

Acupuncture on the day of embryo transfer significantly improves the reproductive outcome in infertile women: a prospective, randomized trial

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Objective: To evaluate the effect of acupuncture on reproductive outcome in patients treated with IVF/intracytoplasmic sperm injection (ICSI). One group of patients received acupuncture on the day of ET, another group on ET day and again 2 days later (i.e., closer to implantation day), and both groups were compared with a control group that did not receive acupuncture.

Design: Prospective, randomized trial.

Setting: Private fertility center.

Patient(s): During the study period all patients receiving IVF or ICSI treatment were offered participation in the study. On the day of oocyte retrieval, patients were randomly allocated (with sealed envelopes) to receive acupuncture on the day of ET (ACU 1 group, n = 95), on that day and again 2 days later (ACU 2 group, n = 91), or no acupuncture (control group, n = 87).

Intervention(s): Acupuncture was performed immediately before and after ET (ACU 1 and 2 groups), with each session lasting 25 minutes; and one 25-minute session was performed 2 days later in the ACU 2 group.

Main Outcome Measure(s): Clinical pregnancy and ongoing pregnancy rates in the three groups.

Result(s): Clinical and ongoing pregnancy rates were significantly higher in the ACU 1 group as compared with controls (37 of 95 [39%] vs. 21 of 87 [26%] and 34 of 95 [36%] vs. 19 of 87 [22%]). The clinical and ongoing pregnancy rates in the ACU 2 group (36% and 26%) were higher than in controls, but the difference did not reach statistical difference.

Conclusion(s): Acupuncture on the day of ET significantly improves the reproductive outcome of IVF/ICSI, compared with no acupuncture. Repeating acupuncture on ET day +2 provided no additional beneficial effect. (Fertil Steril® 2006;xx:xxx. ©2006 by American Society for Reproductive Medicine.)

Key Words: Acupuncture, ET day, IVF, pregnancy

Acupuncture is an ancient therapeutic art, which has been given renewed attention in light of recent scientific research and current integration with modern medical practice in the treatment of a wide range of diseases, including infertility.

The mechanisms through which acupuncture influence female fertility are believed to involve [1] central stimulation of β -endorphin secretion (1), which in turn impacts on the GnRH pulse generator and thereby on gonadotrophin and steroid secretion (2, 3), and [2] a general sympathoinhibitory effect through increased blood flow to the uterus and ovaries

(4), resulting in uterine conditions favoring implantation (for a recent review, see Chang et al. [5]).

Many reports in the literature claim positive effects of acupuncture in the treatment of female infertility, but only a few of them satisfy the requirements of rigorously conducted prospective, randomized trials (6). In a prospective, randomized study comparing electro-acupuncture and alfentanil as anesthesia during oocyte aspiration in IVF, Stener-Victorin et al. (7) found, unexpectedly, a significantly higher implantation rate and "take-home baby" rate per ET in the electro-acupuncture group. Later and larger studies using electro-acupuncture on the day of oocyte retrieval, however, did not confirm these positive effects on reproductive outcome (8, 9). Using conventional manual acupuncture on the day of ET, Paulus et al. (10) demonstrated a significantly increased clinical pregnancy rate in a group receiving acupuncture

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TABLE 1**Patients included or excluded after randomization into the control group, ACU 1, or ACU 2.**

Group	Total no. randomized	Included	Excluded ^a	Excluded ^b
Control group	100	87	6	7
ACU 1	100	95	5	0
ACU 2	100	91	8	1
Total	300	273	19	8

^a No ET owing to total failure of fertilization or poor embryo development.^b Declined participation after randomization.Westergaard. Acupuncture on ET day improves IVF outcome. *Fertil Steril* 2006.

(n = 80) as compared with a control group (n = 80) not receiving acupuncture.

Accepting the above notion that the positive effects of acupuncture on the day of oocyte retrieval and on the day of ET might be mediated through effects on local ovarian and uterine blood flow, enhancing the quality of the endometrium, we hypothesized that applying acupuncture 5 days after oocyte retrieval (i.e. closer to the day of implantation, 6–12 days after oocyte retrieval) might further optimize endometrial conditions for the embryo to implant.

In the present prospective study, women undergoing IVF/ intracytoplasmic sperm injection (ICSI) treatment were randomly allocated to one of three groups: [1] no acupuncture (control group), [2] acupuncture on the day of ET (i.e., 3 days after oocyte retrieval) (ACU 1 group), and [3] acupuncture on the ET day as above and again 2 days later (i.e., 5 days after oocyte retrieval) (ACU 2 group).

The aims of the study were to evaluate the effects of acupuncture on the reproductive outcome of IVF/ICSI treatment by comparing the rates of positive pregnancy tests, clinical pregnancy, and ongoing pregnancy/delivery in these three groups.

MATERIALS AND METHODS

Setting and Design

This prospective, randomized trial was carried out in a large, private IVF clinic in Copenhagen, Denmark from March 1, 2003 to June 30, 2004. During that period, all couples admitted to the clinic for IVF or ICSI treatment of infertility were consecutively invited to participate. At the start of hormonal stimulation for IVF/ICSI, all patients were informed orally and in writing about the aims and practical details of the project, and willingness to participate was confirmed in writing. The study was approved by the institutional review board of the cities of Copenhagen and Frederiksberg (no. 01-203/02).

By design, the study population comprised an unselected average of couples seeking infertility treatment in our clinic.

The only inclusion criterion for participation in the study was the couples' consent to be randomized to one of three groups (see below), independent of infertility diagnosis, number of previous ART attempts, and hormonal treatment in the actual cycle. Patients who after randomization did not achieve ET or who for personal reasons did not want to participate further were excluded from the study (Table 1).

On the basis of the data previously published by Paulus et al. (9), combined with an average 25% clinical pregnancy rate per ET in our clinic during the previous 5 years, power calculations (Medcalc software, Mariakerke, Belgium) anticipated that a significant difference in clinical pregnancy rate of 11% between no acupuncture and acupuncture would require approximately 100 patients in the control group and 200 patients in the acupuncture groups.

Randomization

During the study period of 16 months, a total of approximately 1000 couples underwent IVF or ICSI treatment in our clinic. Of these, a total of 300 couples accepted participation in the study and were randomized to one of three groups by the drawing of a sealed envelope on the day of oocyte retrieval. After randomization, 27 patients were excluded for various reasons (Table 1). Of the remaining 273 patients, 87 were allocated to no acupuncture (control group), 95 to acupuncture on the day of ET (ACU 1 group), and 91 to receive acupuncture on the day of ET and 2 days later.

Acupuncture

The acupuncture points used in the present study were, as in the study by Paulus et al. (10), chosen in agreement with the concepts of traditional Chinese medicine. According to these, the kidney system dominates the reproductive system, the liver (LR) regulates *Qi* (vital force and energy), and spleen (SP) and stomach (ST) are sources of *Qi* and blood. Spleen 6 (SP6) is the crossing point of the spleen, kidney, and liver meridians and is considered the key point in treating infertility. Needling SP6, SP8, SP10, ST36, and ST29 aims to provide improved blood perfusion and more energy

to the uterus. Large intestine 4 (LI4) and LR3 are the so-called “four gates points,” which are commonly used to open relevant meridians and calm the mind. Combining them with pericardium 6 (PC6) and DU20 would relax the patient.

In the ACU 1 group, acupuncture was given on the day of ET (i.e., 3 days after oocyte retrieval) in two sessions lasting 25 minutes immediately before and after ET. Acupoints before ET included DU20 (*Baihui*), ST29, SP8, PC6, and LR3. Acupoints after ET were ST36, SP6, SP10, and LI 4.

Needles were inserted into the above points and manipulated until needle-sensation was obtained, (i.e., *Deqi*—a feeling of, for example, soreness or numbness, distension or pain). After 10 minutes of retention, the needles were again manipulated to maintain *Deqi*. Fifteen minutes later, the needles were removed.

For the ACU 2 group, the same acupuncture protocol as for the ACU 1 group was applied on the day of ET. In addition, this group received one acupuncture session of 25 minutes' duration 2 days after ET (i.e., 5 days after oocyte retrieval), to the following acupoints: DU20, Ren 3, ST29, SP10, SP6, ST36, and LI 4. The acupoints chosen for this session aimed at general relaxation and improvement of uterine blood perfusion, to further enhance endometrial receptivity for implantation. Manipulation and retention was the same as in the previously described procedure.

The patients in the control group followed the clinic's routine procedure (i.e., had bed rest for 1 hour after ET before leaving the clinic).

All acupuncture procedures in the present study were administered by nurses who, before the initiation of the project, were instructed carefully by two professional acupuncture practitioners (Q.M. and S.S.), who supervised the procedures by frequent visits throughout the study period. One of the nurses (M.K.), who was working daily in the clinic, performed approximately half of all acupunctures (94 of 186), whereas the other eight nurses, assisting during weekends and holidays, performed from 3 to 24 acupunctures each.

Hormone Treatments and IVF/ICSI Procedures

Apart from the acupuncture, all patients were treated according to well-established standard regimens of the clinic. These included [1] long protocol GnRH agonist down-regulation from the midluteal phase, followed by gonadotropin stimulation after down-regulation had been ascertained by ultrasound and serum E₂ levels <200 pmol/L, or [2] a short protocol including gonadotropin stimulation from day 2 of the cycle combined with a flexible antagonist protocol, or [3] in a few cases, no hormone stimulation at all.

In all cases, an ovulatory dose of hCG (Pregnyl; Organon, Skovlunde, Denmark) was administered 36 hours before oocyte retrieval. Oocytes were retrieved by ultrasound-guided transvaginal aspiration with automated suction. In

cases of male factor or idiopathic infertility, ICSI was used for fertilization. A maximum of three embryos was transferred back to the uterus after 3 days of culture. Surplus transferable embryos (i.e. more than six even blastomeres and <20% fragmentation) were cryopreserved.

Luteal support was given to all patients, administered as intravaginal P pessaries (Cyclogest; Alpharma, Barnstaple, United Kingdom; 400 mg three times daily) and oral E₂ tablets (Nycomed Danmark, Roskilde, Denmark; 2 mg twice daily) from the day of ET until 12 to 13 days after ET, when a pregnancy test was performed by measurement of serum hCG. Patients with a positive pregnancy test result (serum hCG >10 IU/L) were scanned by ultrasound 3 weeks later, and a clinical pregnancy was diagnosed by the presence of at least one intrauterine gestational sac on that occasion. An ongoing pregnancy was defined as the presence of a viable intrauterine fetus beyond 12 weeks' gestation.

Statistical Methods

Data were expressed as mean \pm SEM. Student's *t*-test was used to test for possible imbalances between the groups regarding the following variables: patient age, body mass index, duration of infertility, mean number of stimulation days, consumption of FSH during stimulation, and mean number of oocytes retrieved, fertilized, cryopreserved, and transferred, and number of transferable embryos. Fisher's exact test was applied to compare frequencies between groups, such as rates of pregnancy, clinical pregnancy, and ongoing pregnancy and delivery. A *P* value <.05 was considered significant.

RESULTS

A total of 273 women were included in the study (Table 1). Of these, 87 were allocated to no acupuncture (control group), 95 to acupuncture on the day of ET only (ACU 1 group), and 91 to acupuncture on the day of ET day and again 2 days later (ACU 2 group). Demographic characteristics were comparable among the groups, with no significant differences with regard to age (median, 37 years; range, 24–45 years), body mass index, duration of infertility, proportion of primary fertility, number of previous IVF/ICSI attempts, and cause of infertility (Table 2). The distribution of ovarian stimulation regimens (i.e., no hormonal stimulation or short [antagonist] protocol or long [agonist] protocol) was not significantly different among the three groups (no stimulation: 2%, 1%, and 3%; short protocol: 24%, 19%, and 18%; long protocol: 74%, 80%, and 79% in the control, ACU 1, and ACU 2 groups, respectively). In addition, the mean (\pm SEM) number of stimulation days and mean (\pm SEM) total consumption of gonadotropin in the three groups was similar (stimulation days: 11.2 \pm 0.3, 11.5 \pm 0.2, and 11.6 \pm 0.3; total consumption of gonadotropin (IU): 2543 \pm 118, 2598 \pm 103, and 2660 \pm 123, respectively, in the control, ACU 1, and ACU 2 groups).

TABLE 2

Demographic characteristics of the study population.			
Characteristic	Control group (n = 87)	ACU 1 (n = 95)	ACU 2 (n = 91)
Age (y), median (range)	37 (27–45)	37 (24–45)	37 (27–45)
BMI (kg/m ²), median (range)	23 (18–32)	23 (16–40)	22 (18–34)
Duration of infertility (y), median (range)	4 (1–9)	3 (1–9)	4 (1–10)
Primary infertility (%)	37	44	45
Previous IVF attempts (%)			
0	36	37	30
≥1	64	67	70
Causes of infertility (%)			
Tubal	19	15	22
Anovulatory	19	11	14
Endometriosis	0	1	4
Male	20	24	19
Mixed	16	14	14
Idiopathic	26	30	26

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Table 3 shows the mean (\pm SEM) number of oocytes retrieved and fertilized and the number of transferred and transferable embryos in the three groups. There were no significant differences between the groups.

The reproductive outcomes in the three groups are shown in Table 4. Of the 273 women, 100 (37%) became pregnant; of these, 91 (33%) had clinical pregnancy and 77 (28%) had ongoing pregnancy (beyond 12 weeks' gestation) or delivery. The rates of positive pregnancy test results, clinical pregnancies, and ongoing pregnancy or delivery were all significantly higher in the ACU 1 group than in the control group (positive pregnancy test: 40 of 95 (42%) vs. 24 of 87 (28%), $P=.044$; clinical pregnancy: 37 of 95 (39%) vs. 21 of 87 (24%), $P=.038$; ongoing pregnancy or delivery: 34 of 95 (36%) vs. 19 of 87 (22%), $P=.049$). The numbers and rates of positive pregnancy tests, clinical pregnancy, and ongoing

pregnancy or delivery in the ACU 2 group were all higher than in the control group, but none of these differences were statistically significant. The rate of early pregnancy loss (expressed as percentage of positive pregnancy tests) was higher in the ACU 2 group (33%) than in the control (21%) and ACU 1 (15%) groups, but the differences were not statistically significant.

DISCUSSION

This prospective, randomized study demonstrates that acupuncture administered on the day of ET significantly improves the reproductive outcome in women undergoing IVF or ICSI treatment for infertility. Thus, our results confirm and extend those of the only comparable prospective, randomized study previously reported (10). The acupuncture procedures used in the ACU 1 group of the present study

TABLE 3

Oocytes and embryos retrieved per cycle			
Variable	Control group (n = 87)	ACU 1 (n = 95)	ACU 2 (n = 91)
Oocytes retrieved	10.6 \pm 0.7	10.4 \pm 0.3	10.7 \pm 0.6
ICSI, n (%)	36 (37)	42 (44)	35 (38)
Oocytes fertilized	7.4 \pm 0.6	7.0 \pm 0.4	7.6 \pm 0.6
Embryos transferred	2.0 \pm 0.1	2.1 \pm 0.05	2.1 \pm 0.06
Embryos cryo preserved	2.4 \pm 0.4	1.9 \pm 0.3	2.4 \pm 0.4
Transferable embryos (transferred + cryopreserved)	4.3 \pm 0.4	4.0 \pm 0.3	4.5 \pm 0.4

Note: Data are mean \pm SEM unless otherwise noted.

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TABLE 4

Reproductive outcomes per ET.			
Reproductive outcome	Control group (n = 87)	ACU 1 (n = 95)	ACU 2 (n = 91)
Positive pregnancy test	24 (28) ^a	40 (42) ^a	36 (40)
Clinical pregnancy	21 (24) ^b	37 (39) ^b	33 (36)
Early pregnancy loss, n (% of positive pregnancy tests)	5 (21)	6 (15)	12 (33)
Ongoing pregnancy/delivery	19 (22) ^c	34 (36) ^c	24 (26)
Implantation rate, % (no. of gestational sacs/no. of transferred embryos)	18 (32/178)	21 (42/200)	19 (36/192)
Data are n (%), unless otherwise noted. Fisher's exact test (two-tailed):			
^a P = .044.			
^b P = .038.			
^c P = .049.			
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were very similar to those used in the Paulus et al. study (10), except for the additional use of auricular acupuncture in the latter.

In the present study, an additional group of patients were randomized to receive acupuncture twice, on the day of ET and on ET day +2 (ACU 2 group). Although the clinical and ongoing pregnancy rates were higher in the ACU 2 group than in the control group, the differences did not reach statistical significance. However, this might relate to the relatively small size of the groups. Combining the acupuncture groups resulted in a significant improved reproductive outcome as compared with the control group, suggesting a beneficial effect on the day of ET, whereas acupuncture on ET day +2 (i.e. closer to the day of implantation) was without additional beneficial effect.

With the application of modern Western scientific principles, the underlying physiologic mechanisms of acupuncture are now increasingly being documented (5). Effects of acupuncture in relation to female infertility might be mediated through central effects on the release of neurotransmitters, including β -endorphin and serotonin, which in turn influence GnRH release and thereby impact on pituitary gonadotropin secretion, ovarian follicular growth, ovulation, and fertility (2, 3, 5).

In addition to this central effect on the hypothalamic-pituitary-ovarian axis, acupuncture exerts a general sympathoinhibitory effect, which locally might reduce uterine artery impedance and thus increase uterine and ovarian blood flow. With Doppler ultrasound, this effect of serial electroacupuncture was documented in 10 infertile women who were down-regulated by GnRH analogue to avoid the effect of endogenous hormones (4). It was proposed that the effect on uterine blood flow might improve the growth and thickness of the endometrium, rendering it more receptive to implantation of the early embryo (4). This notion seemed supported by the results of a later prospective, randomized

study by the same group, in which electroacupuncture was compared with alfentanil for analgesia during oocyte retrieval. Although the effect on fertility was not the primary objective of that study, a significantly higher implantation rate and ongoing pregnancy rate was found in the electroacupuncture group as compared with the alfentanil group (7). Later and larger prospective, randomized studies by the same group, however, were not able to confirm this positive effect on the reproductive outcome of electroacupuncture administered on the day of oocyte retrieval (8, 9).

Measuring uterine artery pulsatility index on the day of acupuncture (i.e. the day of ET), Paulus et al. (10) could not demonstrate significant differences between the acupuncture and control groups, although as mentioned above there was a significant difference in reproductive outcome between the two groups (10). The discrepancy between this finding and the above might relate to differences in setup (i.e., electroacupuncture vs. manual technique and administration of acupuncture on the day of oocyte retrieval vs. the day of ET).

In the present study, we did not try to measure uterine blood flow during ET or before, and the present results therefore do not contribute to the discussion regarding whether the positive effects of acupuncture could be attributed to improved endometrial blood flow (and oxygen tension?). Another possibility could be that acupuncture indirectly, through effects on ovarian and endometrial blood flow, or directly impacts on local humoral factors (hormones, peptide growth factors) that are involved in the regulation of implantation. Experiments with administration of acupuncture during the preovulatory phase of the menstrual cycle have shown that the amount of LH and P in the circulation is increased after needling, and 2–6 hours later the LH peak occurs (11). Whether such effects of acupuncture are also reflected in variations in the circulating levels of hormones and other substances in the luteal phase is not

known, but the question is being approached in an ongoing study in our clinic.

The above-described physiologic, neuroendocrine effects of acupuncture do not rule out that psychological factors (reduction of stress) or placebo effects of the acupuncture procedure might significantly contribute to improve female infertility. Acupuncture certainly has the attributes of a good placebo (Oriental mystique, skin penetration, novelty). Use of placebo acupuncture has been controversial owing to difficulties in designing a method not affecting the acupoints (12). Recently, however, a promising placebo acupuncture technique validated in a prospective, randomized study was described (13), but to our knowledge it has so far not been used in studies on acupuncture effects in female infertility.

In the present study, placebo acupuncture was not used in the control group, and it could be argued that the improvement of the reproductive outcomes found in the acupuncture group could be ascribed to a placebo effect. A number of observations in this study, however, tend to argue against placebo effects explaining the significant differences in reproductive outcomes between the acupuncture and control groups. For instance, if placebo effects were significantly associated with the atmosphere around the acupuncture procedures, one might expect that administering acupuncture in more sessions on different days, as in the ACU 2 group, would further improve the reproductive outcome, but this was not the case. In addition, in a post hoc analysis of our data, we related the reproductive outcomes to acupuncture and to the age of the patients below and above the median of the whole population (37 years). We found that the significantly higher clinical and ongoing pregnancy rates in the ACU 1 group compared with the control group were restricted to patients younger than 38 years (clinical and ongoing pregnancy rates in controls 23% (8 of 35) and 20% (7 of 35) vs. 49% (26 of 53) and 47% (25 of 53) in the ACU 1 group; $P=.015$ and $=.012$, respectively). By contrast, in women aged ≥ 38 years the outcomes were not significantly different (controls: 25% (13 of 52) and 23% (12 of 52) vs. 26% (11 of 42) and 21% (9 of 42), respectively). Although these age-related differences are difficult to explain, they can hardly be ascribed to placebo effects of acupuncture.

Notwithstanding these arguments against a significant influence of placebo on our results, we acknowledge that these beneficial effects of acupuncture in assisted reproductive technologies ought to be confirmed in future prospective, randomized trials including a control group subjected to a

reliable, reproducible placebo acupuncture technique, for instance the one recently reported by Park et al. (13).

In conclusion, the present study confirms that acupuncture administered on the day of ET significantly improves the reproductive outcome of IVF/ICSI. It is also concluded that adding acupuncture on ET day +2 (i.e., closer to the day of implantation) does not further improve the reproductive outcome.

To finally settle the role and relevance of acupuncture in fertility treatment, future prospective, randomized trials including use of a good placebo acupuncture technique are needed.

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This prospective, randomized trial showed a significantly improved reproductive outcome in infertile women who underwent acupuncture on the day of ET as compared with women who did not receive acupuncture.