Curative effect of assisted reproduction technology by Traditional Chinese Medicine multi-channel interventional therapy on 95 cases of in vitro fertilization and embryo transfer failure

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Abstract

OBJECTIVE: To evaluate the curative effect of Traditional Chinese Medicine (TCM) multi-channel interventional therapy on women with Assisted Reproductive Technology (ART) failure, to compare the curative effect of the dual therapy and triple therapy on women with ART Failure, and to choose the best TCM interventional therapeutic plan.

METHODS: The 95 cases with ART Failure from West China second University Hospital of Sichuan University meeting the inclusion criteria were randomly divided into three groups: dual therapy group (31 cases), triple therapy group (33 cases) and control group (31 cases). According to the intervene treatment of in vitro Fertilization and Embryo Transfer (IVF-ET) long cycle scheme, the control group wait naturally for 3 months before IVF-ET. The dual therapy group take TCM prescription II of cultivating emotion and assisting reproduction and auricular acupuncture for 3 months before IVF-ET, then Western Medicine treatment progestin supporting as well as auricular application and Antai Recipe after IVF-ET transplantation. The triple therapy group take TCM prescription II of cultivated emotion and assisted reproduction, auricular acupuncture and retention enema of TCM, and combination treatment the same as dual therapy group after transplantation. The natural pregnancy number, the period condition of secondary IVF-ET and the improvement of the kidney deficiency, liver depression and blood stasis syndrome among those three groups were compared.

RESULTS: It was showed from analysis in 95 cases with ART failure that the number of natural preg-
nancy was as follows: 3 patients from the dual therapy group, 10 patients from the triple therapy group, and no patient from the control group. The comparison among three groups have statistical significance (P < 0.05). The treatment group is superior to the control group, while the triple therapy is superior to the dual therapy. The comparison of the condition of the fertility rate, clinical pregnancy rate, biochemical pregnancy rate and early abortion rate during the period of secondary IVF-ET between pre-therapy and post-treatment of both the dual therapy group and the triple therapy group have statistical significance (P < 0.05). The comparison of the improvement of the kidney deficiency, liver depression and blood stasis syndrome between pre-therapy and post-treatment of both the dual therapy group and the triple therapy group have statistical significance (P < 0.05). The comparison between three groups after treatment have statistical significance (P < 0.05).

CONCLUSION: TCM multi-channel interventional therapy can increase the natural pregnancy rate of patients with ART Failure (the triple therapy is superior to the dual therapy); it can increase the fertility rate, clinical pregnancy rate, and decrease the early abortion rate during the period of secondary IVF-ET; it can improve syndromes of kidney deficiency, liver depression and blood stasis.

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Keywords: Infertility; Fertilization in vitro; Embryo transfer; Kidney deficiency and blood stagnation; Stagnation of liver Qi; Medicine, Chinese Traditional

INTRODUCTION

Infertility is the absence of pregnancy after 12 months or more of regular unprotected sexual intercourse. It becomes an important public health issue for the high prevalence, it has significant influence on the quality of life (QoL) of patients and the family as well as the stability of society. It is reported that Infertility has been ranked as the third biggest disease after tumor and cardiovascular diseases so far, approximately 15% of all human couples are diagnosed with infertility, and nearly 11% in our country, which has a tendency to rise year by year. Represented by in vitro fertilization and embryo transfer (IVF-ET), the rise of assisted reproductive technology, which has improved the cure rate of infertility, brought hope for the majority of infertility patients. However, by IVF-ET, it is far from achieving doctor-patient satisfaction in terms of clinical pregnancy rate. It is estimated that the average clinical pregnancy rate is 40%-50%. There are complications, such as poor ovarian response, ovaries high reaction or even Ovarian hyper-stimulation syndrome (OHSS), high cancellation rate and abortion rate and so on. Recently, TCM multi-channel interventional therapy on IVF-ET failure cases have attracted increasing attention world widely. Our previous studies had demonstrated that the application of TCM therapies in IVF-ET could increase the endometrial thickness of patients, improve the fertilization rate and clinical pregnancy rate and decrease the early abortion rate, thus improving the success rate of IVF-ET. Based on the preliminary research, this study aims to evaluate the curative effect of TCM multi-channel interventional therapy on women with ART failure and compare the curative effect of the dual therapy and triple therapy in order to choose the best TCM interventional therapeutic plan.

MATERIALS AND METHODS

General information

Ninty-five cases with ART failure (long cycle scheme) from December 2012, to March 2014 in reproductive center of West China second University Hospital of Sichuan University, who decide to do IVF-ET (long cycle scheme) again and meet the inclusion criteria were selected. No statistical significances were found in age, BMI, menstruation, the number of pregnancy, infertility and related factors, past IVF-ET condition and TCM syndrome integral, etc. among those three groups (P > 0.05). This study get the ethics committee’s approval, all participants know and voluntarily signed informed consent (the Teaching Hospital of Chengdu University of Traditional Chinese Medicine Central ethics review: 2013KL-022). Clinical trial has been registered.

Diagnostic criteria

Western Medicine diagnostic criteria: infertility diagnostic criteria refers to the Obstetrics and Gynecology. Infertility, defined clinically as the absence of pregnancy after 12 months or more of regular unprotected sexual intercourse.

Primary infertility is defined as no contraception or pregnancy while secondary infertility is being conceived once and then no contraception for one year. According to the relevant content from Notice of the Ministry of Health on revision of human ART and human sperm bank related specifications, basic standards and ethical principles, IVF-ET indications: (a) gametes transport barriers caused by female factors; (b) ovulation obstacle; (c) endometriosis; (d) oligospermia and asthenospermia; (e) unexplained infertility; (f) immune infertility.
The criteria of TCM syndrome differentiation (kidney deficiency, liver depression and blood stasis): Clinical research guiding principles for new medicine of the Chinese medicine and gynecology of Traditional Chinese Medicine were referred,\textsuperscript{11,12} and then the content was formulated. The main symptoms included: (a) sorerness and weakness in waist and knee; (b) irritability and depression; (c) dysmenorrhea or dark and clotted blood. The secondary symptoms included: (a) dizziness and tinnitus; (b) dispiritedness and lassitude; (c) insomnia or amnesia; (d) sexual hypoactivity; (e) alopecia or gomphiasis; (f) feverish sensation in the five centers (palms, soles, and chest) or tidal fever and night sweat; (g) urination much at night; (h) painful chest and hypochondrium and breast; (i) sign often; (j) fullness of lower abdomen; (k) no desire for diet; (l) hypogastrium prickling; (m) dark purple tongue body with ecchymosis and petechiae. Tongue and pulse manifestations included: light red or red tongue body or dark purple tongue with ecchymosis and petechiae; thin white coating or less, and thin wiry pulse or slippery wiry pulse or deep wiry pulse or unsmooth pulse. As mentioned above, one might be diagnosed who meets two no primary symptoms or and one meets one or more symptom from 1-7 minor items, 8-11 minor items and 12-13 minor items respectively, and then refers to the tongue and pulse manifestations.

Inclusive criteria included: (a) within 20 to 42 years old; (b) meet IVF-ET indications; (c) after the failure of the long cycle scheme; (d) meeting the TCM differentiation standard of kidney deficiency liver depression and blood stasis syndrome; (e) know and sign the informed consent voluntarily.

Exclusion criteria included: (a) does not meet the inclusion criteria; (b) allergic constitution; (c) combined with severe primary disease such as cardiovascular, liver, kidney and hematopoietic system and psychopath; (d) combined with acute infections disease and physical organic disease; (e) diagnosed with genital malformations, reproductive system inflammation or tumor; (f) hereditary disease recorded in the "maternal and infant health care law"; (g) serious bad habits such as drug abuse; and (h) exposure to the deformity amount of radiation, poison, drugs and in action period.

**Therapeutic plan**

According to the interntentional treatment of in vitro IVF-ET long cycle scheme, the control group wait naturally for 3 months before IVF-ET. The dual therapy group take TCM prescription II of cultivating emotion and assisting reproduction and auricular acupoint therapy for 3 months before IVF-ET then Western Medicine treatment progesterin supporting as well as auricular application and Antai Recipe after IVF-ET transplantation. The triple therapy group take TCM prescription of cultivated emotion and assisted reproduction, auricular acupoint therapy and retention enema of TCM, and combination treatment the same as dual therapy group after transplantation.

First stage: between the date of the secondary IVF-ET cycling down regulation and after the former failure. The control group: wait naturally. The dual therapy group: TCM prescription of cultivated emotion and assisted reproduction + auricular acupoint therapy. The triple therapy group: TCM prescription of cultivated emotion and assisted reproduction + auricular acupoint therapy + retention enema of TCM.

Second stage: from the day of down regulation to the day of embryo transfer.

The control group: Western Medicine routine regulation, ovulation induction. The dual therapy group: TCM prescription of cultivated emotion and assisted reproduction + auricular acupoint therapy + Western Medicine routine regulation, ovulation induction. The triple therapy group: TCM prescription of cultivated emotion and assisted reproduction + auricular points therapy + retention enema of TCM + Western Medicine routine regulation, ovulation induction. The control group: from the 14th day after embryo transfer and the pregnancy confirmed to 70 d after embryo transfer.

The control group: routine corpus luteum support by Western Medicine. The therapy groups (the dual therapy group, the triple therapy group): TCM prescription of miscarriage prevention + auricular acupoint therapy + routine corpus luteum support by Western Medicine.

TCM prescription of cultivated emotion and assisted reproduction: Tusizi (Semen Cuscuae) 15 g, Gouqizi (Fructus Lycii) 10 g, Chahu (Radix Bupleuri Chinesis) 10 g, Baihe (Bulbus Lilii Lancifolii) 20 g, Fupenzi (Fructus Ruby Chingii) 15 g, Baishao (Paoniae Alba) 15 g, Zhiqiao (Fructus Aurantii Submaturus) 10 g, Gancao (Radix Glycyrrhizae) 5 g, Prepared Dihuang (Radix Rehmanniae) 10 g, Danggui (Radix Angelicae Sinensis) 10 g, Chuanxiong (Rhizoma Chuanxiong) 10 g.

TCM prescription of miscarriage prevention: Tusizi (Semen Cuscuae) 15 g, Chuanxuduan (Radix Dipsaci Asperoidis) 15 g, Nanshashen (Radix Adenophorae Tetraphyllae) 30 g, Shenpi (Pericarpmum Citri Reticulatae) 10 g, Sangiisheng (Herba Taxilli Chinensis) 15 g, stir-frying Baizhu (Rhizoma Atractylodis Macrocephalae) 20 g, stir-frying with honey Gancao (Radix Glycyrrhizae) 5 g, Gouqizi (Fructus Lycii) 10 g.

Decocotion method: soak 30 min, high heat boiling, switch to gentle heat 10-15 min, filtrate 200-300 mL; add water, the same method 2 times, mix up, 1 dose for 2 days, 100 mL, t.i.d., 0.5 h after meal.
Retention enema of TCM
Consist: Danshen (Radix Salviae Miltiorrhizae) 10 g, Sanleng (Rhizoma Spargani) 10 g, Ezhu (Rhizoma Curcumae Phaeocaulis) 10 g, Shanyao (Rhizoma Dioscoreae Opposita) 15 g, Daxuueteng (Gaulis Sargentodae) 20 g, Baijiangcao (Herba Patriniae Sabinaefoliae) 15 g, Pu-gongying (Herba Taraxaci Mongolici) 15 g, Yanhusuo (Rhizoma Corydalis Yanhuso) 15 g, Chishao (Radix Paeoniae Rubra) 15 g.
Method: intelligent from decoction, 50 mL hot water mix, 1 dose for 3 days, q.n., Stop when basic body temperature ascend for 7 days.
Disposable sputum suction tube *1, 50 mL drop stitch *1, the liquid temperature 39-41 °C; empty stool and urine lateral position, Sterilizing anus; lubricate tube, then put into anus for about 10-15 cm, slow push by hollow needle, keep for at least 2 h.

Auricular treatment
Points: liver (CO 12), kidney (CO 10), Shenmen (TF 4), endocrine (CO 18), Spleen (CO 13).
Methods: disinfecting auricular and cochlea with alcohol, pressing ear points with small boxes fixed cowherb seed, gently rubbing stimulation for 1-2 min, 4-6 times a day, indwelling for 4-5 d.
Conventional long plan for IVE-ET: to regulate the pituitary gland with gonadotropin releasing hormone agonist (GnRH-a) at the middle of luteal phase of the former menstrual cycle before entering the IVF treatment cycle; start gonadotropin (Gn) after the regulation criteria reached, and adjust the dosage of Gn according to the actual circumstances of the patients, serum sex hormone levels and transvaginal ultrasound to monitor follicle, when bilateral ovaries contain one or more follicles average 18 mm in diameter or 3 and above follicles reach 17 mm average in diameter, then combine serum sex hormone levels, intramuscular injection of HCG 10000 IU to induce ovulation at 21:00 in the evening, conduct ultrasound-guided transvaginal oocyte retrieval after 34-36 h. Conventionally conduct fertilization in vitro and embryo culture after retrieving the follicles. To the third day after cultivation, transplant 1-3 embryos, support corpus luteum with HCG or progesterone, and then test HCG of blood to confirm whether pregnant or not 12 d after transplantation. If gestational sac, embryo bud and heart beat could be seen in the womb by ultrasound 4-5 weeks after the transplantation, we call it clinical pregnancy.

Curative effect observation index
Stage 1: natural pregnancy; improvement of TCM syndrome before and after treatment.
Stage 2: No. of mature eggs; number of fertilized eggs; fertility rate (fertilization No./No. of oocytes × 100%); number of fresh embryos; endometrial thickness in HCG day and ET day; cycle cancellation rate (cancellation No./start period No. × 100%); start period transplantation rate (transplant cycles No./initial period No. × 100%); oocyte retrieval cycle rate (transplant cycles No./oocyte retrieval cycle × 100%).
Stage 3: initial cycle pregnancy rate (pregnancy No./initial period No. × 100%); cycle pregnancy rate (pregnancy No./transplant cycles No. × 100%).
Stage 4: clinical pregnancy rate (clinical pregnancy/Initial period No. × 100%); clinical pregnancy rate (clinical pregnancy No./transplant cycles No. × 100%); biochemical pregnancy rate (biochemical pregnancy No./pregnancy No. × 100%); ectopic gestation rate (ectopic gestation No./ pregnancy No. × 100%); early abortion rate (early abortion No./clinical pregnancy No. × 100%).

Evaluation standard of TCM syndrome integral therapeutic effect
Reference to Chinese medicine new medicine clinical research guiding principles,1 obstetrics and gynecology and gynecology of Traditional Chinese Medicine.5,6
Healing: symptoms disappear or basically disappear after treatment, syndrome integral reducing 95% or higher; remarkably effective: symptoms significantly reduced after treatment, syndrome integral reducing 70% or higher, < 95%; effective: symptoms reduced after treatment, syndrome integral reducing 30% or higher, < 70%; invalid: no ease or get worse after treatment, syndrome integral reducing < 30%.

RESULTS
Completed situation of the cases brought into three groups
One case missed in the dual therapy group (be out of touch), 3 cases missed in the triple therapy group (one was out of touch and other two went to other reproductive centers after treatment), one case missed in the control group (be out of touch) (Table 1). All the cases in groups enter the FAS.

Comparison of the condition of natural pregnancy among three groups
Both the comparisons among three groups and between any two groups have statistical significance (P < 0.05). Treatment groups are superior to the control group, the triple therapy is superior to the dual therapy (Table 2). FAS is consistent with PPS in conclusion.
Comparison of TCM syndrome among three groups

The comparisons of TCM syndrome integral among three groups have statistical significance ($P < 0.05$). The treatment groups are superior to the control group. Comparisons between the treatment groups have no statistical significance ($P > 0.05$). The comparisons between pre-therapy treatment group and post-treatment group have statistical significance ($P < 0.05$). The post-treatment is superior to the pre-therapy. The comparison between the control group pre-therapy and post-treatment has no statistical significance ($P > 0.05$) (Table 3).

There are statistical significant differences in TCM syndrome therapeutic effect among three groups after treatment ($P < 0.05$). The treatment groups are superior to the control group. Comparisons between the dual therapy group and the triple therapy group have statistical significance ($P < 0.05$) (Table 4).

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<th>Table 1 Distribution of the cases in groups [n (%)]</th>
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<th>Table 3 Comparison of TCM syndrome integral among three groups between pre-therapy and post-treatment and between groups after therapy (scores, $\pm s$)</th>
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<td>Pre-therapy</td>
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<td>Difference between pre-therapy and post-treatment</td>
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<th>Table 4 Comparison of TCM syndrome therapeutic effect among three groups [n (%)]</th>
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Conditions of secondary IVF-ET cycle among three groups

The comparisons of fertilization rate among three groups after treatment have statistical significance ($P < 0.05$). The treatment groups are superior to the control group. The comparisons between the treatment groups pre-therapy and post-treatment have statistical significance ($P < 0.05$). The post treatment is superior to the pre-therapy group. The comparison between the control group pre-therapy and post-treatment has no statistical significance ($P > 0.05$) (Table 5).

The comparisons among three groups after treatment have statistical significance ($P < 0.05$). Clinical pregnancy rates in treatment groups are higher than the control group, while biochemical pregnancy rates and early abortion rates in treatment groups are lower than the control group. The comparison between the dual therapy group and the triple therapy group has no sta-
tistical significance ($P > 0.05$). The comparison between pre-therapy group treatment group and post-treatment group have statistical significance ($P < 0.05$). Clinical pregnancy rates after treatment are higher than pre-therapy group, while biochemical pregnancy rates and early abortion rates after treatment are lower than before. The comparison of the control group pre-therapy and post-treatment has no statistical significance ($P > 0.05$) (Table 6).

**DISCUSSION**

In this study, 3 cases of spontaneous abortion in dual therapy, 10 in triple therapy. Compared with control group, natural pregnancy rate is improved after TCM multi-channel interventional therapy. TCM prescription may tonify kidney and soothe liver, promote blood circulation to remove blood stasis: keeping the balance of Yin and Yang, and affecting the luteal function, leading to the endogenous Gn in a low level and insufficient during normal menstrual cycle. However, in order to improve the success rate of IVF-ET, controlled ovarian hyperstimulation requires that multiple follicles mature synchronously, which leads to the relative lack of blood-supply and sufficient kidney essence of the single follicle. Those follicles with poor quality are not maturing healthily, resulting in forming high quality embryos difficultly and leading to weakness of embryo implantation.

On the one hand, in the process of IVF-ET, GnRH-a and Gn have adverse effects in different degree on the development of follicles and sex hormone secretion, directly or indirectly affecting the endocrine environment, which influences the formation of follicular development and maturation, and interferes the uterine endometrial proliferation with embryonic development synchronously. On the other hand, in the IVF-ET falling-regulation period, GnRH-a promotes the pituitary in desensitization and suppresses the ovarian endocrine function, leading to the endogenous Gn in a low level and affecting the luteal function. In addition, a large number of granulosa cells are lost with suction, and hormone secretion are insufficient during the retrieval follicular process, by which clinical pregnancy rate is decreased and early abortion rate increased.

In this study, the TCM multi-ways treatment can increase the fertilization rate, pregnancy rate and early
abortion rate in IVF-ET cycle.23-26 The reason for research results is that the insufficient of kidney Yin and Yang are the fundamental reason for failure of IVF-ET patients, in which patients with liver stagnation is prominent manifestation, blood stasis is the pathological product of kidney deficiency and liver stagnation, and also an important pathogenic factor. Tonifying-kidney herbs improve the status of kidney deficiency, balance the kidney Yin and Yang, provide the material basis for follicular development and ensure blood supply of Chong-ren meridians, which provides a suitable intrauterine environment for embryo implantation. And smoothing-liver and promoting-blood herbs make liver section and blood unobstructed, resulting in harmonious of Qi and blood, improvement of blood circulation and luteal function, by which the fertilization rate and clinical pregnancy rate increases and the rate of early abortion reduces.23-26

In conclusion, TCM multi-channel interventional therapy can increase the natural pregnancy rate in patients with ART failure (the triple therapy is superior to the dual therapy). It can increase the fertility rate, clinical pregnancy rate and decrease the early abortion rate during the period of secondary IVF-ET as well as improving syndromes of kidney deficiency, liver depression and blood stasis.

REFERENCES