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Auricular Acupressure Reduces Anxiety Levels and Improves Outcomes of in Vitro Fertilization: A Prospective, Randomized and Controlled Study

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The study was to explore whether auricular acupressure (AA) can relieve anxiety during the period from trans-vaginal oocyte retrieval to the embryo transfer in IVF treatment and whether AA can improve the outcomes of IVF. 305 infertile patients with tubal blockage who were referred for IVF were included. The women were randomized into a control group with 102 cases, a Sham-AA group with 102 cases and an AA group with 101 cases. The anxiety levels were rated with Spielberger's State Trait Anxiety Inventory and the Amsterdam Preoperative Anxiety and Information Scale. Data of clinical pregnancy rate (CPR), implantation rate (IR) and live birth rate (LBR) were obtained. The levels of neuropeptide Y (NPY) and transforming growth factor alpha (TGF-alpha) in the follicular fluids were detected with ELISA. After treatment, in AA group, the levels of state anxiety, preoperative anxiety and need-for-information were significantly lower, whereas CPR, IR, LBR and NPY levels in the follicular fluids were markedly higher than Sham-AA group and control group. We concluded that AA could help to reduce anxiety levels associated with IVF and improves the outcomes of IVF partly through increasing the levels of NPY in the follicular fluids.

Auricular acupressure (AA), a micro-acupuncture technique similar to reflexology, is effective in reducing state anxiety in both healthy volunteers and the adult patients awaiting their surgery^{1,2}. It was also effective in improving functions of the endocrine system³⁻⁷. AA was first described in French and Chinese medicine, and rapid progress has been made on the theory and practice of AA since Paul Nogier, a French practitioner, discovered the inverted-fetus shape distribution pattern of auricular acupoints. AA works by stimulating the central nervous system through the cranial nerves/spinal nerves on the auricle of the ear and it can be easily accepted by people for its effectiveness and non-invasion.

In vitro fertilization (IVF), as a choice for over 1,000,000 infertile couples each year, represents the culmination of medical, scientific and social evolution, which is linked to over 3,000,000 babies born worldwide⁸. However, according to the reports of European Society of Human Reproduction and Embryology (ESHRE), no significant increase in the implantation rate (IR) of cleaving embryos has been obtained in the last years, although there have been many relevant techniques emerging to improve the IVF outcomes⁹⁻¹¹.

IVF is a multidimensional stressor and the treatment itself constitutes the primary stressor, which is most likely to evoke anxiety¹². As early as in 1990, anxiety was found in 10.6% of the infertile patients prior to IVF using the Spielberger's State Trait Anxiety Inventory (STAI)¹³. Anxiety disorders were found in 23.2% of Chinese patients undergoing IVF or ICSI¹⁴. In Sweden, approximately 30% of the infertile patients undergoing IVF fulfilled criteria for a depressive and/or anxiety disorder, including sub-threshold diagnosis¹⁵. The anxiety may be caused by the invasive nature of the therapy itself, the knowledge that IVF is often the last hope for a biological child, and the high cost of treatment. The highest stress and anxiety levels during an IVF treatment existed shortly before trans-vaginal oocyte retrieval (TVOR), which might negatively influence the implantation phase^{16,17}. Stress, anxiety and depression contribute to lower pregnancy rates among patients undergoing IVF¹⁸. Both anxiety and depression negatively influenced the clinical pregnancy rate (CPR) of IVF treatment in patients with tubal factor infertility¹⁹.



Recently, complementary and alternative medicines, especially acupuncture, have been chosen by numerous couples before they commenced infertility treatment and some of them chose to use it as an adjunct while undergoing infertility treatment. More than 40 studies have been conducted to explore the effects of acupuncture on IVF outcomes since 1996. However, the conclusions were inconsistent and few of them have systematically evaluated the effects of acupuncture on the psychological parameters of IVF patients. Moreover, none has been done to explore the effects of AA as an adjunct to improve the outcomes of IVF. We therefore designed a prospective, randomized and controlled trial to determine whether AA can relieve anxiety for infertile patients with tubal blockage during the period from TVOR to embryo transfer (ET) in IVF treatment and improve the outcomes of IVF. As neuropeptide Y (NPY) and transforming growth factor alpha (TGF-alpha) play important roles in regulation of ovarian functions and follicular growth²⁰⁻²⁵, we also measured the levels of NPY and TGF-alpha in the follicular fluids of the patients. In our study, a relatively large number of patients were included and two different psychological measurements were used.

Results

The baseline characteristics. As shown in Table 1, there were no significant differences among the control group, Sham-AA group and AA group in all the baseline characteristics, including ages, body mass index (BMI), duration of infertility, the serum levels of luteinizing hormone (LH), follicle stimulating hormone (FSH), total testosterone (TT) and estradiol (E₂) on the 3rd day of spontaneous menstrual cycle, the cycle length, the dosage of recombinant FSH administered, induction length, number of follicles, number of follicles with diameters of more than 14 mm, number of embryos

transferred per cycle, embryo cleavage rate, good-quality embryo rate, fertilization rate, the education levels, employment type and living area of the patients ($P>0.05$).

The levels of state anxiety, preoperative anxiety and need-for-information. No significant differences existed among the three groups on the levels of state anxiety, preoperative anxiety (including anesthesia related anxiety and surgery related anxiety) and need-for-information at T1 (Table 2). At T2, after AA treatment for five days, the levels of state anxiety, preoperative anxiety (including anesthesia related anxiety and surgery related anxiety) and need-for-information in AA group were markedly decreased compared with Sham-AA group and control group ($P<0.05$) (Table 2). There were no significant differences between Sham-AA group and control group on the levels of state anxiety and preoperative anxiety (including anesthesia related anxiety and surgery related anxiety) at T2, however, the need-for-information levels of Sham-AA group were markedly lower than those of the control group ($P<0.05$) (Table 2).

IVF outcomes. As shown in Table 2, the CPR, IR and live birth rate (LBR) of AA group were significantly higher than those of the Sham-AA group and control group respectively ($P<0.017$), and there was no significant difference between Sham-AA and control groups ($P>0.017$).

NPY and TGF-alpha levels in follicular fluids. As shown in Figure 1, NPY levels in the follicular fluids of the AA group were significantly higher than those of Sham-AA and control groups respectively ($P<0.05$), and no marked difference existed between Sham-AA and

Table 1 | The baseline characteristics of the participants

Items	Control	Sham AA	AA
	(n=102)	(n=102)	(n=101)
Ages (years)	30.95 ± 4.78	30.87 ± 4.12	31.65 ± 4.30
BMI (kg/m ²)	23.87 ± 6.14	21.01 ± 4.25	22.08 ± 3.55
Duration of infertility (year)	4.97 ± 4.17	4.16 ± 3.21	5.19 ± 3.39
Day-3 LH	5.17 ± 2.64	4.74 ± 2.55	5.01 ± 2.84
Day-3 FSH (nmol/L)	6.13 ± 2.45	6.89 ± 2.03	6.80 ± 2.42
Day-3 TT (pmol/L)	1.45 ± 0.14	1.31 ± 0.07	1.20 ± 0.02
Day-3 E ₂ (pmol/L)	141.18 ± 68.02	149.27 ± 59.96	135.89 ± 70.92
Cycle length (days)	32.90 ± 9.02	33.09 ± 8.97	32.72 ± 7.99
rFSH administered (IU)	2245.18 ± 600.97	2090.75 ± 644.29	2117.32 ± 849.71
Induction length (days)	9.61 ± 1.87	9.98 ± 2.08	9.78 ± 2.06
Number of oocytes	14.80 ± 7.45	15.19 ± 6.89	14.97 ± 6.31
Number of follicles >14 mm	11.59 ± 6.50	12.42 ± 6.72	12.15 ± 4.98
Fertilization rate	1078/1510 (71.39%)	1092/1549 (70.50%)	1090/1512 (72.09%)
Embryo cleavage rate	1052/1078 (97.59%)	1059/1092 (96.98%)	1054/1090 (96.70%)
Good-quality embryo rate	719/1078 (66.70%)	742/1092 (67.95%)	718/1090 (65.87%)
Embryos transferred per cycle	2.08 ± 0.66	2.04 ± 0.63	1.98 ± 0.54
Education level			
High	26/102 (25.49%)	29/102 (28.43%)	27/101 (26.73%)
Medium	32/102 (31.37%)	33/102 (32.35%)	30/101 (29.70%)
Low	44/102 (43.14%)	40/102 (39.22%)	44/101 (43.56%)
Living Area			
City	62/102 (61.39%)	61/102 (59.80%)	59/101 (58.42%)
Urban	4/102 (3.92%)	5/102 (4.90%)	4/101 (3.96%)
Country	36/102 (35.29%)	36/102 (35.29%)	38/101 (37.62%)
Employment Type			
Full-time	56/102 (54.90%)	53/102 (51.96%)	54/101 (53.47%)
Part-time	13/102 (12.75%)	16/102 (15.69%)	16/101 (15.84%)
Non-employment	33/102 (32.35%)	33/102 (32.35%)	31/101 (30.69%)

Note: Data were shown as mean ± S.D. * $P<0.05$, compared with the Control group; # $P<0.05$ compared with Sham AA group (For Chi-Square tests among three groups, the adjusted significance level for each comparison was set at $P=0.017(0.05/3)$ to give a significance level of 0.05 over all three tests: * $P<0.017$, compared with the Control group; # $P<0.017$ compared with Sham AA group). AA: auricular acupressure; BMI: body mass index; Day-3: the 3rd day of spontaneous menstrual cycle; LH: luteinizing hormone; FSH: follicle stimulating hormone; TT: total testosterone; E₂: estradiol; rFSH: recombinant FSH.



Table 2 | The levels of state anxiety, preoperative anxiety and need-for-information at T1 and T2, and the IVF outcomes

Items	Control	Sham AA	AA
	(n=102)	(n=102)	(n=101)
STAI (T1)			
State anxiety	42.91 ± 9.70	43.12 ± 8.01	44.01 ± 8.93
APAIS (T1)			
Anesthesia related anxiety	3.51 ± 1.42	3.39 ± 1.39	3.59 ± 1.33
Surgery related anxiety	3.99 ± 1.21	3.81 ± 1.36	4.06 ± 1.36
Need-for- information	5.32 ± 1.54	5.25 ± 1.12	5.20 ± 1.51
STAI (T2)			
State anxiety	41.11 ± 9.96	42.29 ± 9.55	34.79 ± 14.01 * #
APAIS (T2)			
Anesthesia related anxiety	3.12 ± 1.12	2.91 ± 1.07	1.97 ± 0.81 * #
Surgery related anxiety	3.87 ± 1.21	3.56 ± 1.52	2.72 ± 1.17 * #
Need-for- information	5.01 ± 1.62	4.71 ± 1.23 *	4.03 ± 1.76 * #
Clinical pregnancy rate (%)	40/102 (39.21%)	41/102 (40.20%)	59/101 (58.42%) * #
Implantation rate (%)	51/202 (25.25%)	52/206 (25.24%)	78/211 (36.97%) * #
Live birth rate (%)	31/102 (30.39%)	32/102 (31.37%)	53/101 (52.48%) * #

Note: Data were shown as mean ± S.D. *P<0.05, compared with the Control group; #P<0.05 compared with Sham AA group. [For Chi-Square tests among three groups, the adjusted significance level for each comparison was set at P=0.017(0.05/3) to give a significance level of 0.05 over all three tests: *P<0.017, compared with the Control group; #P<0.017 compared with Sham AA group]. AA: auricular acupressure; T1: the time-point on the morning of one day before trans-vaginal oocyte retrieval; T2: the time-point on the morning of the day of embryo transfer; STAI: state trait anxiety inventory; APAIS: Amsterdam preoperative anxiety and information scale.

control groups. There were no significant differences on TGF- α levels in the follicular fluids among the three groups.

Adverse result. No side-effect was reported in either group during the period of the research.

Discussion

The present study found that AA applied at three specific points is effective in relieving anxiety levels during the period from TVOR to

ET and notably improves the outcomes of IVF partly through increasing the levels of NPY in the follicular fluids.

Acupressure involves stimulation of acupoints and meridians without use of needles. Stimulating a particular acupoint with acupressure can activate small myelinated nerve fibers that send impulses into the spinal cord, midbrain, pituitary, and hypothalamus²⁶. AA, a treatment method derived from acupressure and ear acupuncture, is a safer, more effective, economic and simple approach^{3,6,27–31}. The practical advantages of AA include^{32,33}: (1) only minimal equipment is needed and any physicians or nurses can perform this treatment; (2) physicians as well as other health care providers who have rare or no experience in traditional Chinese medicine can learn this technique within a few hours and can use it whenever indicated; (3) it is almost a “no-cost” intervention; (4) AA is non-invasive and does not affect the integrity of the epidermis. Acupressure, including AA, has been found to be safe and effective in decreasing prehospital anxiety³², reducing preoperative anxiety^{34,35}, alleviating stress³³ and improving quality of life^{36,37}.

IVF is usually the final treatment option for infertile couples, and failure will probably mean their remaining childless³⁸. It is therefore not surprising that both patients and men always demonstrate elevated levels of anxiety during IVF treatment^{39,40}. In IVF treatment, the state anxiety is caused by the fear of TVOR, or the strain of the emotional moment at ET, which can be measured by the levels of anxiety at a certain time-point⁴¹. Reviews have suggested distress including anxiety has adverse effects on IVF outcomes^{42–44}, however, the conclusions were different in some other researches⁴¹. The differences of these findings may be caused by the variance on the population studied, time-points of assessments, details of the inclusion criteria, size of the studies, definition of indicators of IVF outcomes, types of psychological tests and stages of sampling. In the study, STAI was used to rate the levels of state anxiety, and Amsterdam Preoperative Anxiety and Information Scale (APAIS) was applied to measure the levels of preoperative anxiety (including anesthesia related anxiety and surgery related anxiety) and need-for-information. STAI has been widely used as a standardized tool for assessment of state anxiety in acute settings^{45,46}. To date, STAI has been used in more than 1,000 peer-reviewed medical publications⁴⁶ and has recently been used as a control for other scales^{47,48}. As a simple and reliable measure of preoperative anxiety and need-for-information, APAIS is easy to use and correlates well with other scales^{47–49}. Here we translated APAIS into Chinese and compared it with a back-

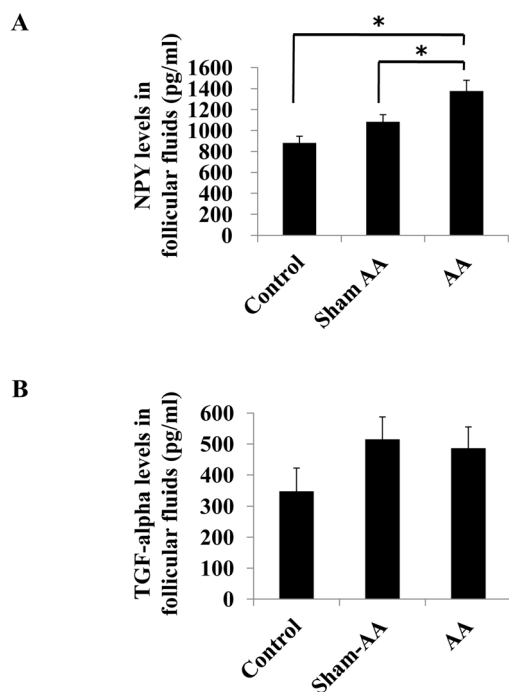


Figure 1 | (A) Neuropeptide Y (NPY) levels in follicular fluids of auricular acupressure (AA) group, Sham-AA group and control group. (B) Transforming growth factor alpha (TGF- α) levels in follicular fluids of AA group, Sham-AA group and control group. For all panels, results were expressed as mean ± S.D. *P<0.05 (ANOVA).



ground questionnaire (STAI) to validate its use. In the present study, the correlation coefficient between the state anxiety items in STAI and the preoperative anxiety items in APAIS was 0.569 ($P < 0.001$), which was consistent with the previously reported correlation between the state anxiety component of STAI and the preoperative anxiety component of APAIS in patients in the preoperative period. Factor analysis with oblique rotation revealed two factors: anxiety (with an eigenvalue of 2.877) and need-for-information (with an eigenvalue of 1.154), which explained 67% of the total variance. In this study, we found AA at the three auricular acupoints--Shenmen (TF4), Endocrine (CO18) and Internal Genitals (TF2) notably decreased the levels of state anxiety, preoperative anxiety (including anesthesia related anxiety and surgery related anxiety) and need-for-information of the patients undergoing IVF treatment. Consequently, AA at the three acupoints can be performed on the patients who feel anxiety during the period from TVOR to ET in IVF treatment. In the study, all the three acupoints used for the subjects of Sham-AA group have not been documented to relieve anxiety, improve functions of the endocrine system or regulate functions of the female reproductive system.

In our study, the first psychological measurement was performed on the morning of one day before TVOR, as we wished to explore the baseline levels of the patients before TVOR. The time-point of the second measurement was chosen based on our clinical experiences that patients often express eminent feelings of anxiety during the period from one day before TVOR to the day of ET. It is valuable to measure the levels of state anxiety, preoperative anxiety and need-for-information on the morning of the day of ET because in that occasion, AA has been applied for five days and the ET operation will start shortly.

In the present study, no significant differences on the baseline characteristics existed among the three groups. The patients' age, BMI and other known confounding factors such as duration of infertility, the serum levels of LH, FSH, TT and E_2 on the 3rd day of spontaneous menstrual cycle, the cycle length, the dosage of recombinant FSH administered, induction length, number of follicles, number of follicles with diameters of more than 14 mm, number of embryos transferred per cycle, embryo cleavage rate, good-quality embryo rate and fertilization rate have been well controlled in the present study. These factors have been shown important in large prospective studies^{50,51}. The lifestyle factors such as smoking and alcohol intake were also well controlled in the study. The factors associated with cultural settings, such as the education levels, employment type and living area of the patients were also well controlled.

In the study, CPR, IR and LBR were selected as the parameters to evaluate the IVF outcomes. We found that AA at the three specific auricular acupoints eminently improves CPR, IR and LBR of the IVF patients, suggesting that AA may have promises to improve the outcomes of IVF. To further explore the possible mechanism underlying AA improving the outcomes of IVF, we detected the levels of NPY and TGF- α in the follicular fluids of the IVF patients. As the most widely distributed neurohormone in the body, NPY can regulate the functions of the hypothalamic-pituitary-ovarian axis. It reaches the ovary via the plexus ovarian nerve, and high concentrations of NPY have been found in follicular fluids^{20–22,52}. The concentration of NPY are finely tuned by fluctuations in the concentration of ovarian steroid hormones⁵². NPY is supposed to play important roles in regulating ovarian functions, follicular growth and ovulation. A prospective, randomized and multicentre clinical trial including 286 patients undergoing IVF treatment found electro-acupuncture significantly increased the levels of NPY in follicular fluids, which may be important for human ovarian steroidogenesis⁵³. TGF- α , as a 50 amino acid polypeptide present predominantly in theca cells of antral follicle, has been found to regulate the growth of follicles^{23,24}. TGF- α in the follicular fluids plays a pronounced role in oocyte maturation

during late follicular phase and it simulates the action of LH in preovulatory follicles⁵⁴. TGF- α also plays an important role in preventing apoptosis of granulosa cells in preovulatory follicles⁵⁵. We found NPY levels in the follicular fluids of the AA group was significantly higher than those of Sham-AA and control groups respectively ($P < 0.05$), and no marked difference existed between Sham-AA and control groups. There were no significant differences on TGF- α levels in the follicular fluids among the three groups. Consequently, NPY may be partly involved in AA improving outcomes of the IVF patients.

In the present study, no side-effect was reported in either group during the period of the research, demonstrating that AA is a safe and non-invasive intervention to improve the outcomes of IVF patients and to reduce the associated anxiety levels.

The present research was conducted in Reproductive Medicine Center of Zhejiang Province, China and more than 4000 IVF/ICSI cycles are performed in the Reproductive Medicine Center per year. Findings from this study have implications for health care providers to consider incorporating AA in their clinical practices when caring for infertile patients with IVF treatment. However, some further relevant indicators should be included, such as fertilization rate, embryo cleavage rate, good-quality embryo rate and incidence of birth defects. As some potential factors may influence the applicability of Sham-AA, including the patient's knowledge, expectation and experience of AA, these factors should be considered in the future studies. As multi-centre trials using the same study design may reduce the variation caused by the different protocols for IVF and differences in baseline CPR and LBR observed between different centers, more large-size, randomized, multicentre, double-blinded and placebo-controlled trials are supposed to be conducted in the future and further research should also be done on the mechanism involved.

Methods

Subjects. In a six-month period (January, 2011 to June, 2011), we screened 405 infertile patients with tubal blockage who were referred to Department of Reproductive Endocrinology, Women's Hospital, School of Medicine, Zhejiang University, China (Reproductive Medicine Center of Zhejiang Province, China) for IVF. Among the patients screened, 94 patients did not meet the inclusion criteria. For four patients, consents were not obtained, and two patients were excluded for other reasons. Finally, 305 infertile patients with tubal blockage were included in the study. The randomization was done two days before TVOR, when the women came to the centre to prepare for TVOR. Having been randomized, the control group had 102 cases, Sham-AA group 102 cases and AA group 101 cases. The ET treatment was conducted three days after TVOR, and all the patients had completed both of TVOR and ET. In the statistical analysis, all of the patients were included without missing cases during the research (Fig 2).

All experimental protocol was approved by the Institutional Review Board of Women's Hospital, School of Medicine, Zhejiang University. The aim and methodology of the study were explained to the patients. The methods were carried out in accordance with the approved guidelines. The study was undertaken with the understanding and appropriate informed consent of each participant. The infertile patients with tubal blockage who were referred to our department for IVF were included in the study. They were otherwise healthy women with regular menstrual cycles and normal sex hormone levels and no other pelvic pathology existed. No structural abnormalities of uterus and ovaries were found by vaginal ultrasound or laparoscopy. None of the patients had received salpingectomy or assisted reproductive technology (ART) therapy before. We excluded all the patients who were not fluent in Chinese, the patients with neurologic or psychiatric disorders, the patients who were taking any tranquilizer, acupuncture or acupunctive therapy, patients with an ear deformity, or the patients with a history of smoking or drinking. All the partners of the patients had normal spermograms and sperm morphology. The present study adheres to the Consolidated Standards of Reporting Trials (CONSORT) statement and Standards for Reporting Interventions in Clinical Trials of Acupuncture (STRICTA). The trial was registered at Australian New Zealand Clinical Trials Registry (ANZCTR) with the identifier ACTRN 12611000899943 (<http://www.ANZCTR.org.au/ACTRN12611000899943.aspx>).

Subjects were randomized into an AA group, a Sham-AA group or a control group with the use of a randomization chart constructed in Microsoft Excel that randomized numbers into three groups. The random allocation sequence was concealed until interventions were assigned. A nurse enrolled the participants and assigned participants to their groups. The IVF clinicians and laboratory staff were blinded to the group assignment. The AA protocol was developed based on the clinical experiences of our hospital, literatures and the consultation with 10 experts in Chinese medicine.

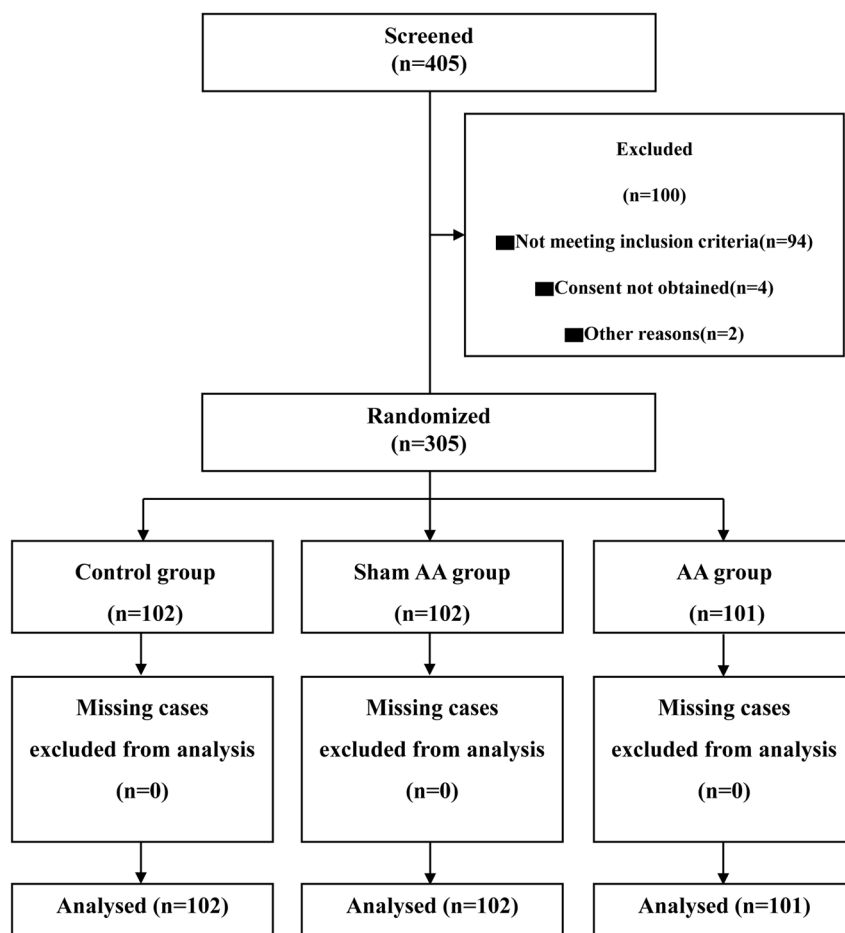


Figure 2 | The flow chart of the progress of participants through the trial. The subjects of auricular acupressure (AA) group and Sham-AA group were asked to press the acupoints four times a day (08:00, 12:00, 16:00 and 20:00 h respectively) with 15 min each time by themselves. The AA treatment consisted of six days (from 12:00 h of one day before trans-vaginal oocyte retrieval to 20:00 h of the next day of embryo transfer) and was conducted on the two ears simultaneously.

The AA treatment was performed by two independent trained nurses who were not involved in the study, and they were blinded to the group assignment.

Group and administration. AA group: Shenmen (TF4, located at the bifurcation of the crura of antihelix), Endocrine (CO18, located at the bottom part of the incisura intertragica), and Internal Genitals (TF2, located at the middle point of the anterior portion of the triangular fossa) were selected as the auricular acupoints (Fig 3). Shenmen (TF4) functions to relieve anxiety. Endocrine (CO18) and Internal Genitals (TF2) improve functions of the endocrine system and regulate functions of the female reproductive system. After the above acupoints were sterilized with 75% alcohol, AA was performed by using acupressure seeds named *Semen vaccariae*. The seed was kept in place by a piece of opaque adhesive patch and fixed onto the acupoint selected. The subjects were asked to press the acupoints four times a day (08:00, 12:00, 16:00 and 20:00 h respectively) with 15 min each time by themselves. The strength should make the local auricle congestive, flushed and ache. The AA was conducted on the two ears simultaneously. The AA treatment consisted of six days (from 12:00 h of one day before TVOR to 20:00 h of the next day of ET). All the subjects were instructed on: the procedure, duration and intensity of AA; the methods of keeping acupressure patches in the right place and protecting them; and recording the time of application of AA and any side-effects in a diary.

Sham-AA group: Triple Energizer (CO17, located in the cavum conchae), Stomach (CO4, located at the commissure of superior concha and inferior concha, just below the disappearance of the crus of the helix) and Large Intestine (CO7, located at the inner 1/3 of the crus of the helix, lying at the lower portion of the superior concha) were selected as the “sham” auricular acupoints (Fig 3). All the manipulation and process were exactly the same as the AA group above.

Control group: The patients in the control group only followed the routine procedure of IVF treatment and no AA was applied on them.

Collection of follicular fluids. The long agonist protocol for controlled ovarian hyperstimulation (COH) was used as previously described⁵⁶. The follicular fluids were sampled by trans-vaginal ultrasound-guided puncture and aspiration of the follicles with diameters of 16–18 mm.

Index and method. The levels of state anxiety, preoperative anxiety (including anesthesia related anxiety and surgery related anxiety) and the need-for-information were evaluated respectively on the morning of one day before TVOR (defined as time-point T1) and on the morning of the day of ET (defined as time-point T2) by an independent gynecologist and two independent psychologists who were both out of the research team. They were all blinded to the group assignment. The levels of state anxiety were measured with the Chinese version of STAI, which is characterized by high reliability and good construct validity^{57,58}. STAI consists of 20 short items measuring state anxiety which refers to transitory emotional arousal or situational distress at a particular moment in time⁵⁹. These items reflect experiential, cognitive and behavioral aspects of state anxiety⁵⁹. The score of each item ranges from 1 to 4 and a higher score reflects a higher level of state anxiety⁵⁹. In the present study, the STAI’s reliability was 0.899 and 0.911 respectively at T1 and T2. The levels of preoperative anxiety and the need-for-information were measured with the APAIS, a simple and reliable measure with good validity^{48,49,60}. The score of each item ranges from 1 to 4 and higher scores reflect higher levels of preoperative anxiety and the need-for-information. APAIS’s reliability with the present samples was 0.794 and 0.738 respectively at T1 and T2. Data of CPR (defined as the presence of at least one gestational sac or fetal heartbeat, confirmed by trans-vaginal ultrasound), IR (defined as the number of gestational sacs per number of transferred oocytes) and LBR (defined as the presence of a baby born alive after 24 weeks gestation) for all the patients were obtained. The levels of NPY and TGF-alpha in the follicular fluids were detected with ELISA (R&D Systems, MN, U.S.A.).

Sample size and Data analysis. The sample size was analyzed using Power Analysis and Sample Size (PASS 11.0). Based on the records of our hospital, a CPR of 0.35 was estimated in the control population and a CPR of 0.41 and 0.53 was respectively estimated in Sham-AA population and AA population. A sample size of 298 achieves 80% power to detect an effect size (W) of 0.18 using a 2 degrees of freedom Chi-Square Test with a significance level (alpha) of 0.05. Based on the records of our hospital, an IR of 0.20 was estimated in the control population and a CPR of 0.22 and 0.39 was respectively estimated in Sham-AA population and AA population. A sample size of

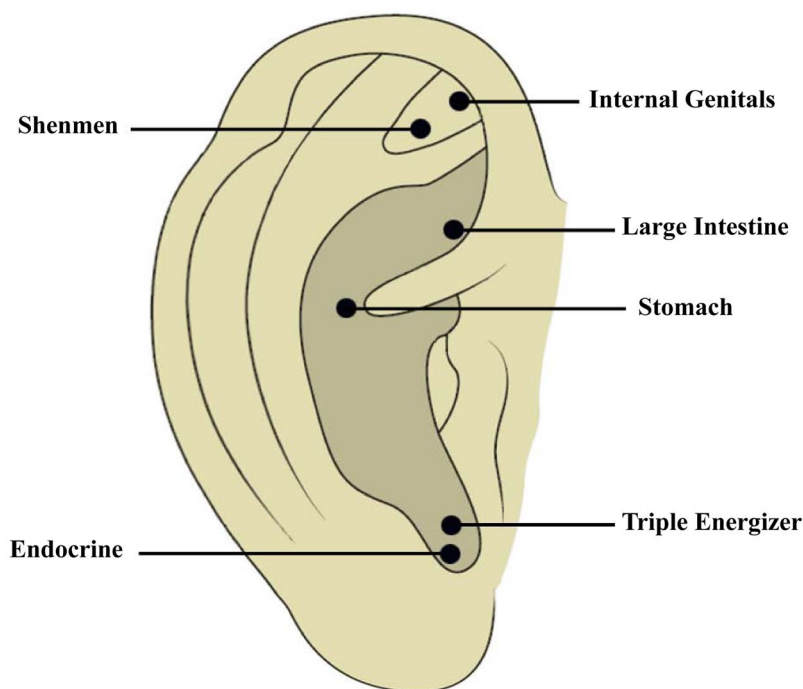


Figure 3 | The auricular acupoints selected. Shenmen (TF4, located at the bifurcation of the crura of antihelix), Endocrine (CO18, located at the bottom part of the incisura intertragica), and Internal Genitals (TF2, located at the middle point of anterior portion of the triangular fossa) were selected in auricular acupressure (AA) group; Triple Energizer (CO17, located in the cavum conchae), Stomach (CO4, located at the commissure of superior concha and inferior concha, just below the disappearance of the crus of the helix) and Large Intestine (CO7, located at the inner 1/3 of the crus of the helix, lying at the lower portion of the superior concha) were selected in Sham-AA group.

267 achieves 80% power to detect an effect size (W) of 0.19 using 2 degrees of freedom Chi-Square Test with a significance level (α) of 0.05.

Data were analyzed using the Statistical Package for Social Sciences (SPSS 19.0 for Windows). The data of ages, BMI, duration of infertility, the serum levels of LH, FSH, TT and E_2 on the 3rd day of spontaneous menstrual cycle, the cycle length, the dosage of recombinant FSH administered, induction length, number of follicles, number of follicles with diameters of more than 14 mm, number of embryos transferred per cycle, STAI scores, APAIS scores and the levels of NPY and TGF- α were found normally distributed using the Kolmogorov-Smirnov (KS) test. The comparisons among different groups were performed with one-way analysis of variance (ANOVA) and multiple comparison tests were conducted with Bonferroni correction procedure. Chi-Square tests were used to detect the data of CPR, IR, LBR, embryo cleavage rate, good-quality embryo rate, fertilization rate, the education levels, employment type and living area of the patients. For Chi-Square tests among three groups, the adjusted significance level for each comparison was set at $P=0.017(0.05/3)$ to give a significance level of 0.05 over all three tests. For all the other hypothesis tests, significance level was set at $P=0.05$ and two-tailed tests were used.

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Author contributions

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Additional information

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