

Increase of success rate for women undergoing embryo transfer by transcutaneous electrical acupoint stimulation: a prospective randomized placebo-controlled study

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Objective: To evaluate the effect of transcutaneous electrical acupoint stimulation (TEAS) on pregnancy rates (PR) in women undergoing ET.

Design: Prospective, randomized, single-blinded placebo-controlled clinical trial.

Setting: Research and laboratory facilities.

Patient(s): A total of 309 patients, less than 45 years old, undergoing cryopreservation embryos transplant or fresh cycle IVF with or without intracytoplasmic sperm injection (ICSI).

Intervention(s): The subjects were randomly allocated to three groups: mock TEAS treatment: 30 minutes after ET (group I, n = 99); single TEAS treatment: 30 minutes after ET (group II, n = 110); and double TEAS treatments: 24 hours before ET and 30 minutes after ET (group III, n = 100).

Main Outcome Measure(s): Clinical PR, embryos implantation rate, live birth rate.

Result(s): The clinical PR, embryos implantation rate, and live birth rate of group I (29.3%, 15.0%, and 21.2%, respectively) were significantly lower than those in group II (42.7%, 25.7%, and 37.3%, respectively) and group III (50.0%, 25.9%, and 42.0%, respectively).

Conclusion(s): Transcutaneous electrical acupoint stimulation, especially double TEAS, significantly improved the clinical outcome of ET. (*Fertil Steril*® 2011;96:912–6. ©2011 by American Society for Reproductive Medicine.)

Key Words: Acupuncture, transcutaneous electrical acupoint stimulation (TEAS), pregnancy rate (PR), in vitro fertilization (IVF), embryo transfer (ET), intracytoplasmic sperm injection (ICSI)

Acupuncture is an important part of traditional Chinese medicine. Acupuncture treatment of female infertility has a long history in China. The mechanisms of acupuncture treatment have been purported to be the modulation of the autonomic nervous, endocrine, and neuroendocrine systems (1).

The birth of the first “test tube baby” in 1978 opened the field of assisted reproductive technology (ART), but the low pregnancy rate (PR) remained an issue in clinical practice. In the recent decade, researchers worked hard to explore complementary therapies in the IVF-ET, including acupuncture (2). However, at present the results

are controversial. Paulus et al. (3) first reported in 2002 that acupuncture treatment improved PR in patients who undergo ART. This was confirmed by several lines of evidence showing similar results. It was further reported that acupuncture was especially effective when it was applied on the day of ET (4–6), but not in other time periods (7, 8). In addition, acupuncture placed at correct sites (acupoints) was regarded as a key point (4). However, several recent publications contradicted previous findings. Andersen et al. (9) applied verum or placebo acupuncture on the day of ET in 635 patients and found no difference of PR in the two groups. There were also reports showing that acupuncture has only a placebo effect, yet they confirmed that acupuncture was safe for women undergoing ET (10, 11). To investigate whether acupuncture plays a role in increasing the success rate of IVF-ET, we conducted a prospective, randomized, single-blinded placebo-controlled clinical trial.

To increase the reproducibility of acupuncture-like stimulation, we chose electrical stimulation instead of manual needling. To reduce the invasiveness, we chose skin electrodes placed on the acupoints instead of piercing the skin with needles. As a result, transcutaneous electrical acupoint stimulation (TEAS) was adopted. To ensure an electrical nerve stimulation without pain and fear, transcutaneous delivery of the stimulation is often adopted in clinical research and application, including obstetrics and gynecology for pain relief during labor (12) or dysmenorrhea (13). To our knowledge, TEAS has not been reported to be used to improve the clinical efficacy of ET. The aim of the present study was to evaluate the

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R.Z. and X.-J.F. contributed equally to this work.

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Project was registered at Chinese Clinical Trial Registry, which was World Health Organization International Clinical Trial Registration Platform (<http://www.chictr.org>; Registration No. ChiCTR-TRC-10000993).

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efficacy of a standardized protocol of TEAS in terms of the pregnancy outcome for women undergoing ET. Among the parameters of the electrical stimulation, the frequency was the key issue for consideration. Stener-Victorin et al. (14–16) reported that low but not high frequency electroacupuncture was effective in modulating the ovarian blood flow and that low frequency was also reported to be more powerful for modulating the function of the hypothalamus-pituitary-ovary system. Because of these advantages, we selected 2 Hz TEAS as the treatment modality.

MATERIALS AND METHODS

Setting and Design

This prospective, randomized, controlled trial was conducted at the Reproductive Medicine Center of the Second Affiliated Hospital, Shandong University of Traditional Chinese Medicine, from July until December 2009. The inclusion criteria were infertile women aged 21–44 years, undergoing frozen- cryopreservation embryos transplant or fresh cycle IVF with or without intracytoplasmic sperm injection (ICSI). The exclusion criteria were not eligible for ET, adverse ovarian reserve, or previous acupuncture experience. The common cause for ET cancellation was high risk of ovary hyperstimulation syndrome (OHSS). Adverse ovarian reserve or poor ovarian response (POR) prediction indicated the antral follicle count ≤ 5 and level of FSH in the serum ≥ 12 IU/L or FSH/LH ≥ 3.6 on cycle day 2. The “previous acupuncture” indicated those who have had the experience of acupuncture before. Information and consent forms regarding the study were provided to all patients during their initial IVF consultation. Informed

consent was obtained from all study participants. This clinical trial was approved by The Second Affiliated Hospital, Shandong University of Traditional Chinese Medicine Institutional Review Board and completed register at Chinese Clinical Trial Registry, which was World Health Organization International Clinical Trial Registration Platform (<http://www.chictr.org>; Registration No. ChiCTR-TRC-10000993).

Randomization

Using a computerized randomization method, patients were assigned into single TEAS treatment group, double TEAS treatment group, or mock TEAS group. Eligible women were identified by a research doctor and were provided with information about the study.

Information Collection and IVF/ICSI Procedures

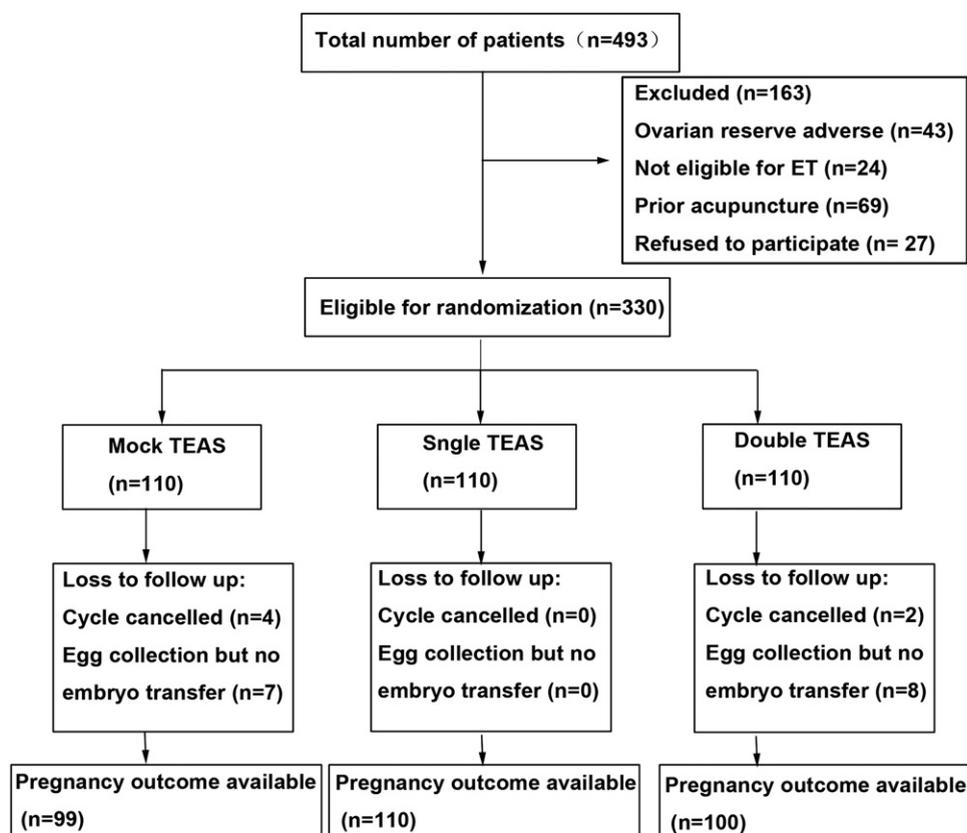
Information on demographics, fertility history, and health status was collected from the subjects. Details of our controlled ovarian hyperstimulation (COH) regimen, oocyte retrieval, and in vitro culture gamete handling, ICSI, ET, and luteal support were performed as described by Braude and Rowell (17).

Blinding

The women undergoing ET and the physician who were performing the transfer were blinded in the whole process. The operators who did TEAS treatment were not blinded, as the small muscle contraction near the stimulation sites were visible.

FIGURE 1

Flow chart of the randomized placebo controlled single blind study of patients undergoing transcutaneous electrical acupoint stimulation (TEAS) during ET.



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TEAS and Placebo Treatment

For women allocated to the single TEAS treatment group, TEAS was administered 30 minutes after ET. The following acupoints were used on both sides (18): ST 36 (Zusanli) and K 13 (Taixi), BL 23 (Shenshu) and RN 4 (Guan-yuan), where self sticky skin electrodes of 2.9 × 2.9 mm were placed. The parameters of TEAS were frequency 2 Hz, pulse width 0.6 ms, intensity 10–12 mA, lasting for 30 minutes. For women in the double TEAS treatment group, extra TEAS was given 24 hours before ET and the following acupoints were used on both sides: Sp 8 (Diji) and S 29 (Guilai), EX-CA 1 (Zigong) and Sp 10 (Xuehai). The parameters were 2 Hz, 15–20 mA, and 30 minutes. For women in the mock TEAS group, 2 Hz intermittent (10 seconds on and 20 seconds off) TEAS at 5 mA (designated as i5mA) was used for 30 minutes. The acupoints used were the same as for the single treatment group. The i5mA intensity represents an extremely weak (minimal), but still sensible stimulation, come and go, which has been approved as a successful placebo (psychologically effective yet physiologically inert) treatment modality in a smoke abatement trial taking place in Singapore (19).

Main Outcome Measures

To assess treatment outcome in all subjects, urinary β -hCG was measured 14 days after retrieval. The primary outcome measurement was clinical pregnancy confirmed by ultrasound observation of fetal cardiac activity 6 weeks after retrieval. Secondary outcomes were biochemical PR, embryos implantation rate, abortion rate, and live birth rate.

Statistical Methods

Clinical PR was used for sample size calculation. According to Paulus et al. (3), a PR of 42.5% in the treatment group and 26% in the control group were reported. The PR in our IVF Center in 2008 was 30%, which was used for the PR of the control group. A trial of 110 women per group would detect a difference of clinical pregnancies between study group and control group of 15% at a power of 80% according to a type II error (β) of 20% and a type I error (α) of 5%.

Data were analyzed with commercial software (Statistical Package for the Social Sciences 11.5.1; SPSS). The initial analysis examined the demographic and baseline characteristics of women randomized to the trial. One-way analysis of variance (ANOVA) and Bonferroni's multiple comparison test was performed for comparison of continuous parameters between the single TEAS, double TEAS, and the placebo group. Comparison of discrete parameters was made by χ^2 analysis. A level of significance of 5% was chosen for both tests.

RESULTS

Of the 330 subjects randomized, an intention-to-treat analysis was performed on the primary end point—the pregnancy outcome. Twenty-one women (6.4%) were unable to comply with the treatment protocol because their cycle was cancelled or the ET was not undertaken. Of the remaining 309 women, 99 were allocated to the mock TEAS group, 110 to the single TEAS treatment group, and 100 to the double TEAS treatment group (Fig. 1).

As demonstrated in Table 1, there were no significant differences in patient's age, body mass index (BMI), duration of infertility, number of previous IVF/ICSI attempts, duration of infertility, percentage of ICSI, percentage of primary infertility, percentage of ET after embryo cryopreservation, and number of transferred embryos.

The reproductive outcomes in the three groups are shown in Table 2. Of the 309 women, 137 (44.3%) had a biochemical pregnancy, 126 (40.8%) had a clinical pregnancy, and 104 (33.7%) had a delivery. The rates of clinical pregnancy, embryos implantation, and live birth were all significantly higher in the single TEAS group than those in the placebo group (clinical pregnancy: 42.7% vs. 29.3%, $P=.044$; embryos implantation: 15.0% vs. 25.7%, $P=.005$; live birth: 37.3% vs. 21.2%, $P=.011$). The addition of TEAS treatment at 24 hours before ET further increased the rates of clinical pregnancy (50.0%) and live birth (42.0%) than single TEAS treatment, but the differences were not statistically significant. The rates of

TABLE 1

Participant demographic and baseline characteristics.

Characteristic	Mock TEAS treatment (n = 99)	Single TEAS treatment (n = 110)	Double TEAS treatment (n = 100)
Age (y)	31.5 (5.2)	31.9 (5.3)	32.6 (4.9)
No. of cycles			
1	96 (97.0)	102 (92.7)	93 (93.0)
2	3 (3.0)	5 (4.5)	7 (7.0)
3	0 (0)	2 (1.8)	0 (0)
4	0 (0)	1 (0.09)	0 (0)
BMI	22.6 (3.5)	23.2 (3.0)	22.6 (3.4)
Duration of infertility (y)			
≤2	29 (29.3)	37 (33.6)	27 (27.0)
2–4	27 (27.3)	22 (20.0)	29 (29.0)
≥5	43 (43.4)	51 (46.4)	44 (44.0)
Reason for infertility	15 (15.2)	15 (13.6)	9 (9.0)
Male factor	48 (48.5)	52 (47.3)	56 (56.0)
Female factor	36 (36.4)	43 (39.1)	35 (35.0)
Both factors	44 (44.4)	34 (30.9)	36 (36.0)
ICSI	57 (57.6)	55 (50.0)	44 (44.0)
Primary infertility	17 (17.2)	23 (20.9)	37 (37.0)
ET after cryopreservation	2.1 (0.5)	2.2 (0.5)	2.2 (0.5)
Embryos transferred			

Note: Values are mean (SD) or n (%). BMI = body mass index; ICSI = intracytoplasmic sperm injection; TEAS = transcutaneous electrical acupoint stimulation.

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TABLE 2

Reproductive outcomes per ET.						
Reproductive outcome	Mock TEAS treatment (n = 99)	Single TEAS treatment (n = 110)	Double TEAS treatment (n = 100)	P value a vs. b	P value a vs. c	P value b vs. c
Biochemical pregnancy	34 (34.3)	49 (44.5)	54 (54.0)	.132	.005	.171
Clinical pregnancy	29 (29.3)	47 (42.7)	50 (50.0)	.044	.003	.291
Early pregnancy loss (% of biochemical pregnancy tests)	3 (8.8)	4 (8.2)	6 (11.1)	1.000	1.000	.864
Implantation rate (% of gestational sacs/no. of transferred embryos)	15.0 (32/213)	25.7 (64/249)	25.9 (56/216)	.005	.005	.956
Live birth rate	21 (21.2)	41 (37.3)	42 (42.0)	.011	.002	.484

Note: Values are n (%), unless otherwise noted. TEAS = transcutaneous electrical acupoint stimulation.

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early pregnancy loss (expressed as percentage of biochemical pregnancy) were similar in the three groups (8.8% in mock TEAS, 8.2% in single TEAS, and 11.1% in double TEAS).

DISCUSSION

Our data show that TEAS administered on the day of ET significantly improved clinical pregnancy outcome (29.3% vs. 42.7%, +13.4%), and TEAS on the day before ET seemed to have an added beneficial effect (50.0% vs. 42.7%, +7.3%). Therefore we suggest a double TEAS treatment for the future studies.

The discrepancy between the present study and previous negative outcome reports (9–11) might be related to differences in setup. We summarize three key points in this trial. The first point is the time of intervention. Consistent with previous findings, treatment on the day of ET seems to be important, whereas an added treatment on the day before ET may further increase the PR. The second point is the method of intervention. Although Paulus et al. (3) first declared acupuncture's beneficial effects on pregnancy outcome, it was not supported by more recent studies (9, 11). To explore whether the low reproducibility is due to some methodological inconsistency, we used TEAS in this trial. It is well known that manual needling is an art, which is not easy to master and to quantify. This has been an issue for acupuncture studies. The very important basic finding reported in the 1970s that the analgesic effect of acupuncture could be totally abolished by IM injection of procaine deep into the acupoint before needle insertion strongly suggested that the signals produced by acupuncture is transmitted by peripheral nerves to the central nervous system (20). Therefore it would be rational to use electrical stimulation instead of mechanical stimulation. This has been confirmed by animal studies as well as human observations (21). By using the electrical stimulation one can easily and precisely change stimulation parameters such as frequency and intensity. In terms of frequency, low frequency electroacupuncture was reported to reduce pulsative index of ovarian blood flow (14, 15) and for hypothalamus-pituitary-ovary modulation (16), which would be difficult, if not impossible, to control by manual operations. In terms of intensity, we used strong current (15–20 mA) on the first TEAS session to stimulate uterine and ovarian blood flow. Whereas on the day of ET, we used milder current (12–15 mA) to induce relaxation for the subject. The third point is the selection of acupoints. Dieterle et al. (5) stressed on the importance of choosing the right points for achieving a favorable reproductive outcome. In fact, some acupoints, such as Hegu (LI 4), Sanyinjiao (SP 6), and

Shimen (RN 5, an acupoint close to Guanyuan used in this study), are taboo in women preparing for pregnancy, because of their role in up-regulating myoelectrical activities of the uterus (22). Therefore we chose acupoints related to increasing of blood flow before ET, and the nourishing points after ET. Another key point was the design of the placebo control. The mock TEAS used in the present study can fulfill the requirements of being psychologically active (a feeling of intermittent tingling sensation) and physiologically inactive (too weak to produce a therapeutic effect), which makes the conclusion more convincing and reliable.

Regarding the possible mechanisms underlying these effects, the following points deserve careful consideration. The first point is the uterine blood flow. High arterial blood flow impedance and low uterine perfusion constitute the cause of low implantation rate (23–25), and electroacupuncture has been reported to increase uterine blood flow in infertile women (26, 27). Optimal endometrial circulation is of utmost importance for endometrial receptivity and favorable reproductive outcome.

Second, psychological stress (both anxiety and depression) (28) or stressful life events (29) may negatively influence the clinical PR. Acupuncture was well known for its effect of releasing β -endorphin and enkephalin in the central nervous system, which is beneficial for reducing pain and anxiety (30). The use of acupuncture makes the subject undergoing IVF more relaxed and optimistic, which might be another mechanism related to the high PR (31, 32). In the present study, we found that many patients fell asleep when receiving TEAS treatment, whereas most patients in the mock TEAS group appeared nervous at the ET stage, suggesting TEAS's antianxiety effect.

Third, the stimulation produced by tube placement in the ET stage and the accompanying nervous feeling might cause uterine and endometrium contraction, which is unfavorable for embryo implantation (33). The previous study of Kim et al. (34) suggested that acupuncture could inhibit uterine motility through suppression of the COX-2 enzyme, therefore it is beneficial for embryo implantation.

The last point is the endometrial receptivity. Asynchrony between embryo implantation and endometrium development may serve as the most important reason for lost embryos. The possibility should be considered if acupuncture can promote the yield of some useful cytokines, such as integrin (35), leukemia inhibitory factor (LIF) (36), and heparin-binding epidermal growth factor (EGF)-like growth factor (37), thereby regulating the time window of implantation.

These are our speculations on the possible mechanisms of action of TEAS on pregnancy outcome in women undergoing ET. Studies are underway on the possible changes of endometrial receptivity (expression of pinopode and related cytokines) produced by TEAS of different parameters.

There are some limitations in the present study. We used eight acupoints for stimulation at a time, but the two-channel TEAS device can only provide four electrodes, therefore we had to use two units simultaneously. Although the two units were started at the same time, the outputs of the two units cannot be guaranteed to be absolutely synchronous. Therefore the real frequency of stimulation perceived by the subject may be 2 Hz (synchronous) or 4 Hz (asynchronous between the 2 units). To guarantee the uniformity of

stimulation, in the future study, we will use a TEAS unit with four channels (8 electrodes) driven by the same generator, or to reduce the number of acupoints from eight to four.

We conclude that, from the favorable effects on the pregnancy outcome of women undergoing IVF-ET, a large scale multicenter clinical trial is warranted to confirm the efficacy of TEAS. Meanwhile, exploration on the mechanisms of action is very much desirable.

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