

# Effect of acupuncture on assisted reproduction treatment outcomes

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## ABSTRACT

**Background** Acupuncture has recently been used as a complementary technique in the management of infertility. It has physiological and psychological effects and may be considered an alternative for stress reduction in women undergoing infertility treatments.

**Objective** To examine the hypothesis that acupuncture treatment may increase the pregnancy rate in patients undergoing intracytoplasmic sperm injection cycles.

**Methods** Patients enrolled in the study were stratified according to age and randomised to either a control group (n=208) or acupuncture group, (n=208). The pregnancy, implantation and abortion rates of the two groups were compared.

**Results** No influence of acupuncture treatment on clinical outcomes was seen; however, when cycles in which the causes of infertility were exclusively tubal-uterine or idiopathic were evaluated separately, a positive influence of acupuncture on pregnancy (OR=5.15, 95% CI 1.03 to 34.5; p=0.048) was noted. Moreover, trends toward an increase in implantation were seen when acupuncture was performed (regression coefficient: 0.645; p=0.092).

**Conclusion** The results suggest that acupuncture treatment had no influence when performed immediately before and immediately after embryo transfer, on clinical outcomes overall. In a subgroup analysis, when the embryo was not affected by an ovarian or seminal influence, a benefit was noted.

## INTRODUCTION

Acupuncture is an important element of traditional Chinese medicine, which can be traced back for at least 3000 years. Both physiological and psychological benefits of acupuncture have been scientifically demonstrated in recent years. Acupuncture has been shown to alleviate nausea and vomiting,<sup>1</sup> postoperative pain,<sup>2</sup> addiction,<sup>3,4</sup> and general pain syndromes.<sup>5-7</sup>

This ancient traditional Chinese treatment technique has an empirical basis on the energy flow of *Qi* and consists of the manipulation and insertion of needles in specific anatomically defined points, with systemic effects.<sup>8</sup>

Traditionally, disease was considered an imbalance of *Qi*, which can be treated by stimulating specific points on the body's surface, thus re-establishing the energy flow balance. There are around 400 acupoints connected with 20 energy channels (meridians) related to organs.<sup>9</sup> Nowadays, acupuncture involves modes of stimulation, such as acupressure, transcutaneous electrical nerve stimulation, moxibustion, ear acupuncture and the use of lasers.

Since the first healthy child was conceived via intracytoplasmic sperm injection (ICSI),<sup>10</sup> this technique has become increasingly popular as a means of infertility treatment. The main challenge for the success of ICSI is production of viable embryos having high implantation potentials, and both the embryo and the endometrium are essential for the implantation process.<sup>11</sup>

Acupuncture has recently been proposed as a complementary technique in the management of infertility. It has physiological and psychological effects and may be considered an alternative for stress reduction,<sup>12</sup> especially in women undergoing infertility treatments. Indeed, many infertile patients undergoing in vitro fertilisation (IVF) treatment are under stress,<sup>13-16</sup> and high levels of stress can reduce fertility.<sup>17</sup> In addition, it has been shown that the receptivity of the endometrium and the uterine contraction frequency at the time of embryo transfer are critical for embryo implantation.<sup>18,19</sup>

A possible mechanism through which acupuncture may influence uterine receptivity would be through increased blood flow to the uterus and ovaries.<sup>20</sup>

However, the influence of acupuncture in assisted reproduction techniques (ARTs) is still controversial. While some have suggested that there is no effect or even a negative effect,<sup>21</sup> other studies have demonstrated a positive effect of acupuncture on ART outcomes.<sup>22,23</sup>

A recent systematic review and meta-analysis of acupuncture in IVF has demonstrated that the currently available literature does not provide sufficient evidence that adjuvant acupuncture improves assisted reproduction clinical pregnancy rate.<sup>24</sup> Conversely, other meta-analyses have suggested that acupuncture given with embryo transfer improves rates of pregnancy and live birth among women undergoing IVF.<sup>25</sup> In another systematic review, Cheong *et al*<sup>26</sup> suggested that acupuncture performed on the day of embryo transfer has a beneficial effect on the live birth rate; however, with the present evidence this could be attributed to placebo effect.

The hypothesis for this study was that acupuncture treatment may contribute to the clinical outcomes in patients undergoing assisted reproduction treatments. Therefore, the purpose of this study was to evaluate whether acupuncture treatment before and after embryo transfer increases the pregnancy and implantation rates in patients undergoing controlled ovarian stimulation for ICSI.

## MATERIALS AND METHODS

### Experimental design

During the recruitment period, all patients aged  $\leq 35$  years, and undergoing ICSI cycles for the first time, were offered a place in the study. Those patients who agreed to participate were stratified according to age and randomised either to a control group which did not receive acupuncture ( $n=208$ ) or an acupuncture group ( $n=208$ ). Patients were randomised before the beginning of the ovarian stimulation according to computer-generated randomised numbers. Patients were told their treatment group on the embryo transfer day.

Patients in the control group received the traditional assisted reproduction treatment with no additional interventions.

All laboratory tests and outcomes were recorded, and collection of data was carried out without knowledge of the patient's treatment group.

The pregnancy, implantation and spontaneous abortion rates of the two groups were compared.

Clinical pregnancy was defined as the presence of a gestational sac visualised by ultrasound 4–6 weeks after embryo transfer, the implantation rate was defined as the total number of gestational sacs divided by the total number of embryos transferred and spontaneous abortion was defined as the spontaneous loss of a pregnancy before 24 weeks' gestation.

Written informed consent was obtained, in which patients agreed to share the outcomes of their own cycles for research purposes, and the study was approved by the local institutional review board.

### Controlled ovarian stimulation

Controlled ovarian stimulation was achieved by long pituitary downregulation using a GnRH agonist (Lupron Kit; Abbott S A Société Française des Laboratoires, Paris, France), followed by ovarian stimulation with recombinant *follicle-stimulating hormone (FSH)* (Gonal-F, Merk-Serono, Geneva, Switzerland). Follicular stimulation was followed with ultrasound starting on day 4 of gonadotrophin administration. When adequate follicular growth and serum oestradiol levels were observed, recombinant human chorionic gonadotrophin (Ovidrel, Merk-Serono, Geneva, Switzerland) was administered to trigger final follicular maturation. Oocytes were collected 35 h after recombinant human chorionic gonadotrophin administration by transvaginal ultrasound ovum pick-up.

### Sperm samples

Ejaculated spermatozoa were obtained by masturbation after 3–5 days of ejaculatory abstinence. After liquefaction at room temperature, sperm samples were prepared by discontinuous density-gradient centrifugation or 'swim up'. For discontinuous density gradients, the bottom fraction was aspirated and washed twice at 300 g for 8 min. For swim up, sperm samples were diluted 1:1 with a HEPES-buffered medium (Irvine Scientific, Santa Ana, California, USA) and incubated at 37°C for 1 h, allowing spermatozoa to move from the plasma seminal to the overlaid culture medium.

### Preparation of oocytes

After retrieval, oocytes were incubated in culture medium (Global, LifeGlobal, Guilford, Connecticut, USA), which was covered with mineral oil (Ovoil, Vitrolife, Kungsbacka, Sweden), at 37°C and 6% CO<sub>2</sub> for 5 h. Cumulus cells were removed with 30 s exposure to a HEPES-buffered medium containing 80 IU/ml

hyaluronidase (Irvine Scientific, Santa Ana, California, USA). Coronal cells were then manually removed using a finely drawn glass Pasteur pipette (Humagen Fertility Diagnostics, Charlottesville, Virginia, USA).

Denuded oocytes were then assessed for nuclear status. Oocytes that were found to have released the first polar body were considered mature and used for ICSI.

### ICSI, assessment of fertilisation, embryo transfer

For ICSI, oocytes were placed individually in 5  $\mu$ l droplets of buffered medium (HTF w/HEPES, LifeGlobal, Guilford, Connecticut, USA). Sperm were placed in a central 5  $\mu$ l droplet of polyvinylpyrrolidone solution (Irvine Scientific) in a 50  $\times$  40 mm glass culture dish (WillCo-dish, New Jersey, USA) and covered with warm mineral oil (Ovoil, Vitrolife). Spermatozoa were retrieved from the central droplet and used for ICSI.

Fertilisation was assessed 18 h after ICSI and normal fertilisation was declared when two clearly distinct pronuclei were present. Embryo quality was evaluated under an inverted microscope (Eclipse TE 300; Nikon, Tokyo, Japan) on the third day of development, and one to four embryos were transferred for each couple on the same day.

### Acupuncture treatment

In the acupuncture group, acupuncture was given on the day of embryo transfer (ie, 3 days after oocyte retrieval) in two sessions lasting 25 min immediately before and immediately after embryo transfer. The acupoints were chosen according to the previous study of Paulus *et al.*<sup>27</sup> The depth of needle insertion was about 10–20 mm, depending on the region of the body undergoing treatment. Before embryo transfer, we used the following locations: PC6 (Inner Pass), SP8 (Earth's Crux), LR3 (Great Rushing), GV20 (Hundred Convergences) and ST29 (Return). After embryo transfer, the needles were inserted at the following points: ST36 (Leg Three Li), SP6 (Three Yin Intersection), SP10 (Sea of Blood) and Li4 (Joining Valley).

According to traditional Chinese medicine, stimulation of the *Taiying* meridians (spleen—SP6, SP8 and SP10) and *Yangming* meridians (stomach—ST29, ST36; colon—Li4) results in better blood perfusion and more energy to the uterus. Stimulation of the body points PC6, LR3 and GV20, relaxes the patient during the procedure.<sup>27</sup>

Sterile disposable stainless steel needles 0.25  $\times$  40 mm (Dong Bang Acupuncture, Korea) were inserted with a guide tube to tissue level and manipulated until needle sensation was obtained, (ie, *de qi*—a feeling of, for example, soreness or numbness, distension or pain). All the procedures were performed by the same professional (CM).

### Statistical analysis

For the sample size calculation we estimated a pregnancy rate of 20% in the control group, with a minimal difference of 12% (based on previous studies) in the treated group, in an unconditional exact test, and a power of 80%. This was performed in a one-sided test, with a requirement for 200 patients in each group.

Results are expressed as the mean  $\pm$  SD for numeric variables and proportions (%) for categorical variables. Mean values were compared by a Student *t* or Mann–Whitney test as appropriate and proportions were compared by  $\chi^2$  test.

To study the influence of acupuncture treatment on pregnancy and spontaneous abortion, binary logistic regressions

were performed, and to study the influence of acupuncture treatment on implantation, linear logistic regression was conducted.

All regression analysis was adjusted for maternal age, number of retrieved oocytes, endometrium thickness and total dose of FSH and fertilisation rate, as these would be considered potential confounders of the association between the factors evaluated and the ICSI outcomes.

Results were expressed as OR, regression coefficients (RCs), 95% CI and p value.

Results were considered to be significant at the 5% critical level ( $p < 0.05$ ). Data analysis was carried out using the Minitab (version 14) statistical program.

## RESULTS

### Cycle's characteristics

The causes of infertility were ovarian disorders (27.9% vs 27.8%,  $p = 0.980$ ), endometriosis (8.5% vs 12.9%,  $p = 0.108$ ), tubal-uterine factor (5.4% vs 2.4%,  $p = 0.092$ ), polycystic ovarian syndrome (4.1% vs 4.3%,  $p = 0.899$ ), male factor (23.2% vs 26.9%,  $p = 0.342$ ), idiopathic causes (11.2% vs 11.0%,  $p = 0.943$ ) and combined causes of infertility (19.4% vs 14.4%,  $p = 0.143$ ) for control and acupuncture groups, respectively.

The female's age, body mass index, total dose of administered FSH, number of aspirated follicles, number of retrieved oocytes, fertilisation rate (normal fertilised oocytes/injected oocytes), high-quality embryos rate (number of grade A embryos/normal fertilised embryos) and number of transferred embryos were the same in both study groups (table 1).

### Clinical outcomes

Our results demonstrated that acupuncture treatment had no influence on pregnancy (OR=1.16; 95% CI 0.77 to 1.69;  $p = 0.655$ ), implantation (RC=0.468;  $p = 0.980$ ), spontaneous abortion rates (OR=2.1; 95% CI 0.30 to 12.15;  $p = 0.313$ ) or live birth rate (OR=1.11; 95% CI 0.65 to 1.73;  $p = 0.832$ ).

The values for pregnancy, implantation, spontaneous abortion and live birth rates are described in table 2.

To evaluate whether acupuncture might improve ICSI outcomes in cycles in which the embryo quality was not affected by a maternal or paternal effect, cycles in which the causes of infertility were exclusively tubal-uterine or idiopathic were evaluated. In this group, 32 patients received acupuncture and 45 were allocated to the control group.

**Table 1** Characteristics of patients who did not receive acupuncture treatment (control group) and those who received acupuncture (acupuncture group)

Groups	Control	Acupuncture	p Value
N	208	208	
Female age	34.6 ± 4.6	35.3 ± 4.7	0.103
Body mass index	22.4 ± 2.9	22.4 ± 3.8	0.951
Total dose of FSH	2306 ± 653	2346 ± 597.0	0.516
Follicles aspirated	17.1 ± 12.4	16.1 ± 12.5	0.349
Oocytes retrieved	11.6 ± 8.4	11.4 ± 8.2	0.829
Fertilisation rate (%)	73.5	74.6	0.533
High-quality embryos rate (%)	65.6	58.4	0.898
Embryos transferred	2.2 ± 0.9	2.1 ± 1.0	0.479

Results are shown as mean ± SD unless stated otherwise.

Mann-Whitney and  $\chi^2$  tests.

FSH, follicle stimulating hormone.

In this subgroup, regression analysis showed that acupuncture had a positive influence on pregnancy, increasing the chance of achieving pregnancy by more than fivefold (OR = 5.15; 95% CI 1.03 to 34.5;  $p = 0.048$ ). Furthermore, although it did not reach significance, trends toward increased implantation were observed when acupuncture was performed before and after embryo transfer (RC=0.645;  $p = 0.092$ ). Nevertheless, no effect of acupuncture treatment on the incidence of spontaneous abortion was noted (OR=0.51; 95% CI 0.0 to 3.64;  $p = 0.998$ ).

When other causes of infertility were evaluated separately, no significant influence of acupuncture on clinical ICSI outcomes was seen.

## DISCUSSION

Although traditional Chinese medicine understanding of acupuncture is based on ancient medical theory, a modern and scientific neuroendocrine perspective has begun to evolve in the past two decades.<sup>9</sup> The positive effect of acupuncture in the treatment of subfertility may be related to the central sympathetic inhibition by the endorphin system, the change in uterine blood flow and motility and stress reduction.<sup>28</sup>

A recent meta-analysis evaluated the effects of acupuncture on IVF clinical outcomes and concluded that acupuncture on the embryo transfer day may improve both pregnancy and live birth rates.<sup>25</sup> In addition, three prospective randomised published studies recorded higher pregnancy rates in the acupuncture group than in the control group.<sup>22,23,27</sup>

This study evaluated the effect of 25 min acupuncture sessions immediately before and immediately after embryo transfer in 208 patients undergoing ICSI cycles and no benefit of acupuncture was observed in the clinical outcomes.

Other studies also failed to find effects of acupuncture on IVF outcomes. In another systematic review it was suggested that the currently available literature does not provide sufficient evidence that adjuvant acupuncture improves assisted reproduction clinical pregnancy rate.<sup>24</sup>

Wang *et al*<sup>29</sup> performed 30 min acupuncture sessions starting on day 5 of the cycle until 2 weeks after oocyte retrieval and found no improvement in the pregnancy rate. Most of the studies reporting positive effects of acupuncture on ICSI outcomes performed the sessions immediately before embryo transfer, unlike in Wang's study. However, in another study in which acupuncture sessions were performed on day 9 of controlled ovarian stimulation, immediately before and immediately after embryo transfer, no increase in the pregnancy rate was found in the acupuncture group.<sup>30</sup>

It is well known that both oocyte and sperm quality are variables that influence the implantation potential of derived embryos. Sperm have a key role during fertilisation,<sup>31,32</sup> but it

**Table 2** ICSI cycles outcomes of patients who did not receive acupuncture (control group) and those who received acupuncture (acupuncture group)

	Control	Acupuncture	p Value
N	208	208	
Pregnancy rate (%)	32.2 (67/208)	40.4 (84/208)	0.652
Implantation rate (%)	21.0	23.4	0.898
Spontaneous abortion rate (%)	11.9 (8/67)	13.1 (11/84)	0.877
Live birth rate	27.4 (57/208)	33.7 (70/208)	0.763

$\chi^2$  and Student t tests.

ICSI, intracytoplasmic sperm injection.

has also been shown that human preimplantation embryo development can be compromised by deficiencies in both the sperm nuclear genome or sperm-derived cytoplasmic factors.<sup>35</sup> On the other hand, expression of the embryonic genome, which is a combination of the sperm and the oocyte contribution, starts between the four-cell and eight-cell stage of human embryo development.<sup>34,35</sup> Therefore, the oocyte has a key role in embryo development between fertilisation and the four/eight-cell stage.

Here we examined whether acupuncture might improve ICSI outcomes in cycles in which the embryo quality was not affected by a maternal or paternal effect. Thus, cycles in which the causes of infertility were due exclusively to idiopathic or tubal-uterine factors were evaluated separately. In this group, acupuncture had a positive effect on implantation and pregnancy, and therefore, an effect of acupuncture on endometrium implantation could be argued.

The possible mechanism of acupuncture in the treatment of female infertility is still unclear. Studies have suggested that it has a potential impact on the hypothalamic–pituitary–ovarian axis and on the uterus.<sup>36,37</sup> It is well established that ART success depends on optimal endometrial receptivity at embryo implantation, and pregnancy rates are affected by uterine contractions at the time of embryo transfer.<sup>38</sup> However, even though it has been hypothesised that acupuncture can inhibit uterus contractibility,<sup>36</sup> Paulus *et al*<sup>39</sup> demonstrated that acupuncture treatment does not inhibit uterine motility and suggested that other mechanisms may be responsible for the increase of pregnancy rate after acupuncture in ART.

Together, the above-cited studies suggest a placebo effect of acupuncture on the ICSI outcomes. A recent systematic review,<sup>26</sup> suggested that acupuncture performed on the day of embryo transfer has a beneficial effect on the live birth rate; however, with the present evidence this could be attributed to a placebo effect.

Myers<sup>40</sup> suggested that there are two potential sources of a placebo effect: belief on the part of the patient and/or belief on the part of the practitioner. A well-conducted trial should include a prospective randomised design in which both parties are blind to subject assignment. However, it is impossible to blind the acupuncturist, but, nevertheless, it would be possible for everyone else on the healthcare team to be blind to subject assignment.

In a recent study, all nurses or doctors in the team were blind to patients' assignments, and the patients in the acupuncture groups received the treatment, while the control subjects lay quietly for 25 min. The authors concluded that the use of acupuncture in patients undergoing IVF was not associated with an increase in the pregnancy rate, but the patients were more relaxed and more optimistic.<sup>41</sup> Since in that study the patients knew which treatment they were receiving, the placebo effect could again bias the study.

In our experiment, patients eligible to participate in the study were allocated into acupuncture or control groups and while the control group did not receive any treatment, the acupuncture group was treated before and after embryo transfer. Therefore, as in most of the studies of the effects of acupuncture in ART, patient knowledge is a limitation of our study, and therefore a placebo effect of the treatment on stress reduction is possible.

In conclusion, our results suggest that acupuncture has no influence on clinical outcomes overall, when performed immediately before and immediately after embryo transfer. In a subgroup analysis, when the embryo was not affected by an ovarian

or seminal influence, a benefit was noted. However, this was only an exploratory analysis; therefore a larger trial is necessary to confirm the results.

**Competing interests** None.

**Ethics approval** This study was conducted with the approval of the local institutional review board.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Contributors** CM, designed the manuscript, performed the acupuncture, analysed the data and revised the final version; DPAFB, analysed the data, wrote the manuscript; RDCSF, collected the data, analysed the data and revised the final version of the manuscript; AI analysed the data and revised the final version of the manuscript; EB supervised all the work and revised the manuscript.

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