniform across our study sites and will be presented and explained to you before you agree to participate in this study. You will not be charged for any lab tests related to this study after you sign the consent form. You will receive CC/placebo and acupuncture treatment free of charge. We will refund the weekly progesterone test and hCG test (17 times in total respectively) after you give us the test reports. Similarly and hopefully, if a pregnancy results from your treatment, you will be responsible for paying for the pregnancy and delivery.

What risks/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.

Common side effects of CC are: hot flashes, vomiting, mood changes, constipation, headache, rash, abdominal bloating, pain around time of ovulation. Rarely, visual disturbance may be reported. You should report any visual disturbances because that is a reason to discontinue the study.

What benefits can I expect?

For your infertility during 4 month intervention, you will intensively get measurements of hormones, free treatments and physician care with the aim to induce ovulation and improve your chances (15% on average) of pregnancy/baby. Hormone analyses and questionnaires will give you detailed information about your health status. Such intensive attention to patients is common in clinical trials, but is not possible to provide to routine outpatients in clinics, because this welfare project is being funded by the central government.

Protection of Human Subjects Section

1) Risk to subjects

The major risks to the subject are side effects from CC and acupuncture and the risks of pregnancy. CC is approved as an ovulation induction method by the Food and Drug Administration in the United States and is the first line drug for the treatment of PCOS. The major risk of CC is multiple pregnancies (8-10%). Common side effects include hot flashes, mood changes, bloating, formation of ovarian cysts and abdominal pain. Other major risks which are extremely rare are pituitary apoplexy, deep venous thrombosis and visual disturbances. Individuals with visual disturbances should discontinue use of CC. The major risks of acupuncture are local skin irritation, discomfort, and vasovagal reactions during the procedure.

2) Adequacy of protection against risks

We will be excluding subjects with a history of major medical morbidity including a history of deep venous thrombosis. You will be seen on a weekly basis and we will record at this time any unusual side effects. Both interventions will be applied according to their traditional use; in the case of acupuncture this has been used for several thousand years. The acupuncture needles will be used only once to reduce the risk of cross infection. With regard to the pregnancy complications, you will undergo preconception counseling and we will seek to identify any potential risk factors for a high risk pregnancy. CC is associated with an 8-10% multiple pregnancies rate and has no known fetal teratogenicity. We will be obtaining regular pregnancy tests in both urine and serum and will stop both CC and acupuncture treatments as soon as you have a positive pregnancy test. We will follow up with you and treat any early pregnancy complications including ectopic pregnancy.

We will be recording any adverse events, including serious ones. These will be reviewed on a quarterly basis by the Data Safety Monitor Board, and any serious adverse events will be immediately reviewed. Although there is always the possibility of private information being seen by others, we will take security measures to protect your data and confidentiality as outlined below.

3) Potential benefits to the subjects and others and importance of the knowledge to be gained

You are suffering from infertility and will benefit from receiving treatment, all of which alone and in combination may offer an increased chance for live birth. This study will help guide therapy for future couples with the same issues. Intensive infertility therapies such as ovarian stimulation and in vitro fertilization are invasive and expensive and result in excessive rates of multiple pregnancies. Simpler therapies, including holistic treatments such as acupuncture are now widely utilized by infertile couples in the West and East. Establishing the evidence based nature of these practices is an important public health aim.

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 always maintain 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 understand and agree to all of the above-mentioned conditions and rights. For any query, you should consult the Privacy Commissioner for Privacy Data or his office (0451-82118464) 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 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.

Your husband is required to have normal semen analysis and both of you are requested to have regular intercourses. Therefore, your husband is also requested to sign this consent form.

Subject's signature

Subject's name

Date

Subject husband's signature

Subject husband's name

Date

Site Investigator's signature

Site Investigator's name

Date

Appendix 3: Risk factors for genetic disorders (Based on Chinese textbook of Obstetrics and Gynecology, Second Edition)

Questions	Yes	No	Unknown	Information
1. If you conceive during this study, will you be 35 or older when your baby is born?				
2. Do you, or your child's father or anyone in your family have any of the following abnormalities? Down syndrome Other genetic abnormalities Neural tube defect Anencephaly Hemophilia Muscular dystrophy Cystic fibrosis If yes, please write the relationship between the affected individuals and you or the child's father:				
3. Do you or your child's father have birth defect? If yes, please give details on who has the defect and what the defect is.				
4. In a previous marriage, do you or your child's father have a stillborn situation or diseases, in addition to the birth defects mentioned in Question 2?				
5. Does a close relative in your or your child's father's family have mental retardation? If yes, please write the relationship between the affected individuals and you or the child's father: If you know , write the reasons:				
6. Do you or your child's father or a close relative of your family have birth defects, or any familial abnormalities or chromosomal abnormalities not listed above? If yes, please write the relationship between the affected individuals and you or your child's father:				
7. In a previous marriage, do you or your child's father have a stillbirth or spontaneous abortion during 3 to 4 months of pregnancy? Do you have a chromosome analysis?				
8. After the pregnancy or last menstrual period , in addition to trace elements and vitamins , do you take other drugs or stimulants (including non- prescription drugs) ? If yes, please write the drug name and medication time during pregnancy:				

Appendix 4: SF-36 Health Survey

INSTRUCTIONS: This set of questions asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities. Please answer every question. Some questions may look like others, but each one is different. Please take the time to read and answer each question carefully by filling in the bubble that best represents your response.

1. In general, would you say your health is:

- Excellent
- Very good
- Good
- Fair
- Poor

2. Compared to one year ago, how would you rate your health in general now?

- Much better now than a year ago
- Somewhat better now than a year ago
- About the same as one year ago
- Somewhat worse now than one year ago
- Much worse now than one year ago

3. The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much? (please circle one number on each line)

	Activities	Yes, limited a lot	Yes, limited a little	No, not limited at all
a	Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports.	1	2	3
b	Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf?	1	2	3
c	Lifting or carrying groceries	1	2	3
d	Climbing several flights of stairs	1	2	3
e	Climbing one flight of stairs	1	2	3
f	Bending, kneeling or stooping	1	2	3
g	Walking more than one mile	1	2	3
h	Walking several blocks	1	2	3
i	Walking one block	1	2	3
j	Bathing or dressing yourself	1	2	3

4. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

	(please circle one number on each line)	Yes	No
a	Cut down the amount of time you spent on work or other activities?	1	2
b	Accomplished less than you would like?	1	2
c	Were limited in the kind of work or other activities	1	2
d	Had difficulty performing the work or other activities (for example, it took extra time)	1	2

5. During the past 4 weeks, have you had any of the following problems with your work or other

regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?			
	(please circle one number on each line)	Yes	No
a	Cut down the amount of time you spent on work or other activities?	1	2
b	Accomplished less than you would like	1	2
c	Didn't do work or other activities as carefully as usual	1	2

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

- Not at all
- Slightly
- Moderately
- Quite a bit
- Extremely

7. How much bodily pain have you had during the past 4 weeks?

- Not at all
- Slightly
- Moderately
- Quite a bit
- Extremely

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

- Not at all
- Slightly
- Moderately
- Quite a bit
- Extremely

9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks.

	(please circle one number on each line)	All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
a	Did you feel full of pep?	1	2	3	4	5	6
b	Have you been a very nervous person?	1	2	3	4	5	6
c	Have you felt so down in the dumps nothing could cheer you up?	1	2	3	4	5	6
d	Have you felt calm and peaceful?	1	2	3	4	5	6
e	Did you have a lot of energy?	1	2	3	4	5	6
f	Have you felt downhearted and blue?	1	2	3	4	5	6
g	Did you feel worn out?	1	2	3	4	5	6
h	Have you been a happy person?	1	2	3	4	5	6
i	Did you feel tired?	1	2	3	4	5	6

10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?

- All of the time
- Most of the time
- Some of the time
- A little of the time
- None of the time

11. How TRUE or FALSE is each of the following statements for you?

	(please circle one number on each line)	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
a	I seem to get sick a little easier than other people	1	2	3	4	5
b	I am as healthy as anybody I know	1	2	3	4	5
c	I expect my health to get worse	1	2	3	4	5
d	My health is excellent	1	2	3	4	5

Appendix 5: Questionnaire for Chinese Quality of Life (ChiQOL)

Leung KF, Liu FB, Zhao L, et al. Health Qual Life Outcomes. 2005; 3:26

<p>1. What do you think about the color of your face?</p> <p><input type="checkbox"/> 1. Very bad <input type="checkbox"/> 4. Good</p> <p><input type="checkbox"/> 2. Bad <input type="checkbox"/> 5. Very good</p> <p><input type="checkbox"/> 3. Neither good nor bad</p>
<p>2. Do you think the colour of your face bright and shiny?</p> <p><input type="checkbox"/> 1. Not at all <input type="checkbox"/> 4. Quite a bit</p> <p><input type="checkbox"/> 2. A little bit <input type="checkbox"/> 5. Extremely</p> <p><input type="checkbox"/> 3. Moderately</p>
<p>3. What do you think about the colour of your lips?</p> <p><input type="checkbox"/> 1. Very bad <input type="checkbox"/> 4. Good</p> <p><input type="checkbox"/> 2. Bad <input type="checkbox"/> 5. Very good</p> <p><input type="checkbox"/> 3. Neither good nor bad</p>
<p>4. Do you think your lips are moist and shiny?</p> <p><input type="checkbox"/> 1. Not at all <input type="checkbox"/> 4. Quite a bit</p> <p><input type="checkbox"/> 2. A little bit <input type="checkbox"/> 5. Extremely</p> <p><input type="checkbox"/> 3. Moderately</p>
<p>5. Do you often have insomnia?</p> <p><input type="checkbox"/> 1. None of the time <input type="checkbox"/> 4. Most of the time</p> <p><input type="checkbox"/> 2. A little of the time <input type="checkbox"/> 5. All of the time</p> <p><input type="checkbox"/> 3. Some of the time</p>
<p>6. Do dreams affect the quality of you sleep?</p> <p><input type="checkbox"/> 1. Not at all <input type="checkbox"/> 4. Quite a bit</p> <p><input type="checkbox"/> 2. A little bit <input type="checkbox"/> 5. Extremely</p> <p><input type="checkbox"/> 3. Moderately</p>
<p>7. How is your sleep?</p> <p><input type="checkbox"/> 1. Very bad <input type="checkbox"/> 4. Good</p> <p><input type="checkbox"/> 2. Bad <input type="checkbox"/> 5. Very good</p> <p><input type="checkbox"/> 3. Neither good nor bad</p>
<p>8. Do you walk lightly?</p> <p><input type="checkbox"/> 1. Not at all <input type="checkbox"/> 4. Quite a bit</p> <p><input type="checkbox"/> 2. A little bit <input type="checkbox"/> 5. Extremely</p> <p><input type="checkbox"/> 3. Moderately</p>
<p>9. Do you tire easily?</p> <p><input type="checkbox"/> 1. Not at all <input type="checkbox"/> 4. Quite a bit</p> <p><input type="checkbox"/> 2. A little bit <input type="checkbox"/> 5. Extremely</p> <p><input type="checkbox"/> 3. Moderately</p>
<p>10. Are your body and limbs often lack strength?</p> <p><input type="checkbox"/> 1. None of the time <input type="checkbox"/> 4. Most of the time</p> <p><input type="checkbox"/> 2. A little of the time <input type="checkbox"/> 5. All of the time</p> <p><input type="checkbox"/> 3. Some of the time</p>
<p>11. Do you often feel full of energy?</p> <p><input type="checkbox"/> 1. None of the time <input type="checkbox"/> 4. Most of the time</p> <p><input type="checkbox"/> 2. A little of the time <input type="checkbox"/> 5. All of the time</p> <p><input type="checkbox"/> 3. Some of the time</p>

<p>12. Do you often feel not able to do what you want?</p> <p><input type="checkbox"/> 1. None of the time <input type="checkbox"/> 4. Most of the time</p> <p><input type="checkbox"/> 2. A little of the time <input type="checkbox"/> 5. All of the time</p> <p><input type="checkbox"/> 3. Some of the time</p>
<p>13. Do you pant easily?</p> <p><input type="checkbox"/> 1. Not at all <input type="checkbox"/> 4. Quite a bit</p> <p><input type="checkbox"/> 2. A little bit <input type="checkbox"/> 5. Extremely</p> <p><input type="checkbox"/> 3. Moderately</p>
<p>14. Do you often feel your food tasty?</p> <p><input type="checkbox"/> 1. None of the time <input type="checkbox"/> 4. Most of the time</p> <p><input type="checkbox"/> 2. A little of the time <input type="checkbox"/> 5. All of the time</p> <p><input type="checkbox"/> 3. Some of the time</p>
<p>15. Do you feel indigestion?</p> <p><input type="checkbox"/> 1. None of the time <input type="checkbox"/> 4. Most of the time</p> <p><input type="checkbox"/> 2. A little of the time <input type="checkbox"/> 5. All of the time</p> <p><input type="checkbox"/> 3. Some of the time</p>
<p>16. Is the quantity of your diet normal?</p> <p><input type="checkbox"/> 1. Not at all <input type="checkbox"/> 4. Quite a bit</p> <p><input type="checkbox"/> 2. A little bit <input type="checkbox"/> 5. Extremely</p> <p><input type="checkbox"/> 3. Moderately</p>
<p>17. How is your appetite?</p> <p><input type="checkbox"/> 1. Very bad <input type="checkbox"/> 4. Good</p> <p><input type="checkbox"/> 2. Bad <input type="checkbox"/> 5. Very good</p> <p><input type="checkbox"/> 3. Neither good nor bad</p>
<p>18. Are you able to adapt to changes in seasons and weather?</p> <p><input type="checkbox"/> 1. Not at all <input type="checkbox"/> 4. Quite a bit</p> <p><input type="checkbox"/> 2. A little bit <input type="checkbox"/> 5. Extremely</p> <p><input type="checkbox"/> 3. Moderately</p>
<p>19. Do the changes in seasons and weather affect your body?</p> <p><input type="checkbox"/> 1. Not at all <input type="checkbox"/> 4. Quite a bit</p> <p><input type="checkbox"/> 2. A little bit <input type="checkbox"/> 5. Extremely</p> <p><input type="checkbox"/> 3. Moderately</p>
<p>20. Do the changes of time in a day cause any adverse effect in your body?</p> <p><input type="checkbox"/> 1. None of the time <input type="checkbox"/> 4. Most of the time</p> <p><input type="checkbox"/> 2. A little of the time <input type="checkbox"/> 5. All of the time</p> <p><input type="checkbox"/> 3. Some of the time</p>
<p>21. Do you have a clear mind?</p> <p><input type="checkbox"/> 1. Not at all <input type="checkbox"/> 4. Quite a bit</p> <p><input type="checkbox"/> 2. A little <input type="checkbox"/> 5. Extremely</p> <p><input type="checkbox"/> 3. Moderately</p>
<p>22. Are you able to react to the external world appropriately?</p> <p><input type="checkbox"/> 1. Not at all <input type="checkbox"/> 4. Quite a bit</p> <p><input type="checkbox"/> 2. A little bit <input type="checkbox"/> 5. Extremely</p> <p><input type="checkbox"/> 3. Moderately</p>
<p>23. Are you able to pay attention?</p> <p><input type="checkbox"/> 1. Not at all <input type="checkbox"/> 4. Quite a bit</p> <p><input type="checkbox"/> 2. A little bit <input type="checkbox"/> 5. Extremely</p>

<input type="checkbox"/> 3. Moderately
24. Is your memory good? <input type="checkbox"/> 1. Very bad <input type="checkbox"/> 4. Good <input type="checkbox"/> 2. Bad <input type="checkbox"/> 5. Very good <input type="checkbox"/> 3. Neither good nor bad
25. Are your responses quick ? <input type="checkbox"/> 1. Not at all <input type="checkbox"/> 4. Quite a bit <input type="checkbox"/> 2. A little bit <input type="checkbox"/> 5. Extremely <input type="checkbox"/> 3. Moderately
26. Is your thinking clear when considering a problem? <input type="checkbox"/> 1. Not at all <input type="checkbox"/> 4. Quite a bit <input type="checkbox"/> 2. A little bit <input type="checkbox"/> 5. Extremely <input type="checkbox"/> 3. Moderately
27. Are you able to concentrate on your thinking? <input type="checkbox"/> 1. Not at all <input type="checkbox"/> 4. Quite a bit <input type="checkbox"/> 2. A little bit <input type="checkbox"/> 5. Extremely <input type="checkbox"/> 3. Moderately
28. Is your thought quick ? <input type="checkbox"/> 1. Not at all <input type="checkbox"/> 4. Quite a bit <input type="checkbox"/> 2. A little bit <input type="checkbox"/> 5. Extremely <input type="checkbox"/> 3. Moderately
29. Do you have spirit in your eyes? <input type="checkbox"/> 1. None of the time <input type="checkbox"/> 4. Most of the time <input type="checkbox"/> 2. A little of the time <input type="checkbox"/> 5. All of the time <input type="checkbox"/> 3. Some of the time
30. Do your eyes move lively ? <input type="checkbox"/> 1. Not at all <input type="checkbox"/> 4. Quite a bit <input type="checkbox"/> 2. A little bit <input type="checkbox"/> 5. Extremely <input type="checkbox"/> 3. Moderately
31. Do you speak clearly ? <input type="checkbox"/> 1. Not at all <input type="checkbox"/> 4. Quite a bit <input type="checkbox"/> 2. A little bit <input type="checkbox"/> 5. Extremely <input type="checkbox"/> 3. Moderately
32. Are you able to express your thoughts through your speech? <input type="checkbox"/> 1. Not at all <input type="checkbox"/> 4. Quite a bit <input type="checkbox"/> 2. A little bit <input type="checkbox"/> 5. Extremely <input type="checkbox"/> 3. Moderately
33. Are you happy ? <input type="checkbox"/> 1. Not at all <input type="checkbox"/> 4. Quite a bit <input type="checkbox"/> 2. A little bit <input type="checkbox"/> 5. Extremely <input type="checkbox"/> 3. Moderately
34. Are you living happily ? <input type="checkbox"/> 1. Not at all <input type="checkbox"/> 4. Quite a bit <input type="checkbox"/> 2. A little bit <input type="checkbox"/> 5. Extremely <input type="checkbox"/> 3. Moderately
35. Do you feel peace in mind? <input type="checkbox"/> 1. Not at all <input type="checkbox"/> 4. Quite a bit

<input type="checkbox"/> 2. A little bit <input type="checkbox"/> 3. Moderately	<input type="checkbox"/> 5. Extremely
36. Are you interested in your living? <input type="checkbox"/> 1. Not at all <input type="checkbox"/> 2. A little bit <input type="checkbox"/> 3. Moderately	
<input type="checkbox"/> 4. Quite a bit <input type="checkbox"/> 5. Extremely	
37. Do you become annoyed easily? <input type="checkbox"/> 1. Not at all <input type="checkbox"/> 2. A little bit <input type="checkbox"/> 3. Moderately	
<input type="checkbox"/> 4. Quite a bit <input type="checkbox"/> 5. Extremely	
38. Do you become angry easily? <input type="checkbox"/> 1. Not at all <input type="checkbox"/> 2. A little bit <input type="checkbox"/> 3. Moderately	
<input type="checkbox"/> 4. Quite a bit <input type="checkbox"/> 5. Extremely	
39. Do you become agitated easily? <input type="checkbox"/> 1. Not at all <input type="checkbox"/> 2. A little bit <input type="checkbox"/> 3. Moderately	
<input type="checkbox"/> 4. Quite a bit <input type="checkbox"/> 5. Extremely	
40. Do you often become mad? <input type="checkbox"/> 1. None of the time <input type="checkbox"/> 2. A little of the time <input type="checkbox"/> 3. Some of the time	
<input type="checkbox"/> 4. Most of the time <input type="checkbox"/> 5. All of the time	
41. Are you able to control your emotion? <input type="checkbox"/> 1. Not at all <input type="checkbox"/> 2. A little bit <input type="checkbox"/> 3. Moderately	
<input type="checkbox"/> 4. Quite a bit <input type="checkbox"/> 5. Extremely	
42. Are you worrying about almost anything? <input type="checkbox"/> 1. Not at all <input type="checkbox"/> 2. A little bit <input type="checkbox"/> 3. Moderately	
<input type="checkbox"/> 4. Quite a bit <input type="checkbox"/> 5. Extremely	
43. Do you often feel sad? <input type="checkbox"/> 1. None of the time <input type="checkbox"/> 2. A little of the time <input type="checkbox"/> 3. Some of the time	
<input type="checkbox"/> 4. Most of the time <input type="checkbox"/> 5. All of the time	
44. Do you often feel hopeless and helpless? <input type="checkbox"/> 1. None of the time <input type="checkbox"/> 2. A little of the time <input type="checkbox"/> 3. Some of the time	
<input type="checkbox"/> 4. Most of the time <input type="checkbox"/> 5. All of the time	
45. Are you often unhappy? <input type="checkbox"/> 1. None of the time <input type="checkbox"/> 2. A little of the time <input type="checkbox"/> 3. Some of the time	
<input type="checkbox"/> 4. Most of the time <input type="checkbox"/> 5. All of the time	
46. Do you often want to cry? <input type="checkbox"/> 1. None of the time <input type="checkbox"/> 2. A little of the time <input type="checkbox"/> 3. Some of the time	
<input type="checkbox"/> 4. Most of the time <input type="checkbox"/> 5. All of the time	

<p>47. Are you often sad?</p> <p><input type="checkbox"/> 1. None of the time <input type="checkbox"/> 4. Most of the time</p> <p><input type="checkbox"/> 2. A little of the time <input type="checkbox"/> 5. All of the time</p> <p><input type="checkbox"/> 3. Some of the time</p>
<p>48. Do you often feel fear without cause?</p> <p><input type="checkbox"/> 1. None of the time <input type="checkbox"/> 4. Most of the time</p> <p><input type="checkbox"/> 2. A little of the time <input type="checkbox"/> 5. All of the time</p> <p><input type="checkbox"/> 3. Some of the time</p>
<p>49. Do you often feel unsafe?</p> <p><input type="checkbox"/> 1. None of the time <input type="checkbox"/> 4. Most of the time</p> <p><input type="checkbox"/> 2. A little of the time <input type="checkbox"/> 5. All of the time</p> <p><input type="checkbox"/> 3. Some of the time</p>
<p>50. Are you being scared easily?</p> <p><input type="checkbox"/> 1. Not at all <input type="checkbox"/> 4. Quite a bit</p> <p><input type="checkbox"/> 2. A little bit <input type="checkbox"/> 5. Extremely</p> <p><input type="checkbox"/> 3. Moderately</p>

Appendix 6: Questionnaire for PCOS Quality of Life (PCOSQOL)

Polycystic Ovary Syndrome Questionnaire Given by ENDOCRINE society journals – JCEM.

This questionnaire is designed for patients with Polycystic Ovary Syndrome. In this questionnaire, we will refer to Polycystic Ovary Syndrome by its initials: PCOS. The questions concern your health and health-related issues. Please respond to each question by checking (✓) the number with the rating that best reflects how you feel. For each question you have seven rating options. Option 1 represents the greatest possible impairment and the option 7 represents the least. You must choose only one option for each question. There is no right or wrong answers. Please be sure to answer every question by checking the most appropriate box. Just choose the option that is the closest to how you feel.

To what extent have you felt that growth of visible hair on your chin has been a problem for you during the last two weeks?

1. Growth of visible hair on your chin?
- ₁ A severe problem
₂ A major problem
₃ A moderate problem
₄ Some problem
₅ A little problem
₆ Hardly any problem
₇ No problem

During the past two weeks, how much of the time have you felt?

2. Depressed as a result of having PCOS?
- ₁ All of the time
₂ Most of the time
₃ A good bit of the time
₄ Some of the time
₅ A little of the time
₆ Hardly any of time
₇ None of the time
3. Concerned about being overweight?
- ₁ All of the time
₂ Most of the time
₃ A good bit of the time
₄ Some of the time
₅ A little of the time
₆ Hardly any of time
₇ None of the time
4. Easily tired?
- ₁ All of the time
₂ Most of the time
₃ A good bit of the time
₄ Some of the time
₅ A little of the time
₆ Hardly any of time
₇ None of the time
5. Concerned with infertility problems?
- ₁ All of the time
₂ Most of the time
₃ A good bit of the time

- ₄ Some of the time
- ₅ A little of the time
- ₆ Hardly any of time
- ₇ None of the time

6. Moody as a result of having PCOS?

- ₁ All of the time
- ₂ Most of the time
- ₃ A good bit of the time
- ₄ Some of the time
- ₅ A little of the time
- ₆ Hardly any of time
- ₇ None of the time

In relation to your last menstruation, how much were the following issues a problem for you?

7. Headaches?

- ₁ A severe problem
- ₂ A major problem
- ₃ A moderate problem
- ₄ Some problem
- ₅ A little problem
- ₆ Hardly any problem
- ₇ No problem

8. Irregular menstrual periods?

- ₁ A severe problem
- ₂ A major problem
- ₃ A moderate problem
- ₄ Some problem
- ₅ A little problem
- ₆ Hardly any problem
- ₇ No problem

To what extent has growth of visible hair on your upper lip been a problem for you during the last two weeks?

9. Growth of visible hair on upper lip?

- ₁ A severe problem
- ₂ A major problem
- ₃ A moderate problem
- ₄ Some problem
- ₅ A little problem
- ₆ Hardly any problem
- ₇ No problem

During the past two weeks, how much of the time did you?

10. Have trouble dealing with your weight?

- ₁ All of the time
- ₂ Most of the time
- ₃ A good bit of the time
- ₄ Some of the time
- ₅ A little of the time
- ₆ Hardly any of time
- ₇ None of the time

11. Have a low self-esteem as a result of having PCOS?
- ₁ All of the time
₂ Most of the time
₃ A good bit of the time
₄ Some of the time
₅ A little of the time
₆ Hardly any of time
₇ None of the time
12. Feel frustration in trying to lose weight?
- ₁ All of the time
₂ Most of the time
₃ A good bit of the time
₄ Some of the time
₅ A little of the time
₆ Hardly any of time
₇ None of the time
13. Feel afraid of not being able to have children?
- ₁ All of the time
₂ Most of the time
₃ A good bit of the time
₄ Some of the time
₅ A little of the time
₆ Hardly any of time
₇ None of the time
14. Feel frightened of getting cancer?
- ₁ All of the time
₂ Most of the time
₃ A good bit of the time
₄ Some of the time
₅ A little of the time
₆ Hardly any of time
₇ None of the time

Over the last two weeks, to what extent have the following issues have been a problem for you?

15. Growth of visible hair on your face?
- ₁ A severe problem
₂ A major problem
₃ A moderate problem
₄ Some problem
₅ A little problem
₆ Hardly any problem
₇ No problem
16. Embarrassment about excessive body hair?
- ₁ A severe problem
₂ A major problem
₃ A moderate problem
₄ Some problem
₅ A little problem
₆ Hardly any problem
₇ No problem

During the past two weeks how much of the time have you been?

17. Worried about having PCOS?

- ₁ All of the time
₂ Most of the time
₃ A good bit of the time
₄ Some of the time
₅ A little of the time
₆ Hardly any of time
₇ None of the time

18. Self-conscious as a result of having PCOS?

- ₁ All of the time
₂ Most of the time
₃ A good bit of the time
₄ Some of the time
₅ A little of the time
₆ Hardly any of time
₇ None of the time

In relation to your last menstruation, how much are the following issues were a problem for you?

19. Abdominal bloating?

- ₁ A severe problem
₂ A major problem
₃ A moderate problem
₄ Some problem
₅ A little problem
₆ Hardly any problem
₇ No problem

20. Last menstrual period?

- ₁ A severe problem
₂ A major problem
₃ A moderate problem
₄ Some problem
₅ A little problem
₆ Hardly any problem
₇ No problem

21. Menstrual cramps?

- ₁ A severe problem
₂ A major problem
₃ A moderate problem
₄ Some problem
₅ A little problem
₆ Hardly any problem
₇ No problem

How much of the time during the last two weeks did you?

22. Feel like you are not sexy because of being overweight?

- ₁ All of the time
₂ Most of the time
₃ A good bit of the time
₄ Some of the time
₅ A little of the time
₆ Hardly any of time

- ₇ None of the time
23. Feel a lack of control over the situation with PCOS?
- ₁ All of the time
₂ Most of the time
₃ A good bit of the time
₄ Some of the time
₅ A little of the time
₆ Hardly any of time
₇ None of the time
24. Have difficulties staying at your ideal weight?
- ₁ All of the time
₂ Most of the time
₃ A good bit of the time
₄ Some of the time
₅ A little of the time
₆ Hardly any of time
₇ None of the time
25. Feel sad because of infertility problems?
- ₁ All of the time
₂ Most of the time
₃ A good bit of the time
₄ Some of the time
₅ A little of the time
₆ Hardly any of time
₇ None of the time

To what extent has growth of visible body hair been a problem for you during the last two weeks?

26. Growth of visible body hair?
- ₁ A severe problem
₂ A major problem
₃ A moderate problem
₄ Some problem
₅ A little problem
₆ Hardly any problem
₇ No problem

For the following questions please (✓) the number between 1 and 7 that best applies to you:

27. How would you rate your overall physical well-being over the past month?
- ₁ The worst I've ever been
₂
₃
₄
₅
₆
₇ The best I've ever been
28. How would you rate your overall emotional well-being over the past month?
- ₁ The worst I've ever been
₂
₃
₄
₅

₆
₇ The best I've ever been

29. How would you rate your overall general well-being over the past month? ₁ The worst I've ever been

₂

₃

₄

₅

₆

₇ The best I've ever been

All of the above questions have been answered to the best of my knowledge.

Signature: _____

Date: ____/____/____

Appendix 7: Questionnaire for Anxiety/Depression Symptoms

Zung Self-Rating Anxiety Scale (Zung SAS)

For each item below, please place a check mark () in the column which best describes how often you felt or behaved this way during the past several days. Bring the completed form with you to the office for scoring and assessment during your office visit.

Source: William W.K. Zung. A rating instrument for anxiety disorders. Psychosomatics. 1971

Place check mark () in correct column.	A little of the time	Some of the time	Good part of the time	Most of the time
1. I feel more nervous and anxious than usual.	1	2	3	4
2. I feel afraid for no reason at all.	1	2	3	4
3. I get upset easily or feel panicky.	1	2	3	4
4. I feel like I'm falling apart and going to pieces.	1	2	3	4
5. I feel that everything is all right and nothing bad will happen.	4	3	2	1
6. My arms and legs shake and tremble.	1	2	3	4
7. I am bothered by headaches neck and back pain.	1	2	3	4
8. I feel weak and get tired easily.	1	2	3	4
9. I feel calm and can sit still easily.	4	3	2	1
10. I can feel my heart beating fast.	1	2	3	4
11. I am bothered by dizzy spells.	1	2	3	4
12. I have fainting spells or feel like it.	1	2	3	4
13. I can breathe in and out easily.	4	3	2	1
14. I get feelings of numbness and tingling in my fingers & toes.	1	2	3	4
15. I am bothered by stomach aches or indigestion.	1	2	3	4
16. I have to empty my bladder often.	1	2	3	4
17. My hands are usually dry and warm.	4	3	2	1
18. My face gets hot and blushes.	1	2	3	4
19. I fall asleep easily and get a good night's rest.	4	3	2	1
20. I have nightmares.	1	2	3	4

Zung Self-Rating Depression Scale (Zung SDS)

For each item below, please place a check mark (ü) in the column which best describes how often you felt or behaved this way during the past several days

Source: Zung WW. (1965). A self-rating depression scale. Archives of General Psychiatry 12: 63-70.

Place check mark (ü) in correct column.	A little of the time	Some of the time	Good part of the time	Most of the time
1. I feel down-hearted and blue.	1	2	3	4
2. Morning is when I feel the best.	1	2	3	4
3. I have crying spells or feel like it.	1	2	3	4
4. I have trouble sleeping at night.	1	2	3	4
5. I eat as much as I used to.	4	3	2	1
6. I still enjoy sex.	1	2	3	4
7. I notice that I am losing weight.	1	2	3	4
8. I have trouble with constipation.	1	2	3	4
9. My heart beats faster than usual.	4	3	2	1
10. I get tired for no reason.	1	2	3	4
11. My mind is as clear as it used to be.	1	2	3	4
12. I find it easy to do the things I used to.	1	2	3	4
13. I am restless and can't keep still.	4	3	2	1
14. I feel hopeful about the future.	1	2	3	4
15. I am more irritable than usual.	1	2	3	4
16. I find it easy to make decisions.	1	2	3	4
17. I feel that I am useful and needed.	4	3	2	1
18. My life is pretty full.	1	2	3	4
19. I feel that others would be better off if I were dead.	4	3	2	1
20. I still enjoy the things I used to do.	1	2	3	4

Appendix 8: List of common medications excluded or requiring wash-out period

PPCOS Excluded Medications

A patient will be excluded from study if they are taking any medication that should not be discontinued and this medication would affect reproductive function or metabolism, or would interact with either study medication.

Hormonal Medications requiring 1 month wash-out:

Progestins (Oral or Cyclic)
 Medroxyprogesterone acetate (Provera, Cycrin, Amen, Curretab)
 megestrol (Megase)
 norethindrone (Aygestin)
 progesterone gel (Crinone)
 Micronized progesterone (Prometrium)

Hormonal Medications requiring 2 month wash-out period:

GnRH Agonists/Antagonists

Leuprolide (Lupron)
 nafarelin (Synarel)
 buserelin
 gosarelin (Zoladex)
 ganarelix (Antagon)
 cetorelix (Cetrotide)

Gonadatropins

Pergonal
 Repronex
 Follistim
 Gonal-F
 Fertinex
 Metrodin

Injectable Contraceptives

medroxyprogesterone acetate (Depo Provera)

Oral Contraceptives

Any Brand

Continuous Progestins (Not including cyclic)

Any Brand

Other medications requiring a 2 month wash-out period

Chinese Herbal medicine

By own habits of local PIs at any routines (orally, intravenously, dermatologically, trans-vaginally, trans-rectally, trans-abdominally etc.).

Somatostatin

octreotide (Sandostatin)
 lanreotide

Anti-Acne

isotretinoin (Accutane)

Anti-androgens

cyproterone (Cyprostat)

spironolactone (Aldactone)

flutamide (Eulexin)

finasteride (Proscar, Propecia)

Anti-diabetic

acarbose (Precose)

Insulin

sulfonylureas

acetahexamide (Dymelor)

chlorpropamide (Diabinese)

tolazamide (Tolinase)

tolbutamide (Orinase)

glimepiride (Amaryl)

glipizide (Glucotrol)

glyburide (DiaBeta Micronase)

thiazolidinediones

rosiglitazone (Avandia)

pioglitazone (Actos)

Biguanides

metformin (Glucophage)

Incretins: GLP-1 Analogues/DPP-IV inhibitors

sitagliptin (Januvia)

vidagliptin (Glavus)

exenatide (Byetta)

Anti-obesity

diazoxide (Proglycem)

orlistat (Xenical)

sibutramine (Meridia)

diethylpropion (Tenuate)

phendimetrazine (Bontril)

phentermine (Adipex-P, Fastin, Ionamin)

Calcium Channel Blockers

diltiazem (Cardizem, Dilacor, Tiazac, Diltia-XL)

verapamil (Isoptin, Calan)

amlodipine (Norvase)

felodipine (Plendil)

isradipine (DynaCirc)

nicardipine (Cardene)

nifedipine (Procardia, Adalat)

nifsoldipine (Sular)

Angiotensin Converting Enzyme (ACE) Inhibitors

Prinivil (lisinopril)

Captoten (captopril)

Cozaar (losartan)

Multiple others

Medications with potential longer washouts (Contact DCC)

Contraceptive Implants

Norplant (levonorgestrel implants)

Implanon

Appendix 9: DNA Collection and Shipping with whole blood.

- 1、 Obtain two 7ml purple tops (containing EDTA) tubes from each patient
- 2、 Store labeled tubes in -20°C refrigerator in less than 2 months until shipment or transfer to -70 °C refrigerator
- 3、 Record on this log:
 - Patient study number
 - Total number of tubes for each patient
 - Date sample was obtained
 - Your initials
- 4、 Complete a new log for each shipment
- 5、 Photocopy log for your records and store in your regulatory binder
- 6、 Enclose log with each shipment
- 7、 Notify Xiaoke Wu or Lihui Hou prior to shipping at (0451)82130094
- 8、 Ship or transfer every 2 months by overnight carrier to:

Wei Li, Gynecology Laboratory
 Department of Obstetrics and Gynecology First Affiliated Hospital
 Heilongjiang University of Chinese Medicine
 Heping Road 26, Xiangfang District
 Harbin 150040, P.R.CHINA
 Mobil: +86-136 3361 2790 (Wei Li)
 Phone: 0451-8210 0901

Patient study #	Date of Sample	# of DNA Tubes	Coordinator's Initials
	___/___/___		
	___/___/___		
	___/___/___		
	___/___/___		
	___/___/___		
	___/___/___		
	___/___/___		
	___/___/___		

Ship Date: ___/___/___
 Name: _____
 Phone # _____

Site: _____
 Page __ of __

Appendix 10: List of side effects of Acupuncture

Do you have any following side effects after the acupuncture session? (Please give a tick at the appropriate place)

	(0) None	(1) Mild	(2) Moderate	(3) Severe
■ Nausea	_____	_____	_____	_____
■ Dizziness	_____	_____	_____	_____
■ Fainting	_____	_____	_____	_____
■ Tiredness	_____	_____	_____	_____
■ Drowsiness	_____	_____	_____	_____
■ Headache	_____	_____	_____	_____
■ Chest pain	_____	_____	_____	_____
■ Itching in the puncture regions	_____	_____	_____	_____

Others: _____

Appendix 11: PCOSAct prescreening questionnaire

Site number:

Site:

Patient Number:

Date:

Investigator:

1. Is the patient eligible for recruitment?	<input type="checkbox"/> Yes <input type="checkbox"/> No
2. Is the patient eligible for recruitment but not interested (record reasons below)?	<input type="checkbox"/> Yes <input type="checkbox"/> No
3. Is the patient eligible for recruitment and recruited?	<input type="checkbox"/> Yes <input type="checkbox"/> No
4. If the patient is recruited, patient No. is:	
Ineligibility Reason	Put "X" after right item
1. Has an exclusionary medical condition	
2. Distance too far/Transportation an issue	
3. Patient not interested	
4. Age	
5. More aggressive treatment	
6. Partner semen concentration not enough	
7. On exclusionary meds or acupuncture	
8. Currently going through infertility treatments (such as IVF-ET)	
9. Other reason not specified:	
10. Proof of pregnancies/miscarriages after initiation cutoff period	
11. Time commitment too much	
12. Did not want random selection	
13. Partner reluctant	
14. Does not want to get pregnant	

Appendix 12: Expectation and credibility of treatment rating scale—attitudes to

acupuncture Questions after the third session of the acupuncture treatment

Below are four questions about acupuncture treatment. We ask you to spontaneously consider each statement and mark your position with a cross (X) to the option that best matches your opinion.

<p>1 How confident do you feel that this treatment will induce ovulation and improve the chance to become pregnant?</p> <p><input type="checkbox"/> 1. Not at all confident</p> <p><input type="checkbox"/> 2. Not very confident</p> <p><input type="checkbox"/> 3. Neither</p> <p><input type="checkbox"/> 4. Quite confident</p> <p><input type="checkbox"/> 5. Very confident</p>
<p>2 How confident would you be in recommending this treatment to a friend suffering from similar complaints?</p> <p><input type="checkbox"/> 1. Not at all confident</p> <p><input type="checkbox"/> 2. Not very confident</p> <p><input type="checkbox"/> 3. Neither</p> <p><input type="checkbox"/> 4. Quite confident</p> <p><input type="checkbox"/> 5. Very confident</p>
<p>3 How logical does this treatment seem to you?</p> <p><input type="checkbox"/> 1. Not at all logical</p> <p><input type="checkbox"/> 2. Not very logical</p> <p><input type="checkbox"/> 3. Neither</p> <p><input type="checkbox"/> 4. Quite logical</p> <p><input type="checkbox"/> 5. Very logical</p>
<p>4 How successful do you think the treatment would be in alleviating other complaints?</p> <p><input type="checkbox"/> 1. Not at all successful</p> <p><input type="checkbox"/> 2. Not very successful</p> <p><input type="checkbox"/> 3. No opinion</p> <p><input type="checkbox"/> 4. Quite successful</p> <p><input type="checkbox"/> 5. Very successful</p>

Vincent C, Lewith G. (1995) Placebo controls for acupuncture studies. *J R Soc Med*; 88:199-202

Appendix 13: Questions after the last session of the acupuncture treatment

Below are two questions about acupuncture treatment. We ask you to spontaneously consider each statement and mark your position with a cross (X) to the option that best matches your opinion.

1 How helpful has the treatment been?

- 1. No help
- 2. Some help
- 3. Neither
- 4. Good help
- 5. Very good help

2 Would you like to receive similar treatment if you would need it in the future?

- 1. Not at all
- 2. Not very
- 3. No opinion
- 4. Quite
- 5. Very

Appendix 14: PCOSAct Protocol Revised History

Protocol revised history -V 7.7 and 7.8

-**Inclusion criteria** P15: Semen parameters use total motile sperm count ≥ 10 million. The 10 million is based on previous publications, although according to WHO up to 9 million.

-**Exclusion criteria** P15: Added one item “Those who do not have follicular development after CC 150mg daily for five days or FSH stimulation.”

-**Screening** P20: Fasting blood glucose will be test for screening.

- **Consent form:**

1. P46: State clear that there is no need to use drugs for withdrawal bleeding if patient do not have any period after taking the tablets and acupuncture treatment. And there is no need to use any further treatment, luteal phase support or herbal medication when patient gets pregnant.
2. P47: State clear that patient need to have required tests results before recruited in the study.
3. P48: State clear that patient will not be charged for any lab tests related to this study after they sign the consent form.

Revisions have been made accordingly in the SOP, MOP and CRF parts.

Protocol revised history-V 7.9

CRF

-Age, Occupation, drinking/smoking habits were added. Patients’ exercise habits were added.

Protocol revised history-V 7.10

1. P6-7. Site changes, the following sites were excluded: First Affiliated Hospital of Harbin Medical University, The women and children hospital of Mudanjiang, First Affiliated Hospital Changchun University of Chinese Medicine, Tanggu District Hospital of Chinese medicine, Wenzhou Zhongshan Hospital, Suqian Maternal and Child Health Hospital, Affiliated Hospital of Shanxi University of Chinese Medicine.

2. P13-4.2 Screening Visit (Visit 1: physician): 5. Take blood for testing at the local lab: serum beta hCG concentration.

Change to “Perform urine pregnancy test.”

3. P15-**Inclusion criteria**: Semen parameters use sperm concentration $\geq 15 \times 10^6$ /ml and total motility $\geq 40\%$.

4. P17-10. Informed Consent change to “Prescreening: Subjects who are interested will first be prescreened and recorded by an intake to verify that they meet basic inclusion criteria (i.e. age, oligomenorrhea, partner availability, etc.). The study will be explained in detail and all questions, as well as the written informed consent to help patients understand. The male partner will also be required to read and understand the written informed consent together with participants at the time of the prescreening. It is important to obtain his consent for participation in the study due to the requirements of intercourse, semen analysis for the inclusion criteria and the information collected.”

5. P20-Table 9. Screening labs of PCOS women (run in local labs): Renal profile change to “BUN or Creatinine”, since we found BUN test is not available in some sites.

6. P28-23.6 Pregnancy Visits: The timing of serum progesterone and hCG tests should be fixed in each patient, either all during the first or the second weekly acupuncture visit.

7. P41-34. Financing change to:

The study is funded to Xiaoke Wu/Lihui Hou by

National Public Welfare Projects for Chinese Medicine (201107005) and National Trial Base for Chinese Medicine in Gynecology from State Administration of Traditional Chinese Medicine of People's Republic of China.

Key discipline construction fund on Gynecology of Traditional Chinese Medicine from Ministry of Education of People's Republic of China.

Key discipline construction fund on Gynecology of Traditional Chinese Medicine from State Administration of Traditional Chinese Medicine of People's Republic of China.

Protocol revised history-V 7.11-09/24/2012

1. P10. Objective change to:

1) Acupuncture protocol 1 plus CC (Arm A) is more likely to result in live birth than acupuncture protocol 2 combined with CC (Arm B),

2) Acupuncture protocol 2 plus CC (Arm B) is more likely to result in live birth than acupuncture protocol 1 plus placebo (Arm C),

3) Acupuncture protocol 1 plus placebo (Arm C) is more likely to result in live birth than acupuncture protocol 2 plus placebo (Arm D).

2. P11 - 4. Study procedures 4.1 Pre-screening added" see Appendix 12".

3. P12- 4.5 Weekly Serum Progesterone Level and Pregnancy Test. Add" Further details of determining response or non-response to treatment based on the weekly visit can be found in Section 23 below."

4. P12- 4.6 Pregnancy Visits (only with conception) 2. Change to "Take blood for serum quantitative hCG levels until the level reaches a point where a pregnancy can be visualized by transvaginal ultrasound (1500-2000 IU/mL)."

5. P12- 4.6 Pregnancy Visits (only with conception) 3. Change to "Perform transvaginal ultrasound when the hCG level or clinical presentation dictates. Determine the number of gestational sacs, location, dimensions, presence and size of fetal parts, and documentation of visualization of fetal heart motion, documentation of any pregnancy related abnormalities."

6. P12- 4.7 End of Treatment Visit (all subjects): 1. Chang to "To take place after pregnancy or after completion of the 4th cycle without pregnancy."

7. P13- 8. Exclusion criteria: "1.Those not has follicular development after CC 150mg daily for five days or FSH stimulation." was deleted.

8. P13- 8. Exclusion criteria: "②Patients with known 21-hydroxylase deficiency or other enzyme deficiency leading to the phenotype of congenital adrenal hyperplasia." was deleted.

9. P15 Add "o) Patients with known congenital adrenal hyperplasia."-CRF

10. P17 "Photographic examples of each grade will be provided to investigators as well as training to study personnel." was deleted.

11. P23 change to "If the subject does not have a menstrual bleed, either after progestin or spontaneous ovulation, she will be scheduled for this visit 2 weeks after the initiation of progestin or documentation of ovulation. These subjects will be managed on a case by case basis by the site PI depending on if they are pregnant and the results of the baseline

ultrasound.” The paragraph was deleted since it is not necessary to have a menstrual bleed prior to medication start so long as the subject is not pregnant, is anovulatory, and lacks evidence of a developing follicle.

12. P23-22.2 Baseline visit change to ” If the subject does not have a menstrual bleed, either after progestin or spontaneous ovulation, she will be managed on a case-by-case basis by the site PI depending on if they are pregnant and the results of the baseline ultrasound.”

13. P23-25. Weekly acupuncture visit: 3 add “or time of day”.

14. P25-23.1 Non-Responders: added” unless at discretion of the site investigator.”

15. P42 Add “but this will be minimized by giving each patient a unique identifier and eliminating personal identifying information from any documents and accessible databases”.

13. P72 “Appendix 12: PCOSAct prescreening questionnaire” was added.

Protocol revised history-V 7.11-09/24/2012

1. P14- Delete “6. Acupuncture treatment related to PCOS within the last 6 months.”
2. P45- Delete “7. No acupuncture treatment related to PCOS within the last 6 months.”
3. P45- Delete “2. Received acupuncture related to treatment of PCOS within the past 6 month.”

Protocol revised history-V 8.1 -13/1/2013

1. The title of our trial is changed to “Effect of Acupuncture and Clomiphene Citrate on Live Birth in Anovulatory Women with Polycystic Ovary Syndrome: a Randomized Controlled Trial”
2. P11- 4.2 Screening Visit (Visit 1: physician) Add”2. Ensure the patients agreeing to frequent blood tests and not having frequent business visits.”
3. P12- 4.2 Screening Visit (Visit 1: physician) 3. Take a comprehensive history and perform complete physical examination including vital signs, height, weight, hip and waist measurements, BMI, pelvic exam with Pap smear or thinprep cytological test (TCT) or Human Papillomavirus (HPV) DNA test.
4. P12-Weekly acupuncture Visit: Add 3. “Complete the credibility check questionnaire before the first session of the acupuncture treatment and after the third acupuncture treatment.”
5. P12-4.7 End of Treatment Visit (all subjects): Add “2. Complete the credibility check questionnaire after the last acupuncture treatment.”
6. P12- 4.7 End of Treatment Visit (all subjects): change to “3. Whether or not perform transvaginal ultrasound for subject is determined by the local PI on the case- by-case basis.”
7. P12- 4.7 End of Treatment Visit (all subjects): Add “These samples will be collected and shipped to the core laboratory for final analysis.”
8. P13-6. Subject selection and exclusion Delete“/or”. PCOS is defined by the modified Rotterdam criteria: oligomenorrhea or amenorrhea, together with presence of >12 antral follicles (≤ 9 mm) and ovarian volume >10 ml on transvaginal scanning
9. P13- Inclusion criteria: Semen analysis: (1) a. sperm concentration $\geq 15 \times 10^6$ /ml and b. total motility (a+b+c) $\geq 40\%$ or forward motility (a+b) $\geq 32\%$ in the semen analysis of the husband (based on World Health Organization, 2010). (2) Total motile sperm

- count ≥ 9 million [based on WHO (2010) criteria, volume 1.5 ml; conc. 15 million; motility 40%. $1.5 \times 15 \times 0.4 = 9$ million].
10. P14- 2. Change to “Use of hormonal or other medication including Chinese Herbal prescriptions which may affect the outcome at least in the past 2 months.”
 11. P18- Table 5. Screening labs of PCOS women (run in local labs) PCOS Safety Screen change to “4. Pap smear or thinprep cytological test (TCT) or HPV DNA test (if no recent results available, per ACOG guidelines).
 12. P18- Table 5. PCOS Inclusion/Exclusion labs, add “8. Glycosylated Hemoglobin A1c level”
 13. P19- 16. Exclusion of Other Infertility Factors Add “An HSG test is generally acceptable if it is done within 3 years unless a patient had a history of pelvic surgery or pelvic inflammatory disease after the HSG. In that case, the patient should have an updated HSG test within 1 year. In addition, if the updated HSG report indicates that the tubes are patent but not fluent, the doctor in charge should read the HSG films and make the final determination whether the tubes are patent or not; that is, there is no need to report the fluency of the tubes. For example, the doctor can document the tubes as patent if he or she sees that the dye fills the tubes and spills out from the tubes. In the event that there are discrepancies between the radiologist and the site PI concerning the patency of the tubes, they should discuss the test and reach a consensus for the diagnosis and sign off on the final report.”
 14. P26- 23.3 Pregnancy add “Pregnant women who presents with threatened miscarriage or are considered to have a significant risk for miscarriage can be given oral Duphaston. No other medications including hCG, herbal medications and acupuncture should be given by the investigators. Should patients choose to take any other medications on their own; the medicines should be clearly documented in the CRFs.”
 15. P26- 23.4 Dispensing of Study Drug before Every Cycle Before the beginning of each cycle, each subject will be dispensed the study drug for that cycle at the weekly visit. Change to “Before the beginning of each cycle, each participant will be dispensed the study drug for that cycle on the same day they are supposed to take medications, and the woman should sign off after receiving the medication.”
 16. P29- 25.1 Acupuncture protocols 1 and 2 add “A total of 32 acupuncture treatments should be maintained. If the participant misses two to four scheduled acupuncture treatments (1-2 weeks), she will take additional acupuncture sessions during the following treatments and make up to 32 times in the end. If the participant misses 6-8 acupuncture treatments (3-4 weeks), she should be scheduled not to take study medication CC either and have this treatment cycle “rested” until she can get acupuncture.”
 17. P32- Add “and the average rate among four arms is 15%”
 18. P36- 31.2 Interim Analysis, Added “We propose to do an interim analysis at stage of 1/3 enrollment, to avoid the serious deviation of clinical habits of local PIs from our standard PCOSAct protocol.”
 19. P39- Delete “5. Meizhuo Zhang”, and change the order to “Taixiang Wu, Jianping Liu, Ernest HY Ng”
 20. P44- Appendix 2: Patient information and consent. Add “or Total motile sperm count ≥ 9 million” and “7. Promise of weekly blood test/acupuncture, and no business travels during a four- month intervention”

21. P45- Appendix 2: Patient information and consent. How long will I be in the study?
Add“/4 month cycles”
22. P46- Appendix 2: Patient information and consent. What benefits can I expect?
Added “(15% on average)”and “/baby”
23. P48- delete “Witness’s signature, Witness’s name, Date”
24. P65- Other Medications Requiring a 2 month wash-out period. Add “Chinese Herbal medicine. By own habits of local PIs, at any routines (orally, intravenously, dermatologically, trans-vaginally, trans-rectally, trans-abdominally etc.)”
25. P68- Appendix 9: DNA Collection and Shipping with whole blood. 2 change to “Store labelled tubes in -20°C refrigerator in less than 2 months until shipment or transfer to -70 °C refrigerator” and 8 changed to “ship or transfer every 2 months by overnight carrier to: ”
26. P70- Appendix 11: PCOSAct prescreening questionnaire. Delete “4. Those who do not have follicular development after CC 150mg daily for five days or FSH stimulation.”
And “Currently Pregnant”
27. P71-73 Add three questionnaires for the credibility check
28. P4- change “Site investigators from 26 Hospitals Site investigators from 25 Hospitals” to “Site investigators from 25 Hospitals” and delete “Dianrong Song and the name of the hospital”