Clinical Research

Effect of electroacupuncture on egg quality and tumor necrosis factor -α of patients with polycystic ovarian syndrome

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ABSTRACT

Objective To observe the effect of electroacupuncture (EA) on egg quality of polycystic ovarian syndrome (PCOS) patients, and to explore its mechanism.

Methods Two hundred patients who received in vitro fertilization-embryo transfer (IVF-ET) were divided into an EA group (102 cases) and a control group (98 cases) according to random number table. All the patients in the two groups were given Diane-35 and gonadotropin-releasing hormone agonist (GnRH-a) for ovarian hyperstimulation. Besides, EA intervention was applied to shenshu (BL 23), Qihai (GV 6), Zusanli (ST 36), Sanyinjiao (SP 6), Neiguan (PC 6) and Zigong (EX-CA1), etc. in the EA group. Then egg quality, final outcome of pregnancy and levels of tumor necrosis factor-α (TNF-α) were compared between the two groups.

Results (1) EA intervention significantly improved high quality embryo rate of PCOS patients (P<0.05), and clinical pregnancy rate was increased by 8.36%; (2) EA intervention significantly reduced the TNF-α levels of follicular fluid (13.61±15.46 vs 34.09±93.53, P<0.05); (3) TNF-α levels of serum and follicular fluid in the pregnancy group were lower than those of non-pregnancy group [pregnancy group: (53.91 ± 63.32) pg/mL, (14.93 ± 25.37) pg/mL, non-pregnancy group: (76.82 ± 82.96) pg/mL, (25.04 ± 35.79) pg/mL], and the differences were significant (both P<0.05).

Conclusion EA improves egg quality of PCOS patients and increases the clinical pregnancy rate of IVF-ET, the mechanism may be related to TNF-α levels.

KEY WORDS: polycystic ovarian syndrome (PCOS); electroacupuncture; controlled ovarian hyperstimulation (COH); tumor necrosis factor -α

Polycystic ovarian syndrome (PCOS) is a kind of endocrine disease featured by hyperandrogenism, insulin resistance, chronic anovulation and polycystic ovaries. The incidence rate of PCOS among women at the childbearing age is 6%—10%, and PCOS blames for 75% of anovulatory infertility [1]. PCOS patients with ovulation failure, especially accompanied by tubal factors, get pregnant with aid of in vitro fertilization-embryo transfer (IVF-ET). Because hyperandrogenism and insulin resistance contribute to disorders of endocrine and metabolic system, in vitro fertilization (IVF) encounters a multitude of challenges such as a big dosage of gonadotropin (Gn), long medication time, follicles’ refusal to grow or excessive follicles; increased incidence rate and cycle cancellation rate of ovarian hyperstimulation syndrome (OHSS); reduced rate of fertilization and high qualified embryo rate; high spontaneous abortion rate and so on [2]. Thus, the
pregnancy rate only reaches 30% to 40%. Therefore, it is urgent that the clinical pregnancy rate of PCOS patients undergoing IVF-ET be improved. The author treated PCOS patients undergoing IVF-ET with EA intervention in the course of COH, and positive clinical results were achieved. The report is as follows.

CLINICAL DATA

General data

Two hundred PCOS patients received IVF-ET in Genitourinary Department, Second Affiliated Hospital of Shandong University of TCM from January 2009 to December 2012. They were divided into an EA group (102 cases) and a conventional group (98 cases) according to the random number table, they were aged from 21 to 42 years old with an average of 31 years old, with 1 to 15 years of infertility with an average of 4.52 years.

It was shown in table 1 and table 2 that the differences in general information and levels of blood hormone between the two groups before the treatment were not significant (all \( P>0.05 \)) and were comparable.

Diagnostic criteria

PCOS diagnostic criteria (2003 Rotterdam criteria [31]): ① sporadic ovulation and/or anovulation; ② clinical and/or biochemical indicators reveal hyperandrogenism, excluding other potential pathogenic factors, such as congenital adrenal hyperplasia, androgen-secreting tumors, Cushing’s syndrome and so on; ③ polycystic ovaries, and B-ultrasonic examination showed there were more than 12 follicles with diameter of 2–9 mm at each section and/or ovaries increase > 10 mL in volume. Any patient that met two of the items above was diagnosed with PCOS.

Inclusive criteria

According to IVF-ET indications stipulated in “Notice on the Revision of Related Technical Specifications, Basic Standards and Ethical Principles of Assisted Reproductive Technology and Human Sperm Bank” (WKJP [2003] No. 176) issued by the Ministry of Health, the following patients were selected: ① PCOS patients with a plan for undergoing IVF-ET due to tubal factors or oligospermia or asthenospermia; ② patients compliant with the criteria above, and long protocol of ovarian hyperstimulation was planned to given.

Exclusive criteria

① Endometriosis; ② immune infertility; ③ unexplained infertility; ④ patients undergoing intracytoplasmic sperm injection (ICSI) due to male factors; ⑤ chromosomal abnormalities; ⑥ uterine malformations and/or ovarian surgeries; ⑦ age \( \geq 40 \) years; ⑧ application of steroids in the past three months.

METHODS

Long protocol of ovarian induction adopted by patients in the two groups

On the 3rd day of the previous menstrual cycle, patients were given Diane-35 (produced by Bayer Healthcare Limited), one pill one day for 21 days in total. On the 18th day, they were given subcutaneous injection of decepept, (produced by Ferring Pharmaceuticals, 0.1 mg/bottle), 0.1 mg daily for 10 days in total. Then the dosage was reduced to 0.05 mg. Four days later, the fasting levels of follicle stimulating hormone (FSH), estradiol (E2), luteinizing hormone (LH) and progesterone (P) were tested while endometrium and antral follicles were tested via B-ultrasound. If down-regulation was up to standard, intramuscular injection of 150–225 IU/d of recombinant human follicle stimulating hormone (Gonal-F, produced by Merck Serono GmbH, 75 IU/bottle) was given. Five days later, vaginal B-ultrasound was used to monitor follicle growth and E2 and LH were tested, and the amount of Gn was adjusted appropriately according to age, follicle growth and hormone levels. When B-ultrasound identified at least one follicle with diameter up to 20 mm or two up to

<table>
<thead>
<tr>
<th>Groups</th>
<th>Patients</th>
<th>Age (years)</th>
<th>Duration of infertility (years)</th>
<th>BMI</th>
<th>Type of infertility (case)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EA</td>
<td>102</td>
<td>31±5</td>
<td>4.36±1.16</td>
<td>23.36±6.40</td>
<td>primary: 50; secondary: 52</td>
</tr>
<tr>
<td>Control</td>
<td>98</td>
<td>31±5</td>
<td>4.69±1.41</td>
<td>23.33±3.58</td>
<td>primary: 46; secondary: 52</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Groups</th>
<th>Patients</th>
<th>FSH (IU/L)</th>
<th>LH (IU/L)</th>
<th>E2 (pmol/L)</th>
<th>T (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EA</td>
<td>102</td>
<td>6.43±2.02</td>
<td>4.65±2.96</td>
<td>150.76±149.52</td>
<td>1.47±1.95</td>
</tr>
<tr>
<td>Control</td>
<td>98</td>
<td>6.66±2.16</td>
<td>4.76±2.96</td>
<td>157.86±67.05</td>
<td>1.54±2.36</td>
</tr>
</tbody>
</table>
18 mm, patients should stop taking Gn and decepety. At 9 o'clock that night, intramuscular injection of 5 000–10 000 IU of human chorionic gonadotropin (hCG, produced by Livzon Group, China) was given. Thirty-six h later, transvaginal oocyte retrieval was conducted under B-ultrasound guidance. Seventy-two h later, 2 to 3 high quality embryos were transplanted (2 embryos for women below 35 years old in the first assisted reproductive cycle), and then intramuscular injection of 60 mg/d progesterone was given for luteal support. Fourteen days following the transplantation, urinary hCG was tested to determine the biochemical pregnancy. Five weeks after the transplantation, ultrasound revealed gestational sac and primitive impulse of heart tube to determine clinical pregnancy. If gestational sac and primitive impulse of heart tube terminated within the first 12 weeks of pregnancy, it was identified as early abortion.

**EA intervention protocol**

The patients in the EA group received electroacupuncture therapy during the menstrual cycle prior to COH and during COH (except the menstrual period).

**Acupoints selection:** Shènshū (腎俞 BL 23), Qìhāi (氣海 CV 6), Zǔsānli (足三里 ST 36), Sànyīnjiāo (三陰交 SP 6), Nèiguān (內關 PC 6) and Zhīgōng (子宮 EX-CA 1).

**Manipulation:** Before needling, acupoints area was disinfected with 75% ethanol cotton balls. *Hwato* sterile needles were twisted into the skin and manipulated to the extent of *deqi*. Then needles were connected to the electrode wire of G6805-1 pulse therapy EA apparatus (produced by Qingdao Xin Sheng Industrial Co., Ltd, China). The dilatational wave was applied and the current intensity reached the degree that patients felt comfortable. They were treated once a day, 30 min each time, for 5 days, with 1 to 2 days off, until oocyte retrieval.

**Observation indices**

(1) Blood hormone levels: on the 3rd day of the menstrual phase and on the hCG day, 3 mL of fasting venous blood was collected and analyzed by Bayer ACS-180 automated chemiluminescence immunoassay system.

(2) On the day of oocyte retrieval, 3 mL of venous blood was collected. After the blood was centrifuged at the speed of 3 000 r/min for 10 min, the supernatant was collected and stored in refrigerator at −80 °C. Transvaginal oocyte retrieval was conducted under B-ultrasound guidance to retain mature, clear and non-ensanguined follicular fluid containing oocytes with diameter of ≥18 mm. After the follicular fluid was centrifuged at the speed of 3 000 r/min for 10 min at room temperature, the supernatant was collected and stored in refrigerator at −80 °C.

(3) Gn dosage, days of medication, number of oocytes, fertilization rate, cleavage rate, high quality embryo rate, endometrial thickness on the hCG day, OHSS incidence, cycle cancellation rate, clinical pregnancy rate, rate of early spontaneous abortion were observed.

**Statistical analysis**

**Statistical analysis** was performed using SPSS17.0 software package. Measurement data were expressed as mean ± standard deviation (\( \bar{x} \pm s \)), *t* test was used; enumeration data were expressed as a percentage (%), and \( \chi^2 \) test was used; ranked data were tested by Mann-Whitney *U*; correlation analysis of the two variables was conducted by Spearman rank correlation analysis; if \( P<0.05 \), the difference was significant.

**RESULTS**

(1) **Effect of EA on egg quality and pregnancy rate of IVF-ET (Table 3 and Table 4)**

**Table 3** Comparison of retrieved oocytes of PCOS patients between the two groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Patients</th>
<th>Eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>EA</td>
<td>102</td>
<td>15.22±5.23</td>
</tr>
<tr>
<td>Conventional</td>
<td>98</td>
<td>16.45±6.49</td>
</tr>
</tbody>
</table>

**Table 4** Comparison of egg quality and pregnancy rate of IVF-ET between the two groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Patients</th>
<th>Fertility rate (%)</th>
<th>Cleavage rate (%)</th>
<th>High-quality embryonic rate (%)</th>
<th>Pregnancy rate(%)</th>
<th>OHSS incidence rate (%)</th>
<th>Cancellation rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EA</td>
<td>102</td>
<td>79.12</td>
<td>97.36</td>
<td>50.81(1)</td>
<td>53.19 (50/94)</td>
<td>7.84 (8/102)</td>
<td>7.84 (8/102)</td>
</tr>
<tr>
<td>Conventional</td>
<td>98</td>
<td>76.85</td>
<td>95.89</td>
<td>42.33</td>
<td>44.83 (39/87)</td>
<td>11.22 (11/98)</td>
<td>11.22 (11/98)</td>
</tr>
</tbody>
</table>

Note: compared with that in the conventional group, \(^{1} P<0.05 \).
group \((P<0.05)\).

(2) Effect of EA on TNF-\(\alpha\) levels of PCOS patients (Table 5)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Patients</th>
<th>Serum TNF-(\alpha)</th>
<th>Follicular fluid TNF-(\alpha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EA</td>
<td>102</td>
<td>56.23±74.95</td>
<td>13.61±15.46</td>
</tr>
<tr>
<td>Control</td>
<td>98</td>
<td>79.38±117.45</td>
<td>34.09±93.53</td>
</tr>
<tr>
<td>(t) value</td>
<td></td>
<td>6.423</td>
<td>-2.139</td>
</tr>
<tr>
<td>(P) value</td>
<td></td>
<td>&gt;0.05</td>
<td>0.035</td>
</tr>
</tbody>
</table>

It could be seen that TNF-\(\alpha\) level in follicular fluid in the EA group was significantly lower than that of the control group, and the difference was statistically significant \((P<0.05)\).

(3) Comparison of TNF-\(\alpha\) levels in pregnancy patients and non-pregnancy patients (Table 6)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Patients</th>
<th>Serum TNF-(\alpha)</th>
<th>Follicular fluid TNF-(\alpha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy</td>
<td>89</td>
<td>53.91±63.32</td>
<td>14.93±25.37</td>
</tr>
<tr>
<td>non-pregnancy</td>
<td>92</td>
<td>76.82±82.96</td>
<td>25.04±35.79</td>
</tr>
<tr>
<td>(t) value</td>
<td></td>
<td>-2.083</td>
<td>-2.199</td>
</tr>
<tr>
<td>(P) value</td>
<td></td>
<td>0.039</td>
<td>0.029</td>
</tr>
</tbody>
</table>

It could be seen that TNF-\(\alpha\) levels in serum and follicular fluid of pregnancy patients were lower than those in non-pregnancy, and the differences were statistically significant (both \(P<0.05\)).

(4) Comparison of Gn dosage and medication time in the EA group and in the conventional group (Table 7)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Patients</th>
<th>Gn dosage (IU)</th>
<th>Gn medication time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EA</td>
<td>102</td>
<td>2 062.50±696.06</td>
<td>9.78±1.30</td>
</tr>
<tr>
<td>Control</td>
<td>98</td>
<td>2 271.30±728.91</td>
<td>10.30±1.81</td>
</tr>
<tr>
<td>(t) value</td>
<td></td>
<td>-2.072</td>
<td>-2.290</td>
</tr>
<tr>
<td>(P) value</td>
<td></td>
<td>0.04</td>
<td>0.023</td>
</tr>
</tbody>
</table>

It could be seen that Gn Dosage and medication time in the EA group were significantly less than those in the control group (both \(P<0.05\)).

(5) Correlation between TNF-\(\alpha\) levels and amount and rate of high quality embryos (Figure 1–Figure 4)
It could be seen that TNF-α levels in serum and follicular fluid were significantly negatively correlated with amount and rate of high quality embryos (P <0.05).

**DISCUSSION**

In recent years, acupuncture is used in assisted reproductive technology (ART) to increase the pregnancy rate, which has been widely studied. Studies such as acupuncture-induced ovulation, restored ovarian function and adjustment of the menstrual cycle provide a basis for the therapy combining acupuncture and ART to improve the rate of pregnancy. WANG Shao-jin, et al[10] proposed that the realization of acupuncture effect was closely related to the conduction of cell information by analyzing nerve-endocrine mechanism produced by acupuncture effects, and found that endocrine function of the hypothalamic-pituitary-ovarian axis of PCOS patients had benign adjustment effect, thus normal secretion of gonadal hormone could be achieved and ovulation is improved.

ART involves three stages: COH, transvaginal oocyte retrieval under B-ultrasound guidance and embryo transplantation. The function of acupuncture intervention in transvaginal oocyte retrieval under B-ultrasound guidance and embryo transplantation to increase the pregnancy rate has been reported[6-10], and the mechanism was discussed in various aspects[11-19]. But the effect of EA intervention on egg quality and pregnancy outcome during the COH has not been studied adequately. The study mainly discussed the effect of EA intervention on pregnancy of PCOS patients undergoing IVF-ET during the COH.

Chinese medicine believes that pathogenesis of PCOS is very complex, involving dysfunction of kidney, spleen and liver as well as adjustment disorders of kidney-tiangui-thoroughfare vessel and conception vessel caused by pathological products like phlegmatic hygrosis and blood stasis. The key factor responsible for the disease is kidney deficiency, and phlegmatic hygrosis and blood stasis are its common pathological links. BL 23, CV 6, ST 36, SP 6, PC 6 and EX-CAl were selected as main acupoints. BL 23 belongs to the bladder meridian of foot-taiyang as back-shu point of the kidney, tonifying the kidney and supporting yang, strengthening waist and alleviating water retention. Kidney dominates bones to produce marrow, stores the essence of life and is concerned with reproduction. BL 23 is where channel qi of kidney comes in and out. Needling at BL 23 can help warm kidney and tonify yang and circulate qi-blood. Starting from the uterus, CV 6 belongs to the conception vessel, related to pregnancy and interlinked with vitality, and breeding genuine qi as root of primordial yang. Besides, it is where conception vessel, governor vessel and thoroughfare originate from, and qi-blood in the body gathers, adjusting the vitality. Needling CV 6 can help warm kidney, tonify and consolidate menstruation and help BL 23 to supplement kidney qi and yang. ST 36 is one of the main points of stomach meridian of foot-yangming, regulating spleen and stomach, tonifying middle energizer and qi, clearing and activating the meridians and collaterals, dispelling wind and dampness and strengthening body resistance and eliminating evil; SP 6 governs diseases of the three yin meridians of spleen, liver and kidney, strengthening the spleen and stomach, nourishing the liver and tonifying the kidney, and mild reinforcing-reducing two points makes blood exuberant, and helps dredge channel qi, promote the flow of energy and blood and regulate and invigorate qi activity, thus dredging conception vessel and replenishing qi and blood in great thoroughfare vessel, resulting in menophasia and fertility. PC 6 treats emotional stress and blocked qi activity. Needling at PC 6 regulates qi activity and tonifies qi in liver, spleen and kidney. EX-CAl is an important extra points, which governs birth, thus needling EX-CAl nurses and nourishes the uterus. EX-CAl is a main point for treating infertility.

Profusion blood and vigor is the base of female physiological activities. Blood is controlled by spleen, stored in liver and discharged by kidney, endlessly supplying and irrigating the whole body, indicating physiological characteristics of women are related to liver and spleen and have an even closer relationship with kidney. Thoroughfare can regulate twelve meridians, so with exuberant blood and essence, thoroughfare can nourish the uterus and store and distribute blood to nurture the fetus. Conception vessel is known as “sea of yin meridian”, commanding qi of yin meridians. WANG Bing, a well-known medical scientist in Tang Dynasty, said: “conception vessel plays a dominant role in pregnancy”. Therefore, unobstructed flow of qi in conception vessel can help conceive a baby. In this study, needling BL 23 and CV 6 tonified kidney qi; ST 36 and SP 6 harmonized the spleen and stomach and eliminated phlegm and dampness; SP 6 dominates blood, a confluent acupoint of the three yin meridians of spleen, liver and kidney, and the blood is taken from the three yin meridians to treat infertility; infertile females usually suffer from stagnation of liver qi and poor flow of qi and blood, leading to blood stasis. PC 6 dispels melancholy and
achieves catharsis of liver and gallbladder qi; EX-CA1 is one of main extra points of conception vessel, which governs birth and cures infertility. Needling at all the points regulates liver, spleen and kidney, tonifies kidney qi, regulates spleen and stomach, eliminates phlegm and dampness, regulates the flow of blood, smooths liver-qi stagnation, and regulates the function of the kidney-tianqiu-thoroughfare axis.

TNF-α is a non-glycosylated protein with various biological functions. Because increased concentration of TNF-α of Th1-typed cytokines is associated with adverse pregnancy outcomes, its role in IVF-ET has attracted attention from many scholars and scientists [20]. Studies showed [21-23] that quality of oocyte with significantly increased concentrations of TNF-α in follicular fluid was poor, and TNF-α concentration of follicular fluid was associated with IVF-ET clinical pregnancy outcome, suggesting that increased concentrations of TNF-α in follicular fluid may affect egg quality, thereby affecting embryo implantation and resulting in adverse outcomes. The results of this study showed that: TNF-α levels of serum and follicular fluid in the pregnancy patients were significantly lower than those in non-pregnancy patients (both \( P<0.05 \)), indicating that TNF-α levels have great negative correlation with egg quality and pregnancy outcome.

Cumulus granulosa cells are granule cell layers wrapped around oocytes, which have an extensive and complex intercellular coupling mechanism with oocytes, including gap junctions, regulating the growth of the oocyte. Susan, et al [24] found by in vitro culture of mouse granulosa cells that recombination of TNF-α could enhance the expression of Fas antigen and promote apoptosis of granulosa cells. Basini, et al [25] further proved by in vitro culture of bovine cumulus granulosa cells that recombination of TNF-α could inhibit granulosa cells from secreting progesterone and promote apoptosis of granulosa cells. Host, et al [26] found that apoptosis of granulosa cells was related to maturation and fertilization of oocytes. Ramane, et al [27] found that eggs’ ability to fertilize was related to apoptosis of cumulus granulosa cells; Lee, et al [24] reported that cumulus granulosa cells are related to fertilization rate of patients and IVF outcome. It was found in this study that with EA intervention, TNF-α levels of follicular fluid were significantly lower than that in the control group, and the differences were significant (\( P \leq 0.05 \)); TNF-α level of serum was lower than those of the control group, but the difference was not significant (\( P>0.05 \)), proving that EA intervention improved the quality of eggs by lowering TNF-α level in follicular fluid, and its mechanism may be explained as follows: EA intervention lowered the TNF-α level in follicular fluid, reduced apoptosis of cumulus granulosa cells and improved egg quality, thus improving clinical pregnancy rate of patients receiving IVF-ET, which needed to be confirmed by further studies.

REFERENCES


**ABSTRACT IN CHINESE**

[摘 要] 目的: 观察电针对多囊卵巢综合征 (PCOS) 患者卵子质量的影响, 并探讨其作用机制。方法: 将受体休外受精—胚胎移植 (IVF-ET) 的PCOS患者200例按照随指数表法分为电针组 (102例) 、对照组 (98例), 两组均给予选英—35及促性腺激素释放激素激抗剂促排卵, 电针组在促排卵过程中加胃俞、气海、足三里、三阴交、内关、子宫等穴位电针干预, 比较两组患者卵子质量、最终的妊娠结局及 胚胎坏死因子-α (TNF-α) 水平。结果: (1) 电针干预可显著提高PCOS患者的优胚率 (P<0.05), 并可将临床妊娠率提高8.36%; (2) 电针干预可明显降低卵泡液TNF-α水平 (13.61±15.46 vs 34.09±93.53, P<0.05); (3) 妊娠患者血清、卵泡液TNF-α水平均低于非妊娠患者 [妊娠: (53.91±63.32) pg/mL, (14.93±25.37) pg/mL，非妊娠: (76.82±82.96) pg/mL, (25.04±35.79) pg/mL] 差异有统计学意义 (均P<0.05)。结论: 电针能够改善PCOS患者卵子质量, 提高IVF-ET的临床妊娠率, 其机制可能与TNF-α水平有关。

[关键词] 多囊卵巢综合征 电针 控制下超促排卵 胚胎坏死因子-α