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Original Article

Transcutaneous electrical acupoint stimulation (TEAS) treatment improves pregnancy rate and implantation rate in patients with implantation failure

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ABSTRACT

Objective: Although major advances have greatly improved the outcomes of assisted reproductive technology in the last two decades, there remains significant difficulty in achieving pregnancy for many patients even after repeated attempts of IVF. Interestingly, recent studies have shown that transcutaneous electrical acupoint stimulation (TEAS) can improve the reproductive outcomes of select IVF patients. To determine the utility of TEAS in improving IVF outcomes in patients with a history of implantation failure, we conducted a retrospective study of clinical outcomes of women, who had a prior history of unsuccessful pregnancy outcome after IVF-embryo transfer (IVF-ET), following TEAS treatment. **Materials and methods:** A total of 25 patients, who had failed to conceive after multiple IVF cycles in which good embryos were transferred, received noninvasive low frequency TEAS treatment prior to and during an IVF-ET cycle. The clinical outcomes, including biochemical pregnancy rate, clinical pregnancy rate and implantation rate, were compared to those of prior cycles which received only standard IVF treatment. **Results:** Analysis of reproductive outcomes showed that implantation rate and clinical pregnancy rate increased significantly in IVF cycles that included the TEAS treatment when compared to prior cycles that received only the standard IVF treatment in this cohort of patients.

Conclusions: This surprising finding indicated that TEAS treatment is a promising technique to improve reproductive outcomes in difficult cases of IVF-ET. Because TEAS treatment is noninvasive and has high reproducibility, and can be applied with limited training, further refinement of this procedure would not only substantiate the beneficial effects of TEAS, but also allow the technique to be more effective and reproducible.

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Introduction

Successful embryo implantation requires synchronous development of a competent blastocyst and a receptive endometrium. Implantation failure is a major predicament for many infertile couples and their physicians. Although the potential causes of

implantation failure remain to be fully defined, suboptimal endometrial receptivity is considered a major contributor to this etiology because normal endometrial receptivity, which is gauged by endometrial thickness, morphology, uterine blood flow and endometrial vascularity, is essential for the success of a pregnancy [1].

Earlier studies have shown that low-dose aspirin, or vaginal administration of sildenafil, micronized estradiol or pentoxifylline can partially improve uterine perfusion and endometrial receptivity in patients with thin endometrium [2]. In addition, a number of clinical procedures, including (1) flushing uterine cavity with hCG-supplemented medium before embryo transfer, (2) endometrial perfusion with granulocyte colony-stimulating factor (G-CSF), and (3) endometrial stimulation induced by local injury, have been attempted to improve fertility in patients with implantation failure.

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However, only limited and marginal improvements in implantation rate and pregnancy rate have been achieved with these procedures in the majority of patients with implantation failure [3,4].

Interestingly, recent studies of transcutaneous electrical acupoint stimulation (TEAS), which is a hybrid technique based on a combination of transcutaneous electrical stimulation of physical therapy and acupoint therapy from Traditional Chinese Medicine, indicated that this procedure could be beneficial for improving fertility in *in vitro* fertilization (IVF) patients. Specifically, TEAS has been reported to (1) improve basal endocrine levels in patients, (2) increase the number of oocytes retrieved, and high-quality embryos, and (3) improve endometrial receptivity, and clinical outcome of transferred embryos [5–8]. The interest in applying TEAS treatment to improve fertility in IVF patients is highlighted by several potential advantages of this technique, which applies low frequency pulse current into the body via skin contact. First, the TEAS treatment is noninvasive, and is devoid of discomfort or pain that is associated with traditional acupuncture. Second, the procedure has high reproducibility and can be applied with limited training.

Recent studies have shown that TEAS treatment at acupuncture points (or acupoints), including ST36 (Zusanli), KI3 (Taixi), BL23 (Shenshu) and RN4 (Guanyuan), may improve endometrial receptivity by increasing uterine blood flow and inhibiting uterine motility through the suppression of COX-2 activity [5]. Likewise, Stener-Victorin et al. and others have reported that low frequency electroacupuncture is effective in modulating the ovarian blood flow and the function of the hypothalamus–pituitary–ovary system [9–11]. Furthermore, low-frequency electrical acupuncture could stimulate the release of many neuropeptides such as serotonin, endogenous opioids and oxytocin, and reduce psychological stress through the release of β -endorphins and enkephalin in the central nervous system (CNS) [12,13]. Moreover, it has been reported that TEAS treatment may improve endometrial receptivity by enhancing endometrial HOXA10 expression, and endometrial vascularization [6].

Based on this understanding, we hypothesized that TEAS treatment could be beneficial for the treatment of infertility in patients with repeated implantation failure. In the present study, we applied the TEAS technique to IVF patients with a history of implantation failure, and analyzed its effect on implantation rate and pregnancy rate in a retrospective study.

Materials and methods

Study subjects, ethics, consent and permissions

The retrospective study was conducted with the approval of Institutional Review Board of Chang Gung Memorial Hospital, and was based on the review of assisted reproductive technology (ART) records. A total of 37 patients who had prior histories of unsuccessful pregnancy outcome after IVF-embryo transfer (IVF-ET) (i.e., no successful pregnancy after ≥ 2 times IVF-ET with good quality embryos) were recruited from September 2015 to August 2016 at Chang Gung Memorial Hospital infertility center (Fig. 1). These patients presented with repeated implantation failure and poor endometrial vascularization in which subendometrial vessels penetrated only to the outer hypoechogenic area surrounding the endometrium, but not the hyperechogenic outer margin [14].

All 37 patients received TEAS treatment during the study, and we excluded 12 patients from this analysis due to the presence of severe uterine cavity synechiae ($N = 3$), poor ovarian response ($N = 3$), endometrial cancer ($N = 1$), or personal reasons for not receiving embryo transfer after TEAS treatment ($N = 5$).

Clinical evaluation of embryos

The quality of blastocysts was scored by a modified simplified blastocyst grading system as described by Alison et al., 2015 [15]. Grade A was equal to 5 points, Grade B was equal to 4 points, Grade C was equal to 3 points, Cavitating was equal to 2 points and Compacting was equal to 1 point. A quality score for each embryo transfer was calculated as the means of embryo quality score of the total number of embryos transferred.

TEAS treatment

All patients received TEAS treatment 6–8 times per menstrual cycles by using an electrostimulator (EN-Stim 4, Enraf Nonius) with dispersed-dense waves at 2 Hz frequency via self-adhesive skin electrodes (Fig. 2). The TEAS treatment was repeated at least one cycle before the preparation of endometrium for embryo transfer, and once one week after the embryo transfer procedure.

Before embryo transfer, TEAS treatments were applied to five acupoints, including Guanyuan (RN4), Zhongji (RN3), Zigong (EX-CA1), Tianshu (ST25), and Sanyinjiao (SP6) bilaterally. The intensity was set at 15–20 mA. After embryo transfer, TEAS treatments were given at 30 min and one week after embryo transfer. In these sessions, the procedure was applied to four acupoints, including Zusanli (ST36), Taixi (KI3), Shenshu (BL23), and Guanyuan (RN4) bilaterally. The intensity was set at 8–10 mA.

In each episode of TEAS treatment, these acupoints were stimulated in pairs with the positive and negative electrodes placed on separate acupoints. For stimulation before embryo transfer, the acupoint pairs included (1) ST25 + RN3, (2) ST25 + RN4, and (3) EX-CA1 + SP6; whereas the stimulation after embryo transfer included (4) BL23 + KI3 and (5) RN4 + ST36 (Fig. 2).

Each episode of treatment included stimulation at all selected acupoints and lasted for 30 min. The acupoints were chosen based on protocols reported in earlier studies of TEAS treatment in IVF patients, and the Traditional Chinese Medicine dialectical treatment principle [5,6]. Specifically, the paired acupoints were chosen based on their vertical locations in order to allow electrical current flow in parallel with muscles surrounding the pelvis.

Analysis of biomarkers

To evaluate the effect of TEAS treatment, blood β -hCG levels were measured at 2 weeks after embryo transfer. The morphology of intrauterine gestational sac and fetal cardiac activity were evaluated by sonography procedure (GE Voluson S6) at 5 weeks after embryo transfer. Biochemical pregnancy was defined as having a β -hCG level >30 mIU/ml. Clinical pregnancy was defined as having visual confirmation of fetal cardiac activity based on transvaginal ultrasound examination.

Statistical analysis

Statistical analysis was carried out using SPSS (V.20.0). The data are presented as Mean (SD). Due to the small sample size, we performed non-parametric tests including Wilcoxon signed-rank test to compare two related samples. In all analyses, the difference was considered statistically significant when $p < 0.05$.

Results

Analysis of data from a total of 25 patients who received the full-course IVF-ET procedure and the TEAS treatment showed that the age of patients ranged from 27 to 44 (mean \pm SD = 35.9 ± 4.1 , Table 1), and the average body mass index (BMI) was 22.1 ± 4.7 . The

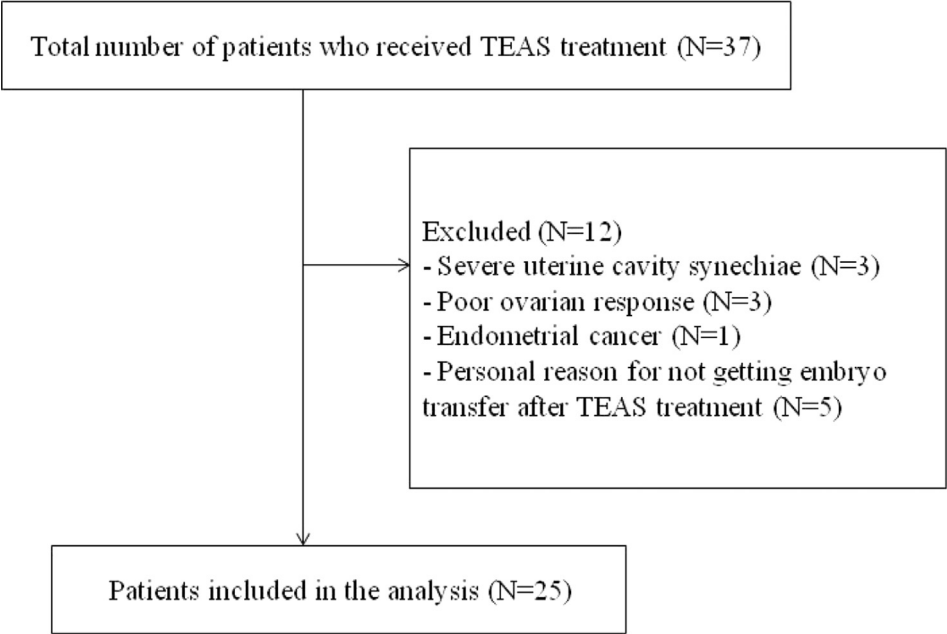


Fig. 1. Flowchart of patients who met the inclusion criteria for the study.

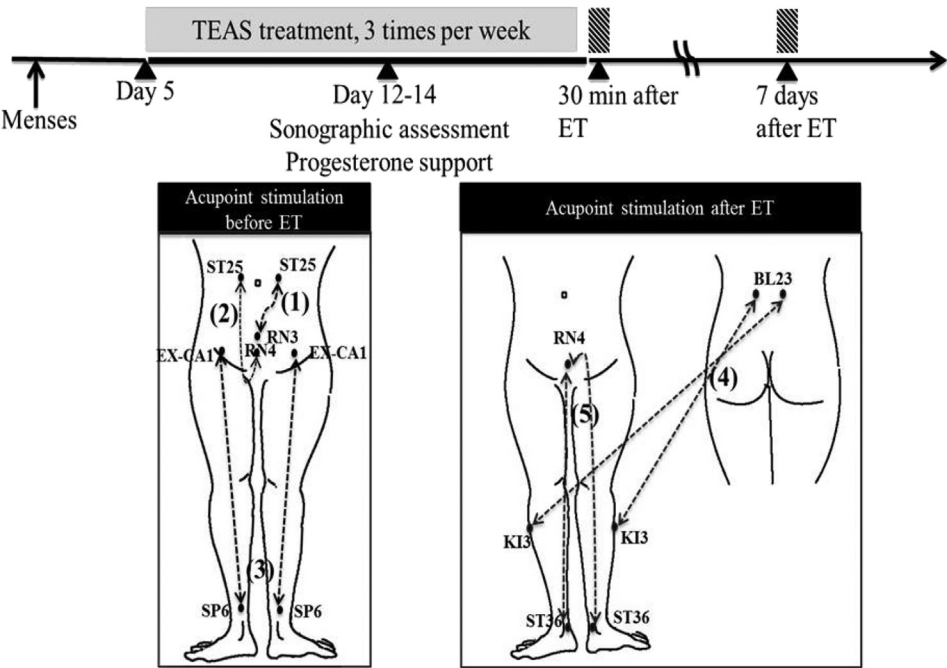


Fig. 2. Timeline of TEAS treatment during the IVF cycle. Schematic representation of acupoint stimulation before and after embryo transfer (ET) is depicted by the horizontal line. The acupoints, including ST25, RN3, RN4, EX-CA1, SP6, BL23, and ST36 are indicated black dots. The acupoint pairs that were stimulated together by opposite electrodes are connected by dotted lines. These acupoint pairs included (1) ST25 + RN3, (2) ST25 + RN4, (3) EX-CA1 + SP6, (4) BL23 + KI3, and (5) RN4 + ST36.

Table 1		
Basic information and fertility history of the patient cohort.		
Age	27–44 (mean ± SD = 35.9 ± 4.1)	
BMI	22.1 ± 4.7	
Dominant diagnosis	Ovulation factor	6 (24%)
	Tubal factor	13 (52%)
	Uterine factor	3 (12%)
	Unexplained factors	3 (12%)

dominant diagnosis for infertility in these patients included ovulation factor (6; 24%), tubal factor (13; 52%), uterine factor (3; 12%), and unexplained factors (3; 12%).

Analysis of prior medical records and embryo scores at the blastocyst stage showed that the blastocyst embryo scores were not significantly different between IVF cycles with or without the TEAS treatment (Table 2). Likewise, biochemical analysis showed that the estradiol (E2) levels at the IVF cycles that did not receive the TEAS

Table 2

Clinical characteristics and reproductive outcomes of IVF-ET procedures after TEAS treatment.

Characteristics	IVF cycle without TEAS (N = 25)	IVF cycle with TEAS (N = 25)	P value ^b
Number of IVF treatment	1.92 (1.47) ^a (N = 25)	1.24 (0.52) (N = 25)	0.06
Embryo Score (Blastocyst)	2.95 (0.99) (N = 20)	3.0 (1.08) (N = 20)	0.77
E2 levels before progesterone treatment (pg/ml)	772.4 (328.8) (N = 5)	507.4 (139.6) (N = 5)	0.12
Biochemical pregnancy rate	9.7% (23.3%) (N = 25)	51.3% (49.1%) (N = 25)	0.003 ^b
Clinical pregnancy rate	4.0% (20.0%) (N = 25)	32.0% (47.6%) (N = 25)	0.020 ^b
Implantation rate	3.9% (11.6%) (N = 23)	22.9% (25.1%) (N = 23)	0.010 ^b

^a Data are presented as Mean (SD).^b Wilcoxon signed-rank test.

treatment and subsequent IVF cycles that received the TEAS treatment did not differ significantly.

By contrast, analysis of reproductive outcomes showed that biochemical pregnancy rate, clinical pregnancy rate, and implantation rate increased significantly in IVF cycles that received the TEAS treatment when compared to prior cycles that received only the standard IVF treatment (Table 2). The biochemical pregnancy rate (51.3% vs. 9.7%) and the implantation rate (22.9% vs. 3.9%) in these patients increased by more than 5-fold, and the clinical pregnancy rate (32.0% vs. 4.0%) increased by more than 7-fold after receiving the accompanying TEAS treatment.

Discussion

In recent years, a number of reports have suggested that acupuncture treatment could have beneficial effects on fertility in IVF patients. Importantly, studies that applied TEAS treatment in IVF patients indicated that this quantifiable and reproducible procedure can improve reproductive outcomes [16]. Consistently, our study indicated that the application of TEAS treatment in patients with prior implantation failure significantly improves clinical pregnancy outcome (32.0% vs. 4.0%) and implantation rate (22.9% vs. 3.9%) in dozens of patients who experienced repeated implantation failure. This surprising finding indicated that TEAS treatment is a promising technique to improve reproductive outcomes in difficult cases of IVF-ET, and provided the basis for further improvement of this accessory fertility-improving procedure.

Successful implantation is a complex process that requires a receptive endometrium, a functional blastocyst, and a synchronized physical and biochemical dialog between the maternal and embryonic tissues. Although major advances have greatly improved the outcomes of assisted reproductive technology (ART) in the last two cascades, there remains significant difficulty in achieving pregnancy for many patients. The situation is particularly frustrating when the patients cannot achieve embryo implantation even after repeated attempts of IVF [17]. Due to the lack of understanding of the exact causes of implantation failure in humans, patients with repeated implantation failure have a minimal option of improving their chance of pregnancy with the current standard of care. Whereas the exact mechanism of action of TEAS remains to be defined, earlier studies indicated that TEAS is a pleiotropic procedure that could improve reproductive outcomes by triggering the release of a number of neuroendocrine and endocrine hormones as well as by regulating ovarian/uterine circulation. Specifically, acupoint stimulation has been shown to activate muscle afferents and modulate signal transmission in the spinal cord (segmental level) and the CNS. Low-frequency electrical acupoint stimulation can lead to the release of serotonin, endogenous opioids, β -endorphins, enkephalin, and oxytocin in the CNS, leading to functional changes in different organs [12,13]. In addition, low frequency electroacupuncture appeared to modulate ovarian blood flow, and regulate the activities of hypothalamus–pituitary–ovary axis [9–11]. Because TEAS is a quantifiable procedure that applies

acupuncture-like stimulation to acupoints without the disadvantages of pain and fear that are associated with traditional invasive acupuncture procedure, it has been adopted for pain relief treatment during labor [18] or in patients with dysmenorrhea [19]. With this background, we hypothesized that TEAS treatment could be particularly beneficial for IVF-ET patients who experienced implantation failure because the causes of reduced endometrial receptivity in these patients could be a combination of aberrant systemic hormonal control and local uterine homeostasis. However, the highly significant outcomes of the present study could be attributed to the careful selection of patient population. We have precluded cases that are likely associated with defective embryonic development and multifactorial effectors, and included only patients whose infertility was associated with repeated implantation failure (Table 1). Thus, the dominant cause of infertility in this study cohort could be mainly associated with suboptimal endometrial receptivity.

Unlike earlier studies of TEAS in IVF patients, which utilized a prospective randomized placebo-controlled design [5,6], we focused on the evaluation of reproductive outcomes in the same patients who received IVF cycles with or without the TEAS treatment. Because implantation failure in these patients could be associated with drastically different causes, this study design allowed us to compare the effects of TEAS treatment within a coherent physiological environment in individual patients, thus minimizing variations that are associated with the underlying causes of infertility. In addition, it is important to note that we have not observed any adverse effects in the patient cohort. Therefore, further refinement of the TEAS treatment would not only substantiate the beneficial effects of TEAS, but also allow the technique to be more effective and reproducible.

Although the exact endometrial volume and endometrial/sub-endometrial vascularity that are optimal for conception in IVF patients remains to be defined [20–23], normal endometrial blood flow and proper endometrial vascularization on the day of embryo transfer is important for a successful pregnancy [24]. Low endometrial/subendometrial vascularization was commonly found in cases with unexplained recurrent pregnancy loss when compared to controls that did not have recurrent implantation failure [1,25,26]. Because earlier studies have reported that TEAS may improve endometrial triple-line pattern, and endometrial/sub-endometrial vascularization in IVF patients [6], the observed positive effects of TEAS in the present study could be related to changes in uterine blood flow and uterine motility, in addition to regulation of the central sympathetic and neuroendocrine factors [5].

The major limitation of this study is associated with the modest size of patient cohort. Study of a large cohort of patients with a well-characterized cause of implantation failure is needed to validate the observed beneficial effects of TEAS treatment, and to optimize this novel accessory fertility treatment (e.g., stimulus frequency, amplitude of stimulation, treatment courses, and time-span of treatment of the TEAS regimens), thereby producing a pleasantly surprising result for many infertile patients.

Overall, our results suggested that TEAS treatment represents a potential noninvasive accessory treatment that may improve fertility in IVF patients with implantation failure. Because TEAS treatment is noninvasive and can be applied with limited training, further improvement of this technique has the potential to facilitate reproduction not only in difficult cases of IVF-ET but also IVF patients in general.

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Declaration of conflicting interests

The authors declare that they have no conflict of interest.

References

- [1] Abdallah Y, Naji O, Saso S, Pexsters A, Stalder C, Sur S, et al. Ultrasound assessment of the peri-implantation uterus: a review. *Ultrasound Obstet Gynecol* 2012;39:612–9.
- [2] Margalioth EJ, Ben-Chetrit A, Gal M, Eldar-Geva T. Investigation and treatment of repeated implantation failure following IVF-ET. *Hum Reprod* 2006;21:3036–43.
- [3] Simon A, Laufer N. Assessment and treatment of repeated implantation failure (RIF). *J Assist Reprod Genet* 2012;29:1227–39.
- [4] Coughlan C, Ledger W, Wang Q, Liu F, Demirel A, Gurgan T, et al. Recurrent implantation failure: definition and management. *Reprod Biomed Online* 2014;28:14–38.
- [5] Zhang R, Feng XJ, Guan Q, Cui W, Zheng Y, Sun W, et al. Increase of success rate for women undergoing embryo transfer by transcutaneous electrical acupoint stimulation: a prospective randomized placebo-controlled study. *Fertil Steril* 2011;96:912–6.
- [6] Shuai Z, Lian F, Li P, Yang W. Effect of transcutaneous electrical acupoint stimulation on endometrial receptivity in women undergoing frozen-thawed embryo transfer: a single-blind prospective randomised controlled trial. *Acupunct Med* 2015;33:9–15.
- [7] Zheng Y, Feng X, Mi H, Yao Y, Zhao Y, Li J, et al. Effects of transcutaneous electrical acupoint stimulation on ovarian reserve of patients with diminished ovarian reserve in in vitro fertilization and embryo transfer cycles. *J Obstet Gynaecol Res* 2015;41:1905–11.
- [8] Zheng CH, Zhang MM, Huang GY, Wang W. The role of acupuncture in assisted reproductive technology. *Evid Based Complement Altern Med* 2012;2012:1–15.
- [9] Stener-Victorin E, Waldenström U, Tagnfors U, Lundberg T, Lindstedt G, Janson PO. Effects of electro-acupuncture on anovulation in women with polycystic ovary syndrome. *Acta Obstet Gynecol Scand* 2000;79:180–8.
- [10] Stener-Victorin E, Kobayashi R, Kurosawa M. Ovarian blood flow responses to electro-acupuncture stimulation at different frequencies and intensities in anaesthetized rats. *Auton Neurosci* 2003;108:50–6.
- [11] Stener-Victorin E, Kobayashi R, Watanabe O, Lundberg T, Kurosawa M. Effect of electro-acupuncture stimulation of different frequencies and intensities on ovarian blood flow in anaesthetized rats with steroid-induced polycystic ovaries. *Reprod Biol Endocrinol* 2004;2:16.
- [12] Stener-Victorin E, Wu X. Effects and mechanisms of acupuncture in the reproductive system. *Auton Neurosci* 2010;157:46–51.
- [13] Maliqueo M, Benrick A, Alvi A, Johansson J, Sun M, Labrie F, et al. Circulating gonadotropins and ovarian adiponectin system are modulated by acupuncture independently of sex steroid or beta-adrenergic action in a female hyperandrogenic rat model of polycystic ovary syndrome. *Mol Cell Endocrinol* 2015;412:159–69.
- [14] Chien LW, Au HK, Chen PL, Xiao J, Tzeng CR. Assessment of uterine receptivity by the endometrial-subendometrial blood flow distribution pattern in women undergoing in vitro fertilization-embryo transfer. *Fertil Steril* 2002;78:245–51.
- [15] Richardson A, Brearley S, Ahitan S, Chamberlain S, Davey T, Zujovic L, et al. A clinically useful simplified blastocyst grading system. *Reprod Biomed Online* 2015 Oct;31(4):523–30.
- [16] Zheng CH, Zhang J, Wu J, Zhang MM. The effect of transcutaneous electrical acupoint stimulation on pregnancy rates in women undergoing in vitro fertilization: a study protocol for a randomized controlled trial. *Trials* 2014 May 9;15:162.
- [17] Shapiro BS, Richter KS, Harris DC, Daneshmand ST. Dramatic declines in implantation and pregnancy rates in patients who undergo repeated cycles of in vitro fertilization with blastocyst transfer after one or more failed attempts. *Fertil Steril* 2001;76:538–42.
- [18] Chao AS, Chao A, Wang TH, Chang YC, Peng HH, Chang SD, et al. Pain relief by applying transcutaneous electrical nerve stimulation (TENS) on acupuncture points during the first stage of labor: a randomized double-blind placebo-controlled trial. *Pain* 2007;127:214–20.
- [19] Proctor ML, Smith CA, Farquhar CM, Stones RW. Transcutaneous electrical nerve stimulation and acupuncture for primary dysmenorrhoea. *Cochrane Database Syst Rev* 2002, CD002123.
- [20] Kupesic S, Bekavac I, Bjelos D, Kurjak A. Assessment of endometrial receptivity by transvaginal color Doppler and three-dimensional power Doppler ultrasonography in patients undergoing in vitro fertilization procedures. *J Ultrasound Med* 2001;20:125–34.
- [21] Wu HM, Chiang CH, Huang HY, Chao AS, Wang HS, Soong YK. Detection of the subendometrial vascularization flow index by three-dimensional ultrasound may be useful for predicting the pregnancy rate for patients undergoing in vitro fertilization-embryo transfer. *Fertil Steril* 2003;79:507–11.
- [22] Jarvela IY, Sladkevicius P, Kelly S, Ojha K, Campbell S, Nargund G. Evaluation of endometrial receptivity during in-vitro fertilization using three-dimensional power Doppler ultrasound. *Ultrasound Obstet Gynecol* 2005;26:765–9.
- [23] Alcazar JL. Three-dimensional ultrasound assessment of endometrial receptivity: a review. *Reprod Biol Endocrinol* 2006;4:56.
- [24] Kim A, Jung H, Choi WJ, Hong SN, Kim HY. Detection of endometrial and subendometrial vasculature on the day of embryo transfer and prediction of pregnancy during fresh in vitro fertilization cycles. *Taiwan J Obstet Gynecol* 2014;53:360–5.
- [25] Chen L, Quan S, Li H, Chen C, Xing F, Yu Y. A comparison of endometrial and subendometrial vascularity assessed by three-dimensional ultrasonography and power Doppler angiography between healthy fertile women and women with unexplained primary recurrent miscarriage. *Fertil Steril* 2011;95:1127–9.
- [26] El-Zenneni H, Moustafa R, Abdel-Hafeez M, El-Salally H, Abdel-Kader A, Elnaggar A. Assessment of uterine, subendometrial blood flows and endometrial gland vascular endothelial growth factor (EG-VEGF) in women with unexplained infertility. *Middle East Fertil Soc J* 2015;20:119–26.