

A randomized double blind comparison of real and placebo acupuncture in IVF treatment

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BACKGROUND: Acupuncture has been used during IVF treatment as it may improve outcome, however, there are concerns about the true efficacy of this approach. This randomized double blind study aimed to compare real acupuncture with placebo acupuncture in patients undergoing IVF treatment. **METHODS:** On the day of embryo transfer (ET), 370 patients were randomly allocated to either real or placebo acupuncture according to a computer-generated randomization list in sealed opaque envelopes. They received 25 min of real or placebo acupuncture before and after ET. The endometrial and subendometrial vascularity, serum cortisol concentration and the anxiety level were evaluated before and after real and placebo acupuncture. **RESULTS:** The overall pregnancy rate was significantly higher in the placebo acupuncture group than that in the real acupuncture group (55.1 versus 43.8%, respectively, $P = 0.038$; Common odds ratio 1.578 95% confidence interval 1.047–2.378). No significant differences were found in rates of ongoing pregnancy and live birth between the two groups. Reduction of endometrial and subendometrial vascularity, serum cortisol concentration and the anxiety level were observed following both real and placebo acupuncture, although there were no significant differences in the changes in all these indices between the two groups. **CONCLUSIONS:** Placebo acupuncture was associated with a significantly higher overall pregnancy rate when compared with real acupuncture. Placebo acupuncture may not be inert. Trial registered with HKClinicalTrials.com: number HKCTR-236.

Keywords: acupuncture; IVF; pregnancy rate

Introduction

IVF-embryo transfer (IVF-ET) is an effective treatment for various causes of infertility. Despite improvement in ovarian stimulation regimens, culture media conditions and the technique of ET, there has not been a significant increase in the implantation rates of cleaving embryos, which have remained steady at 20–25% for a long time (ESHRE, 2001, 2008).

Acupuncture has been used during IVF treatment, either on the day of transvaginal ultrasound-guided oocyte retrieval (TUGOR) (Stener-Victorin *et al.*, 1999, 2003; Humaidan and Stener-Victorin, 2004; Gejervall *et al.*, 2005; Sator-Katzenschlager *et al.*, 2006) or the day of ET (Paulus *et al.*, 2002, 2003; Dieterle *et al.*, 2006; Smith *et al.*, 2006; Westergaard *et al.*, 2006). A meta-analysis (Ng *et al.*, 2008) of these 10 randomized studies revealed a significant improvement of the pregnancy rate in favour of acupuncture treatment [odds ratio (OR) 1.42, 95% confidence interval (CI) 1.17–1.72]. A subgroup analysis detected a significant improvement of the pregnancy rate for acupuncture treatment when it was administered on the day of ET (OR 1.83, 95% CI 1.40–2.39) but no

improvement of the pregnancy rate when acupuncture treatment was administered on the day of TUGOR only (OR 1.07, 95% CI 0.81–1.42). These data suggest that acupuncture improves IVF outcomes only when it is done on the day of ET. The positive effect of acupuncture during IVF treatment may be related to the change in uterine blood flow and uterine contractility, and relaxation of stress (Ng *et al.*, 2008).

Another meta-analysis reveals similar findings (Manheimer *et al.*, 2008). However, there is still concern about the efficacy of acupuncture in IVF (Pinborg *et al.*, 2008) as the improvement in the pregnancy rates of IVF treatment with acupuncture is higher than that for drugs or other procedures given to enhance the success of this treatment and the underlying biological mechanism is difficult to explain. Patients were not blinded in the majority of the above randomized studies because of the difficulty in achieving blindness in the acupuncture treatment. The study of Smith *et al.* (2006) was single blind and only that of Sator-Katzenschlager *et al.* (2006) was double blind. Sator-Katzenschlager *et al.* (2006) found that auricular acupuncture with electric stimulation significantly

increased the IVF pregnancy rate when compared with that of auricular acupuncture without electric stimulation and of the controls. It remains uncertain whether the improvement of the pregnancy rate following acupuncture is related to psychological factors. A double blind study is urgently needed to confirm the efficacy of acupuncture in IVF.

The aim of this randomized double blind study was to compare real acupuncture with placebo acupuncture performed on the day of ET in patients undergoing IVF treatment. The hypothesis was that real acupuncture performed on the day of ET significantly improved the pregnancy rate of IVF treatment.

Materials and Methods

Infertile patients undergoing IVF treatment in the Centre of Assisted Reproduction and Embryology, The University of Hong Kong-Queen Mary Hospital, were recruited if they had a normal uterine cavity shown on ultrasound scanning on the day of TUGOR. Exclusion criteria were: (i) patients who had an abnormal uterine cavity and (ii) cancellation of ET due to failed fertilization or the risk of ovarian hyperstimulation syndrome (OHSS). Indications for IVF included tubal, male, endometriosis, unexplained and mixed factors. ICSI was performed for couples with severe semen abnormalities. Serum basal FSH concentration was checked on Day 2–3 of the cycle within 2–3 months of commencing treatment. Every patient gave an informed written consent prior to participating in the study, which was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (HKClinicalTrials.com–HKCTR-236). Patients were recruited to join the study only once and did not receive any monetary compensation for participation in the study.

The details of the long protocol of ovarian stimulation regimen, gametes handling, standard insemination and ICSI were as previously described (Ng *et al.*, 2000). In short, patients were pre-treated with busserelin (Suprecur, Hoechst, Frankfurt, Germany) nasal spray 150 µg four times a day from the mid-luteal phase of the cycle preceding the treatment cycle. On the second day of the treatment cycle, transvaginal scanning was performed to count the number of antral follicles and blood was then taken for basal serum estradiol (E₂) concentration. Antral follicle count was the sum of antral follicles on the left and right sides. When the ultrasound scanning showed no ovarian cyst and serum E₂ concentrations were below 200 pmol/l, HMG (Menogon, Ferring GmbH, Kiel, Germany) was started for ovarian stimulation. Ovarian response was monitored by serial transvaginal scanning and hCG (Profasi, Serono, Geneva, Switzerland) was given i.m. when the leading follicle reached 18 mm in diameter and there were at least three follicles of ≥ 16 mm in diameter. Serum E₂ concentration was measured on the day of hCG administration.

TUGOR was scheduled 36 h after the hCG injection and ET was performed under transabdominal ultrasound guidance 2 days after the retrieval. Embryos were examined for the number/regularity of blastomeres and the degree of fragmentation and graded according to the criteria by Veeck (1988). Patients were allowed to have a maximum of two embryos replaced into the uterine cavity 2 days after TUGOR. Excess good-quality embryos were frozen on the day of ET. All fresh embryos were cryopreserved if patients had developed symptoms suggestive of OHSS or serum E₂ on the day of hCG injection was >20 000 pmol/l in order to reduce the risks of OHSS. Luteal phase was supported by two doses of hCG (1500 IU on the day of ET and 6 days after ET) or vaginal progesterone, 400 mg two times per day for 14 days after ET (Cyclogest, Cox Pharmaceuticals, Barnstaple, UK). A urine pregnancy test (SureStep™ One step hCG pregnancy

test, Unipath Limited, Bedford, UK) was done 16 days after ET. If it was positive, ultrasound examination was performed 10–14 days later to confirm intrauterine pregnancy and to determine the number of gestational sacs present.

TCM diagnosis

Eligible patients were informed of the study on the day of TUGOR and those who agreed to join the study were further counselled on the day of ET. A registered traditional Chinese medicine (TCM) practitioner (E.W.S.S.) categorized these patients' conditions by the four diagnostic methods: observation, auscultation, interrogation and palpation according to the TCM principles (Maciocia, 1998). They were classified into the related syndromes which included Kidney Yang/Yin deficiency, Liver Qi stagnation with blood stasis, Spleen Qi deficiency with Phlegm and combination of those syndromes.

Assignment and masking

Patients were then randomized according to a computer-generated randomization list in sealed, opaque envelopes into two groups: real acupuncture and placebo acupuncture groups. The randomization sequence was in a block of 10 with 1:1 ratio and was stratified according to the cycle number, i.e. the first cycle and the repeated cycle. The randomization list was generated and kept by a project nurse not involved in the clinical care of these patients. The sequence of randomization was concealed until interventions were assigned. Patients, clinical staff involved in the care of patients and embryologists were blinded to the treatment group assigned. The codes for the treatment groups were revealed only after the completion of the whole study and statistical analysis.

All acupuncture treatments were performed in the same way by the same certified acupuncturist (E.W.Z.S.) who had completed the degree of Chinese Medicine and had 2 years of experience in acupuncture. The acupuncturist followed a standard way of communicating with patients whether they were in the real or placebo acupuncture groups.

Real acupuncture group

Patients in the real acupuncture group received real acupuncture for 25 min before and after ET. Sterile disposable stainless steel needles (Mayfair Medical Supplies Ltd., Hwato, Suzhou, China; 0.30 × 40 mm) were inserted into the acupoints. The acupoints used before ET were PC6 (Neiguan), SP8 (Diji), LR3 (Taichong), GV20 (Baihui) and ST29 (Guilai). After ET, the needles were inserted at ST36 (Zusanli), SP6 (Sanyinjiao), SP10 (Xuehai) and LI4 (Hegu). The designation of acupoints adhered to the 2nd edn of the Standard Acupuncture Nomenclature (World Health Organization Regional Office for the Western Pacific, 1993).

The depth of the needle insertion into the skin depended on the location of the acupoints, ranging from 10 to 20 mm. Needle reaction (soreness, numbness, or distension around the puncture sites or sometimes propagate along the corresponding meridians which termed the DeQi sensation) was elicited during the initial insertion. After 10 min, the needles were stimulated manually by rotating, lifting and thrusting the handle of the needle in order to maintain DeQi sensation. The needles were retained in position for 25 min and then removed.

Placebo acupuncture group

Patients in the placebo acupuncture group received placebo acupuncture for 25 min before and after ET. The Streitberger's placebo needles (Streitberger and Kleinhenz, 1998; Asiamed, Pullach, Germany; 0.30 × 30 mm) were used. The appearance of the placebo needle was the same as the needle used in the real acupuncture group. The placebo needle was not fixed into the copper handle and

the tip of the needle was blunt. When it was pushed forward against the skin, the needle slid into the handle and the whole needle appeared shortened. This also gave patients a pricking penetration sensation. To place the placebo needle in position, the Park's placebo device (Park *et al.*, 1999; Dong Bang AcuPrime, Exeter, UK) was used. The same acupoints and procedures as in the real acupuncture group were applied.

Measurement of endometrial and subendometrial vascularity

The endometrial and subendometrial vascularity was measured by three-dimensional (3D) ultrasound with power Doppler (Voluson 730[®], GE Healthcare, Zipf, Austria) before and after the first real or placebo acupuncture session prior to ET. The details of 3D ultrasound and data analysis were as previously described (Ng *et al.*, 2004). The setting conditions of the 3D power Doppler ultrasound machine for this study were as follows: frequency, mid; dynamic set, 2; balance, G > 140; smooth, 5/5; ensemble, 12; line density, 7; and power Doppler map, 5. The setting conditions for the sub-power Doppler mode were as follows: gain, 6.0; balance, 140; quality, normal; wall motion filter, low 1 and PRF, 0.9 kHz. The resulting truncated sector was adjusted to cover the endometrial cavity in a longitudinal plane of the uterus. The sweep angle was set to 90° to ensure the uterine volume and its subendometrium was scanned completely. During the volume acquisition, the patient and the 3D transvaginal probe was held in position as still as possible. A 3D data set then was acquired and the resulting multi-planar display was then examined to ensure the area of interest was captured entirely. If the image was scanned without power Doppler artefact, the data set was saved for later analysis.

The built-in VOCAL[®] (Virtual Organ Computer-Aided Analysis) Imaging Program for the 3D power Doppler histogram was used in the analysis, along with computer algorithms, to measure the endometrial volume and vascularity. Vascularization index (VI) represents the presence of blood vessels in the endometrium and is expressed as a percentage of the endometrial volume. Flow index (FI) represents the mean power Doppler signal intensity within the endometrium and is expressed as the average intensity of flow. Vascularization flow index (VFI) is a combination of vascularity and flow intensity and is calculated by multiplying VI and FI. During analysis and calculation, the manual mode of the VOCAL Contour Editor was used. Twelve contour planes were analyzed for the whole endometrium of each patient to cover 180° by a 15° rotation step. After the assessment of the endometrium, the subendometrium was analyzed by the application of shell imaging. A variable contour which parallels the originally defined surface contour was then generated. In the present study, the subendometrial region was defined as within 1 mm of the originally defined myometrial-endometrial contour. The VI, FI and VFI of the subendometrial region were calculated accordingly (Ng *et al.*, 2006).

The intra-observer reliability was expressed as the mean intra-class correlation coefficient (ICC) with 95% CI. The ICCs for 3D scanning of VI, FI and VFI were 0.9929 (95% CI 0.9715–0.9982), 0.9810 (95% CI 0.9233–0.9953) and 0.9912 (95% CI 0.9644–0.9978), respectively. The mean ICCs for data acquisition of VI, FI and VFI were 0.9929 (95% CI 0.9821–0.9972), 0.9960 (95% CI 0.9898–0.9984) and 0.9909 (95% CI 0.9770–0.9964), respectively.

Measurement of serum cortisol concentration and the anxiety level

Blood samples were drawn immediately before the first real or placebo acupuncture session and after the second session. Serum was stored at –20°C until assayed. Serum cortisol concentration was then determined by using a chemiluminescent immunoassay (ADVIA Centaur[®] Immunoassay System, Siemens Medical Solutions Diagnostics Ltd.,

Hong Kong, China). The sensitivity of the cortisol assay was 0.2 µg/dl with intra- and inter-assay coefficients of variation 3.69 and 5.45%, respectively.

The anxiety level was assessed before the first real or placebo acupuncture session and after the second session by Trait-State Anxiety Questionnaire (Shek, 1993).

Evaluation of side effects and blindness of the study

At the end of the second real or placebo acupuncture session, the occurrence of side effects was reported by a self-completed questionnaire and the validity of blindness to the group assignment was assessed by asking patients to guess whether they were placed in the real or placebo acupuncture groups.

Statistical analysis

The pregnancy rates in the acupuncture group and the control group were 42.5 and 26.3%, respectively, in a previous study (Paulus *et al.*, 2002). The pregnancy rate per transfer at our unit in 2005 was 35%. Assuming 15% increase in the pregnancy rate after acupuncture, 185 patients in each arm was required at a power of 80% and a significance level of 5% (Sigmastat, Jandel Scientific, San Rafael, CA, USA). Therefore, 370 patients were recruited in this study.

The primary outcome measure was the overall pregnancy rate which was defined by a positive urinary pregnancy test. Secondary outcome measures included the implantation rate, the clinical pregnancy rate, the ongoing pregnancy rate, the live birth rate, the endometrial and subendometrial vascularity, the cortisol concentration and the anxiety level. The implantation rate was the proportion of embryos transferred resulting in an intrauterine gestational sac. Patients with one or more intrauterine gestational sacs on scanning or the histological confirmation of gestational product in miscarriages were considered as having clinical pregnancies. Ongoing pregnancies were those pregnancies beyond 10 weeks of gestation and the patients were at the stage of referral to antenatal care. A baby born alive after 24 weeks gestation was classified as a live birth.

One sample of the Kolmogorov–Smirnov test was used to test the normal distribution of continuous variables. Continuous variables were given as mean ± SD if normally distributed, and as median (interquartile range) if not normally distributed. Statistical comparison was carried out according to the intention-to-treat by Student's *t*-test, Mann–Whitney *U*-test, Wilcoxon signed ranks test for continuous variables and chi-square test for categorical variables, where appropriate. Statistical analysis was performed using the Statistical Program for Social Sciences (SPSS Inc., Version 14.0, Chicago, USA). The two-tailed value of $P < 0.05$ was considered statistically significant.

Results

Participant flow

During the study period between August 2006 and July 2007, 511 couples underwent ovarian stimulation for IVF treatment. A total of 14 patients did not proceed to TUGOR because of poor or no ovarian response after ovarian stimulation. After screening, 115 women were not eligible: 64 with ET canceled because of failed fertilization or risk of OHSS; 37 who had joined the study before and 14 with abnormal uterine cavity. Twelve women declined the invitation because of personal reasons and 370 eligible women agreed to participate in the study. All recruited patients completed the study and their

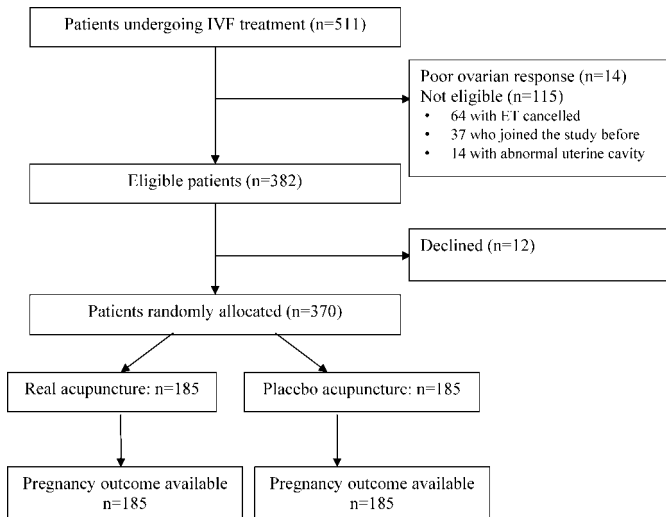


Figure 1: Flow chart of the randomized double blind study of patients undergoing acupuncture during IVF.

pregnancy outcomes were available. The flow chart of subjects is shown in Fig. 1.

Analysis

The real and placebo acupuncture groups were comparable in terms of the age of women, duration of infertility, proportion of primary infertility, smoking habit, previous experience in

acupuncture, cause of infertility, the insemination method, the cycle number, BMI, serum basal FSH concentration, antral follicle count, HMG dosage, HMG duration, number of oocytes obtained, the number of embryos and the type of luteal phase support (Table I). Embryo quality in terms of blastomere number and grading and the distribution of TCM syndromes were comparable for both groups (data not shown).

The overall pregnancy rate was significantly higher in the placebo acupuncture group than that in the real acupuncture group (55.1 versus 43.8%, respectively, $P = 0.038$; Common OR 1.578, 95% CI 1.047–2.378) (Table II). No significant differences in rates of clinical pregnancy, ongoing pregnancy, live birth rate, implantation, miscarriage and ectopic pregnancy were demonstrated between real and placebo acupuncture groups.

The median general anxiety trait scale were similar for the real and placebo acupuncture groups [47.00 (44.00–49.00) versus 47.00 (45.00–49.00), respectively, $P = 0.697$]. There were no significant differences in all 3D power Doppler flow indices of the endometrial and subendometrial regions, serum cortisol concentration and the anxiety level between the placebo and real acupuncture groups, before and after the acupuncture treatment (Table III). The differences of these parameters between before and after the acupuncture treatment were also similar for both groups. However, the 3D power Doppler flow indices of the endometrial and subendometrial regions, serum cortisol concentration and the anxiety level

Table I. Comparison of demographic characteristics and ovarian response in patients undergoing acupuncture during IVF.

	Real acupuncture (n = 185)	Placebo acupuncture (n = 185)
Age of women (years) ^a	36.0 (33–38)	36.0 (34–38)
Duration of infertility (years) ^a	4.0 (2.0–6.0)	4.0 (2.5–7.0)
Primary infertility ^b	118 (64)	109 (59)
Smoker ^b	11 (6)	9 (5)
Previous acupuncture experience ^b	58 (31)	53 (29)
Cause of infertility ^b		
Tuboperitoneal	33 (18)	26 (14)
Endometriosis	22(12)	16 (9)
Male	96 (52)	98 (53)
Unexplained	15 (8)	18 (10)
Mixed	19 (10)	27 (14)
Cycle number ^b		
First cycle	129 (70)	128 (69)
Repeated cycle	56 (30)	57 (31)
Insemination method ^b		
Conventional	131 (71)	128 (69)
ICSI	54 (29)	57 (31)
Body mass index (kg/m ²) ^c	21.6 ± 2.1	21.7 ± 2.7
Basal FSH concentration (IU/l) ^a	8.1 (6.3–9.7)	7.9 (6.5–9.8)
Antral follicle count ^a	8.0 (5–12)	8.0 (6–13)
HMG dosage (IU) ^a	2100 (1650–2700)	2100 (1650–2850)
HMG duration (days) ^a	10.0 (9–12)	11.0 (9–12)
Estradiol on day of hCG (pmol/l) ^a	7410 (5230–11 033)	7348 (4845–11 103)
No. of oocyte obtained ^a	8.0 (5–12)	7.0 (5–11)
No. of embryos transferred ^b		
One	23 (12)	16 (9)
Two	162 (88)	169 (91)
Luteal phase support ^b		
hCG	129 (69.7)	119 (64.3)
Vaginal progesterone	56 (30.3)	66 (35.7)

^aData are given in median (interquartile range).

^bData are given in number (%).

^cData are given in mean ± SD.

Table II. Comparison of pregnancy outcomes.

	Real acupuncture	Placebo acupuncture	P-value
Overall pregnancy rate	43.8 (81/185)	55.1 (102/185)	0.038*
Clinical pregnancy rate	38.9 (72/185)	49.2 (91/185)	0.059
Ongoing pregnancy rate	31.9 (59/185)	40.5 (75/185)	0.105
Live birth rate	29.7 (55/185)	38.4 (71/185)	0.100
Implantation rate	28.0 (97/347)	32.8 (116/354)	0.189
Miscarriage rate	32.1 (26/81)	30.4 (31/102)	0.931
Ectopic pregnancy rate	2.5 (2/81)	1.0 (1/102)	0.585

Data are given in %; *Statistically significant difference.

were significantly ($P < 0.001$) lower after the acupuncture treatment in both real and placebo acupuncture groups, when compared with that before the acupuncture treatment.

No significant differences were found in the occurrence of side effects between the real and placebo acupuncture groups (Table IV). The severity of these side effects was mild to moderate and no serious adverse effects were reported. There was no difference between the two groups in the proportion of women who could correctly guess the treatment allocation (Table IV).

Discussion

All patients in this double blind study followed our standard IVF protocol and all acupuncture procedures were performed by the same certified acupuncturist. The real and placebo acupuncture groups were comparable for demographic characteristics, the cycle number, ovarian responses and the number of embryos transferred. We found a significantly higher overall pregnancy rate following placebo acupuncture when compared with that of real acupuncture. There was a trend of higher rates of clinical pregnancy, ongoing pregnancy, live birth and implantation in the placebo acupuncture group, although the differences did not reach statistical significance. Surprisingly, significant changes in endometrial and subendometrial vascularity, serum cortisol concentration and the anxiety level were documented following both real and placebo acupuncture.

Our findings were clearly contradictory to those of other studies (Paulus *et al.*, 2002, 2003; Dieterle *et al.*, 2006; Smith *et al.*, 2006; Westergaard *et al.*, 2006). The acupoints and acupuncture protocol used in the present study were same as those used in Paulus *et al.* (2002), except auricular acupuncture was not used. Other researchers (Dieterle *et al.*, 2006; Smith *et al.*, 2006; Westergaard *et al.*, 2006) employed similar acupoints and acupuncture protocol. There are two possible explanations for our interesting results: real acupuncture may be associated with a lower pregnancy rate or placebo acupuncture may lead to a higher pregnancy rate. There is so far no evidence to suggest that real acupuncture would adversely affect IVF outcomes because the pregnancy rate was higher in the acupuncture group than in the control group in all of the above studies (Manheimer *et al.*, 2008; Ng *et al.*, 2008).

Acupuncture can produce strong placebo effects (Amanzio and Benedetti, 1999; Birch, 2006). When we planned the present study, we believed that the best way to study the effect of acupuncture in IVF treatment was a double blind

Table III. Comparison of endometrial and subendometrial vascularity, serum cortisol concentration and the anxiety level between real and placebo acupuncture groups.

	Real acupuncture			Placebo acupuncture			P-value ¹	P-value ²	P-value ³
	Before	After	Change ^c	Before	After	Change ^c			
Endometrial VI (%) ^a	0.180 (0.005–0.718)	0.014 (0–0.297)	0.037 (0–0.480)	0.188 (0.004–0.679)	0.021 (0–0.287)	0.036 (0–0.375)	0.878	0.942	0.641
Endometrial FI (0–100) ^a	20.378 (16.847–22.733)	18.346 (0–21.570)	0.844 (–0.259–11.770)	20.397 (16.830–23.309)	18.069 (0–21.075)	1.165 (0–7.512)	0.617	0.824	0.781
Endometrial VFI (0–100) ^a	0.039 (0.001–0.170)	0.003 (0–0.065)	0.007 (0–0.112)	0.041 (0.001–0.145)	0.004 (0–0.079)	0.007 (0–0.079)	0.910	0.996	0.771
Subendometrial VI (%) ^a	0.340 (0.024–1.304)	0.041 (0–0.482)	0.158 (0–0.975)	0.273 (0.024–1.167)	0.076 (0–0.436)	0.075 (0–0.558)	0.434	0.365	0.293
Subendometrial FI (0–100) ^a	21.607 (16.980–24.189)	17.941 (0–22.359)	1.543 (0–16.029)	20.793 (17.306–23.896)	18.791 (0–22.377)	0.403 (–0.919–6.880)	0.656	0.459	0.111
Subendometrial VFI (0–100) ^a	0.078 (0.005–0.317)	0.007 (0–0.108)	0.033 (0–0.235)	0.061 (0.004–0.255)	0.015 (0–0.097)	0.016 (0–0.135)	0.420	0.650	0.284
Cortisol (µg/dl) ^b	14.68 ± 4.13	9.05 ± 3.04	5.62 ± 4.27	14.81 ± 4.58	9.64 ± 4.12	5.18 ± 4.97	0.760	0.120	0.354
Anxiety level ^b	42.90 ± 5.90	41.72 ± 5.80	1.18 ± 4.90	43.59 ± 5.86	42.02 ± 5.62	1.58 ± 5.67*	0.258	0.623	0.468

P-value¹, comparing parameters before acupuncture between real and placebo groups; P-value², comparing parameters after acupuncture between real and placebo groups; P-value³, comparing change in parameters between real and placebo groups.

VI, vascularization index; FI, flow index; VFI, vascularization flow index.

^aData are given in median (interquartile range).

^bData are given in mean ± SD.

^cChange = Before – After.

Table IV. Comparison of side effects and guess of treatment groups.

	Real acupuncture (<i>n</i> = 185)	Placebo acupuncture (<i>n</i> = 185)	<i>P</i> -value
Side effects			
Nausea	2.7 (5)	2.7 (5)	1.000
Dizziness	5.4 (10)	4.3 (8)	0.810
Fainting	5.9 (11)	3.2 (6)	0.321
Tiredness	12.4 (23)	16.2 (30)	0.373
Drowsiness	15.7 (29)	22.2 (41)	0.144
Headache	4.3 (8)	2.2 (4)	0.380
Chest pain	1.6 (3)	0.5 (1)	0.623
Puncture site itching	28.1 (52)	19.5 (36)	0.067
Guess of treatment groups			0.057
Real	60.0 (111)	51.4 (95)	
Placebo	8.1 (15)	15.7 (29)	
Uncertain	31.9 (59)	32.9 (61)	

Data are given as % (*n*).

setting. Various types of controls have been used in acupuncture studies, including no acupuncture, placebo acupuncture and sham acupuncture. For double blind studies, either sham or placebo acupuncture has to be used as controls. Sham acupuncture can be performed by inserting needles into non-acupoints or acupoints with superficial penetration. Sham acupuncture, however, can still elicit responses and might not be an inert control (Vincent and Lewith, 1995; Birch, 2006). Placebo acupuncture is a non-invasive acupuncture because the needle would not penetrate into skin and can be performed by using blunted needles, fingernails, toothpicks or retractable needles (Goddard *et al.*, 2005). The non-invasive placebo acupuncture appears to be the best control in acupuncture studies (Stener-Victorin *et al.*, 2002).

In the present study, we used Streitberger's placebo needles and the Park's devices in the placebo acupuncture group. Both the Streitberger's placebo needle (White *et al.*, 2003) and the Park's device (Park *et al.*, 2002, 2005) have been confirmed to be reliable controls in randomized studies. Although about 30% of our subjects had previous acupuncture experience, there was no difference in the proportion of correct guessing of the treatment group between the two groups and the validity of blinding was confirmed.

Based on our results, placebo acupuncture may not be an inert control as well. Significant changes in endometrial and subendometrial vascularity, serum cortisol concentration and anxiety level were observed following the placebo and real acupuncture treatment. On the other hand, there were no significant differences in the changes in endometrial and subendometrial vascularity, serum cortisol concentration and anxiety level between the placebo and real acupuncture groups. In order to clarify whether the changes in endometrial and subendometrial vascularity were related to bed rest during acupuncture, prior to ET we measured the endometrial and subendometrial vascularity by 3D power Doppler ultrasound in another 27 IVF patients before and after 25 min of bed rest. We could not observe any significant change in all 3D power Doppler flow indices of endometrial and endometrial regions (unpublished data). This implied that the changes in

endometrial and subendometrial vascularity were due to the effect of real and placebo acupuncture.

Acupressure over acupoints has been shown to be an effective intervention for a number of clinical conditions such as alleviation of dysmenorrhoea, reduction of anxiety and nausea and vomiting during pregnancy (Beal, 1999, Markose *et al.*, 2004, Hsieh *et al.*, 2006, Jun *et al.*, 2007, Mora *et al.*, 2007). Therefore, the non-invasive placebo needle used in the present study may elicit physiological effects. Subjective sensations like DeQi have been regarded as an important component to evaluate whether the effect of acupuncture is properly elicited. Unfortunately, patients were not asked to document this point. Our results indicated that the placebo acupuncture may indeed lead to a higher pregnancy rate. However, we cannot draw a firm conclusion because a control arm without any acupuncture was not included for comparison.

The positive effect of acupuncture during IVF treatment may be related to the change in uterine blood flow and uterine contractility, and relaxation of stress (Ng *et al.*, 2008). Relatively low oxygen concentration was present around the blastocyst during the time of implantation (Graham *et al.*, 2000; Hu *et al.*, 2003) and vascular endothelial growth factor expression was up-regulated by hypoxic conditions in human endometrial stromal cells (Nasu *et al.*, 2004). Thus, hypoxia is suggested to play a beneficial role in implantation (Sharkey *et al.*, 2000). There is a period of relatively reduced perfusion in the immediate post-ovulatory period, extending to the implantation period in spontaneous cycles (Fraser *et al.*, 1987; Raine-Fenning *et al.*, 2004).

We measured endometrial and subendometrial vascularity before and after the first real or placebo acupuncture session only, but not after the second session. This is mainly due to the concerns of adverse effects of power Doppler energy on the embryos after ET. A significant reduction was demonstrated in endometrial and subendometrial 3D power Doppler flow indices following placebo and real acupuncture, although there were no significant differences in the changes of all these indices between the placebo and real acupuncture groups. A reduction of endometrial and subendometrial vascularity following acupuncture may lead to a more hypoxic environment of the endometrium in favour of embryo implantation.

Most of the infertile patients suffered from great stress and anxiety and high stress level may have an adverse impact on IVF outcomes (Chan *et al.*, 2006). We found a significant decrease in serum cortisol concentration and the anxiety level following placebo and real acupuncture. Reduction in stress in both groups may also contribute a better pregnancy rate following placebo and real acupuncture. According to TCM theories, SP6 is the crossing point of the kidney, the spleen and the liver channels and is considered to play a crucial role in treating female infertility. The attribution of SP6, SP8, SP10, ST29 and ST36 are related to the spleen and the stomach channels. The aim of needling them is to strengthen the essence of kidney and liver and adjust Qi and blood perfusion to the uterus. LR3 and LI4 are so-called 'four gates points' and are commonly used to open corresponding meridians, soothe the stagnation of liver Qi and calm the mind (Shen). Combining them with PC6 and GV20 would relieve the stress and anxiety.

The TCM diagnosis in the great majority of patients was kidney and spleen deficiency as well as liver Qi stagnation with blood stasis. Therefore, the selection of acupoints correctly matches the patterns of syndrome differentiation of the participants according to the TCM principles. Acupuncture treatment in this trial was performed by a registered acupuncturist and disposable needles were used each time. Our results also confirmed the safety of acupuncture treatment and the risks of the acupuncture treatment were minimal. Only a small proportion of patients experienced mild to moderate side effects in this trial and no serious adverse effect was identified.

In conclusion, placebo acupuncture was associated with a significantly higher pregnancy rate in IVF treatment when compared with real acupuncture. Reduction in endometrial and subendometrial vascularity, serum cortisol concentration and the anxiety level were noted in both real and placebo acupuncture groups. Further research is required to confirm the efficacy of placebo or non-invasive acupuncture in IVF treatment.

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