

Influence of acupuncture on the outcomes of in vitro fertilisation when embryo implantation has failed: a prospective randomised controlled clinical trial

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Received 23 October 2012
Accepted 25 February 2013

ABSTRACT

Objective To evaluate the effectiveness of acupuncture and moxibustion as an adjuvant treatment in women undergoing in vitro fertilisation (IVF) when embryo implantation has failed.

Methods A prospective, randomised controlled clinical trial was conducted with 84 infertile patients who had had at least two unsuccessful attempts of IVF. The patients were randomised in three groups: control (n=28), sham (n=28) and acupuncture (n=28). The sample size was calculated by assuming a pregnancy rate of 10% when embryo implantation had failed. The pregnancy rates of the current IVF cycle were evaluated by measurement of blood β human chorionic gonadotrophin (β hCG) and subsequent transvaginal ultrasound. Acupuncture was performed on the first and seventh day of ovulation induction, on the day before ovarian puncture and on the day after embryo transfer. In the acupuncture group, patients were treated with moxibustion at nine acupuncture points (BL18, BL22, BL23, BL52, CV3, CV4, CV5, CV7, GV4) and needling at 12 points. In the sham group needles were inserted in eight areas that did not correspond to known acupuncture points.

Results The clinical pregnancy rate in the acupuncture group was significantly higher than that in the control and sham groups (35.7% vs 7.1% vs 10.7%; $p=0.0169$).

Conclusions In this study, acupuncture and moxibustion increased pregnancy rates when used as an adjuvant treatment in women undergoing IVF, when embryo implantation had failed.

INTRODUCTION

Assisted reproduction techniques, including in vitro fertilisation (IVF), have evolved greatly over the past decades.^{1–2} However, pregnancy rates remain near 30%, and are considered low.^{3–4} Many IVF cycles will not result in pregnancy and generally further cycles will be required.^{1–4} Embryo implantation failure is usually defined as occurring when patients have had at least two previous unsuccessful attempts of IVF^{5–6}; it is a major cause of failure of IVF procedures.^{5–7} Possible causes of embryo implantation failure have been widely investigated, but there is no consensus about the reason.^{5–7} It is believed that embryo quality and endometrial receptivity are key factors of IVF failure.^{8–10}

When embryo implantation has failed, pregnancy rates fall to <10%.^{9–11} Most treatments are empirical and do not significantly change pregnancy rates.^{5–8–9}

Acupuncture has been used in the treatment of infertility problems.¹² Studies suggest several mechanisms to explain the role of acupuncture in the IVF procedure,^{12–18} such as increased blood flow that increases endometrial receptivity,^{12–13–19} stimulation of β endorphin levels influencing the secretion of steroid hormones, regulating the menstrual cycle and ovulation^{12–13–15–20} and reducing anxiety of infertile couples.^{14–17–21}

The objective of this study was to evaluate whether acupuncture, as an adjuvant treatment in women undergoing

To cite: Ioyama Manca di Villahermosa D, dos Santos LG, Nogueira MB, et al. *Acupunct Med* Published Online First: [please include Day Month Year] doi:10.1136/acupmed-2012-010269

IVF when embryo implantation has failed, influences pregnancy rates.

METHODS

Study design and subjects

A prospective, randomised controlled clinical trial was conducted with infertile women undergoing treatment at the Clinic for Human Reproduction, Faculty of Medicine of ABC, Santo André SP, Brazil, between April 2009 and October 2011. During this period, all women diagnosed with embryo implantation failure (who had had at least two previous unsuccessful attempts of IVF), aged <38 years (and with partners aged <50 years), with no uterine factor and transfer of at least two good embryos (good quality, with <20% of fragmentation and cell number suitable for the transfer day) were asked to participate in the study. The following exclusion criteria were used: transfer of frozen or poor-quality embryos; previous use of acupuncture; use of some other form of empirical treatment or severe oligospermia (<2 million/ml).

The selected patients were carefully informed of the objectives and procedures of the study and signed an informed consent form. The research project was approved by the ethics and research committee of the Faculty of Medicine of ABC, under number 029/2009.

Eighty-four patients were randomised using sealed envelopes with computer-generated numbers in three groups, to allow comparison of treatments: control group (n=28), sham group (n=28) and acupuncture group (n=28).

Acupuncture treatment

Each patient underwent a total of four treatment sessions, always performed on days: D1 (first day of ovulation induction), D7 (seventh day of ovulation induction), on the day before the day of ovarian puncture and on the day after the day of embryo transfer. In all sessions, the treatment was performed by the same acupuncturist, with more than 5 years of practice in acupuncture.

Ovulation induction started on day 2 of the cycle with a daily dose of 150 IU recombinant follicular stimulating hormone. Co-treatment with a gonadotrophin releasing hormone antagonist (GnRHa; 0.25 mg) was initiated at a follicle size of 13 mm. As soon as three or more follicles had reached a size of 17 mm, a bolus of GnRHa (Triptorelin 0.2 mg) was administered. Ovarian puncture was performed 35 h later. Embryos were transferred on day 3 after ovarian puncture.

Traditional acupuncture was performed in all women of the acupuncture group, according to the principles of traditional Chinese medicine and the classic point localisation, including depth of insertion.

For all patients of the acupuncture group a sequence of points for acupuncture and moxibustion was selected. First, moxibustion was performed

unilaterally at nine acupuncture points (BL18, BL22, BL23, BL52, CV3, CV4, CV5, CV7, GV4), for about 5 min. After moxibustion, 11 points—PC6, KI3, KI6, KI7, KI10, LR3, SP4, SP6, SP10, ST40, LU7—were needled unilaterally together with the extra point *Zigong* bilaterally. The needles were manually inserted and stimulated to obtain *de qi* and left for a period of 20 min.

Unilateral points were preferred in order to reduce the total number of needles inserted. The points were selected by observation of the traditional Chinese medicine diagnosis most often found in infertile women.

For women in the sham group, needle insertion was performed in the arm and thigh, which are known not to correspond with classically described acupuncture points, using a total of eight needles (two needles in each region bilaterally); the needles were inserted superficially without eliciting *de qi*. The needles were also left for 20 min as in the acupuncture group. No moxibustion or any other simulation technique was performed in the sham group.

Evaluations of results

Demographic data, current IVF results (number of oocytes retrieved, endometrial thickness and number of transferred embryos) and the outcomes of pregnancy (β human chorionic gonadotrophin (β hCG) test and ultrasound) were obtained from the electronic medical records of the clinic for human reproduction. Chemical pregnancy was confirmed by the fraction of serum β hCG on the 12th day after embryo transfer. Clinical pregnancy was considered when the intrauterine gestational sac was identified at transvaginal ultrasound examination, after the fourth or fifth week of gestation.

Statistical analysis

The sample size was calculated using the method described by Pocock (1993). We calculated a sample size of 66 patients, with 22 patients in each group, and the type I probability of error being fixed in 5% and the type II probability error in 20%. This sample size was able to detect if acupuncture and moxibustion could change pregnancy rates, assuming an expected 10% of pregnancy when embryo implantation had failed. The sample size calculation assumed a unilateral test and was performed as an unconditional exact test.

Bonferroni corrected analysis of variance test was used to compare data on absolute frequencies, and χ^2 test to compare data on relative frequencies.

A significance level of 5% (p value) was considered. All data were analysed using Stata Statistical Software (StataCorp, 2007).

RESULTS

A total of 84 patients were included in the study. All patients were randomised: 28 received acupuncture

Table 1 Control variables, demographic data

Variables	Control group (n=28)	Sham group (n=28)	Acupuncture group (n=28)	p Value
Age (years)	36.4±2.1	36.2±2.2	36.0±2.7	0.8520*
Duration of infertility (years)	4.7±1.9	4.3±1.4	4.4±1.5	0.6486*
Primary infertility, % (n)‡	89.3 (25)	82.1 (23)	82.1 (23)	0.8311†
Previous IVF cycles (number)	2.7±0.8	2.5±0.5	2.5±0.6	0.3133*

Results are shown as mean±SD unless stated otherwise.

*Analysis of variance; † χ^2 ; ‡relative frequency (n).

IVF, in vitro fertilisation.

treatment according to the principles of traditional Chinese medicine (acupuncture group), 28 patients received placebo or sham acupuncture (sham group) and the other 28 patients underwent only IVF without any intervention (control group). None of the 84 patients dropped out or was excluded from the study.

The three groups were compared with respect to age, duration of infertility, type of infertility and number of previous IVF cycles (table 1). None of the variables showed statistically significant differences.

Data from the current IVF procedure were obtained and compared in the three study groups (table 2). The number of oocytes retrieved for each ovarian puncture was statistically higher in the acupuncture group than in the other two groups (8.4 ± 3.1 ; $p=0.0427$), as was endometrial thickness ($10.3\text{ mm}\pm 1.6$; $p=0.0002$).

The pregnancy rates were also compared in the three groups (table 3). Both the biochemical pregnancy rate (39.3%; $p=0.0327$) and the clinical pregnancy rate (35.7%; $p=0.0169$) were significantly higher in the acupuncture group than in the other two groups, when compared with pregnancy rates of <10% when embryo implantation had failed.

DISCUSSION

This study indicates that when embryo implantation has failed, use of acupuncture and moxibustion as adjuvant treatments, significantly improves pregnancy outcomes. In this study, acupuncture and moxibustion produced better therapeutic results than those obtained with IVF alone (control group) and placebo acupuncture (sham group). Thus, the results confirm and extend the findings obtained in other studies,^{15–17 22–24} although this is the first study of the use of acupuncture specifically when embryo implantation has failed.

The control, sham and acupuncture groups were statistically similar with respect to demographic

characteristics (age, duration of infertility, type of infertility and causes of infertility), number of previous IVF procedures and number of transferred embryos. Thus other known predictors of outcome are balanced among the groups (table 1).

The way in which acupuncture can affect the neuroendocrine system continues to be studied. However, it is well established that both acupuncture and electroacupuncture inhibit the perception of pain by increasing the levels of several neurotransmitters such as β -endorphin.^{12 16 17 19} These substances influence a series of hypothalamic functions, influencing reproductive, autonomic and even immunological functions.^{17 19 17}

Stener-Victorin *et al*²⁰ demonstrated that low-frequency acupuncture (2 Hz) induced ovulation in one-third of women with anovulatory cycles associated with polycystic ovary syndrome.²⁰ Animal studies have shown that acupuncture can normalise the secretion of GnRH and influence peripheral gonadotrophin levels.^{25 26} Other authors have also shown that acupuncture may influence plasma levels of follicular stimulating hormone, luteinising hormone, oestradiol and progesterone, improving ovulation rates.^{12 13 17 19} Other studies have shown negative results,^{27 28} and the reasons for these different outcomes are still unknown.

Endometrial thickness, morphology and uterine artery blood flow are important parameters for successful implantation of human embryos into the uterine cavity.¹⁹ Acupuncture, which can bring about central sympathetic inhibition, can contribute to the reduction of impedance of the uterine artery and, thus, improve the blood flow to the uterus.^{12 14 29} Stener-Victorin and Humaidan¹⁹ demonstrated that effect through the use of acupuncture in 10 infertile women blocked with the use of GnRH analogues to avoid the endogenous hormone effect on the uterine artery blood flow.¹⁹

Table 2 IVF data

Variables	Control group (n=28)	Sham group (n=28)	Acupuncture group (n=28)	p Value*
Oocytes retrieved (number)	6.5±3.5	6.5±3.1	8.4±3.1	0.0427
Endometrial thickness (mm)	8.7±1.6	8.5±1.6	10.3±1.6	0.0002
Embryos transferred (number)	2.0±0.8	2.2±0.7	2.2±0.6	0.5428

Results are shown as mean±SD.

*Analysis of variance.

IVF, in vitro fertilisation.

Table 3 Pregnancy rates

Parameters	Control group (n=28)	Sham group (n=28)	Acupuncture group (n=28)	RR	95% CI	p Value*
Biochemistry pregnancy rates, % (n)	10.7 (3)	10.7 (3)	39.3 (11)	3.28	1.08 to 9.91	0.0327
Clinical pregnancy rates, % (n)	7.1 (2)	10.7 (3)	35.7 (10)	4.20	1.25 to 14.09	0.0169

Results are shown as relative frequency (n).

* χ^2 .

In 2002, Paulus *et al*¹⁵ reported the first prospective randomised controlled study of the use of acupuncture in patients undergoing IVF. They studied 160 patients and observed a significant increase in pregnancy rates in the acupuncture group compared with the control group (42.5% vs 26.3%). Since then the number of infertile patients treated with acupuncture has been increasing, and some human reproduction services advise their patients to undergo IVF in association with acupuncture.^{17 19 29}

Failure of embryo implantation is considered the biggest challenge of reproductive medicine.^{5 8 9} It is estimated that after the second IVF attempt pregnancy rates decrease to <10%.^{9 11} This is the first study to use acupuncture specifically in the treatment of embryo implantation failure. In this study, acupuncture increased pregnancy rates when embryo implantation had failed to a higher rate than expected after the first IVF cycle (35.7%; $p=0.0169$). Further studies are needed to investigate the benefits of acupuncture in this specific group of patients.

Acupuncture in patients undergoing IVF is a relatively simple procedure, without side effects and contraindications.^{13 17 29} However, to ensure greater acceptance of acupuncture in the West it is necessary to demonstrate that its effects and actions are much greater than a possible placebo effect.^{30 31}

It is observed that more and more couples choose acupuncture for treating infertility, alone or as an adjuvant treatment in conjunction with Western assisted reproduction treatments. Most scientists and physicians agree that this is a controversial area and that more studies are needed before acupuncture is definitely accepted as part of assisted reproduction treatment protocols, such as IVF.

The findings of this study demonstrated that acupuncture and moxibustion seem to increase pregnancy rates when used as an adjuvant treatment in women undergoing IVF, when embryonic implantation has failed.

Summary points

- ▶ Evidence on acupuncture's effect on IVF success is inconsistent, which may be due to timing.
- ▶ In this RCT, acupuncture and moxibustion were given four times.
- ▶ The clinical pregnancy rate was increased compared with sham and no acupuncture.

Contributors DIMdV, CPB, FLV: substantial contributions to conception and design; drafting the article and revising it critically for important intellectual content. DIMdV, LGdS, MBN: substantial contributions to acquisition, analysis and interpretation of data; DIMdV, CPB: final approval of the version to be published.

Competing interests None.

Patient consent Obtained.

Ethics approval Ethics committee in research of the Faculty of Medicine of ABC.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

- 1 Evers JL. Female subfertility. *Lancet* 2002;360:151–9.
- 2 Oakley L, Doyle P, Maconochie N. Lifetime prevalence of infertility and infertility treatment in the UK—results from a population-based survey of reproduction. *Hum Reprod* 2008;23:447–50.
- 3 Vahratian A. Utilization of fertility-related services in the United States. *Fertil Steril* 2008;90:1317–19.
- 4 Lintsen AME, Eijkemans MJC, Hunault CC, *et al*. Predicting ongoing pregnancy chances after IVF and ICSI—a national prospective study. *Hum Reprod* 2007;22:2455–62.
- 5 Tan BK, Vandekerckhove P, Kennedy R, *et al*. Investigation and current management of recurrent IVF treatment failure in the UK. *BJOG* 2005;112:773–80.
- 6 Mahutte NG, Arici A. Failed fertilization—is it a predictable? *Curr Opin Obstet Gynecol* 2003;15:211.
- 7 Ola B, Li T. Implantation failure following in-vitro fertilization. *Curr Opin Obstet Gynecol* 2006;18:440–5.
- 8 Kling C, Schmutzler A, Wilke G, *et al*. Two-year outcome after recurrent implantation failure—prognostic factors and additional interventions. *Arch Gynecol Obstet* 2008;278:135–42.
- 9 Laufer N, Simon A. Recurrent implantation failure—current update and clinical approach to an ongoing challenge. *Fertil Steril* 2012;97:1019–20.
- 10 Penzias AS. Recurrent IVF failure—other factors. *Fertil Steril* 2012;97:1033–8.
- 11 Simon A, Laufer N. Repeated implantation failure—clinical approach. *Fertil Steril* 2012;97:1039–43.
- 12 Anderson BJ, Haimovici F, Ginsburg ES, *et al*. In vitro fertilization and acupuncture—clinical efficacy and mechanistic basis. *Altern Ther Health Med* 2007;13:38–48.
- 13 Chang R, Chung PH, Rosenwaks Z. Role of acupuncture in treatment of female infertility. *Fertil Steril* 2002;78:1149–53.
- 14 Huang S, Chen AP. Traditional Chinese medicine and infertility. *Curr Opin Obstet Gynecol* 2008;20:211–15.

- 15 Paulus WE, Zhang M, Strehler E, *et al.* Influence of acupuncture on the pregnancy rate in patients who undergo assisted reproduction therapy. *Fertil Steril* 2002;77:721–4.
- 16 Pinborg A, Loft A, Andersen AN. Acupuncture with in vitro fertilization. *BMJ* 2008;336:517–18.
- 17 Rosenthal L, Anderson B. Acupuncture and in vitro fertilisation—recent research and clinical guidelines. *J Chin Med* 2007;84:28–35.
- 18 Zheng CH, Huang GY, Zhang MM, *et al.* Effects of acupuncture on pregnancy rates in women undergoing in vitro fertilization—a systematic review and meta-analysis. *Fertil Steril* 2012;97:599–611.
- 19 Stener-Victorin E, Humaidan P. Use of acupuncture in female infertility and a summary of recent acupuncture studies relates to embryo transfer. *Acupunct Med* 2006;2:157–63.
- 20 Stener-Victorin E, Lundeberg T, Caiander S, *et al.* Steroid-induced polycystic ovaries in rats: effect of electroacupuncture on concentrations of endothelin-1 and nerve growth factor (NGF), and expression of NGFmRNA in the ovaries, the adrenal glands, and the central nervous system. *Reprod Biol Endocrinol* 2003;1:1–33.
- 21 Isoyama D, Cordts EB, van Niewegen AMBS, *et al.* Effect of acupuncture on symptoms of anxiety in women undergoing in vitro fertilization—a prospective randomized controlled study. *Acupunct Med* 2012;30:85–8.
- 22 Dieterle S, Ying G, Hatzmann W, *et al.* Effect of acupuncture on the outcome of in vitro fertilization and intracytoplasmic sperm injection: a randomized, prospective, controlled clinical study. *Fertil Steril* 2006;85:1347–51.
- 23 So EWS, Ng EHY, Wong YY, *et al.* A randomized double blind comparison of real and placebo acupuncture in IVF treatment. *Hum Reprod* 2009;24:341–8.
- 24 Westergaard LG, Mao Q, Krogslund M, *et al.* Acupuncture on the day of embryo transfer significantly improves the reproductive outcome in infertile women—a prospective, randomized trial. *Fertil Steril* 2006;85:1341–6.
- 25 Manni L, Lundeberg T, Holmaang A, *et al.* Effect of electroacupuncture on ovarian expression of alpha (1) and beta (2) adrenoceptors and p75 neurotrophin receptors in rats with steroid-induced polycystic ovaries. *Reprod Biol Endocrinol* 2005;3:21.
- 26 Zhaohui Z, Yugui C, Yuanming Z, *et al.* Effect of acupuncture on puberal development of rats and rabbits at different developmental stages. *Neuropeptides* 2007;41:249–61.
- 27 Yu EH, So WS, Gao J, *et al.* The role of acupuncture in the management of subfertility. *Fertil Steril* 2008;90:1–13.
- 28 El-Toukhy T, Sunkara SK, Khairy M, *et al.* A systematic review and meta-analysis of acupuncture in vitro fertilization. *BJOG*. 2008;115:1203–13.
- 29 Craig L, Criniti A, Hansen K, *et al.* Acupuncture lowers pregnancy rates when performed before and after embryo transfer. *Fertil Steril* 2007;88(Suppl 1):S40.
- 30 Stener-Victorin E. Acupuncture in vitro fertilization—why do reviews produce contradictory results? *Focus Altern Complement Ther* 2009;14:8–11.
- 31 Stener-Victorin E, Manheimer E. Commentary on the Cochrane review of acupuncture and assisted conception. *Explore* 2011;7:120–3.