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ASSISTED REPRODUCTION TECHNOLOGIES

Acupuncture combined with gonadotropin-releasing hormone agonists improves endometrial receptivity and pregnancy outcome in patients with recurrent implantation failure of in vitro fertilization-embryo transfer

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Abstract

Objective Gonadotropin-releasing hormone agonists (GnRHa), combined with other auxiliary treatments, can improve pregnancy outcomes in in vitro fertilization-embryo transfer (IVF-ET). This research investigated the effect of acupuncture combined with GnRHa in patients with recurrent implantation failure (RIF) of IVF-ET.

Methods A total of 164 patients who intended to undergo frozen-thawed embryo transfer after RIF of IVF-ET were selected for experiments and then divided into the control (received conventional hormone replacement therapy (HRT) for endometrial preparation) and study groups (received a combination of acupuncture, GnRHa, and HRT for endometrial preparation) ($n = 82$). Endometrial thickness (EMT), endometrial morphological classification, submucosal uterine blood flow classification, clinical pregnancy rate, embryo implantation rate, and early abortion rate for each transfer cycle were compared between the two groups.

Results EMT of the study group was higher than that of the control group 1 day before transfer. There were more patients with linear endometrium (A + B type) in the study group on the day of endometrial transformation than in the control group. The number of patients with type I submucosal uterine blood flow in the study group was decreased and the number of patients with type III was increased compared with the control group on the day of endometrial transformation. The clinical pregnancy rate and embryo implantation rate of the study group were higher than those of the control group.

Conclusion Acupuncture combined with GnRHa improves the endometrial receptivity of patients with RIF of IVF-ET, thereby increasing clinical pregnancy rates and improving pregnancy outcomes.

Keywords *In vitro* fertilization-embryo transfer · Recurrent implantation failure · Acupuncture · Gonadotropin-releasing hormone agonists · Endometrial receptivity · Pregnancy outcome

Introduction

In vitro fertilization (IVF)-embryo transfer (ET) is a procedure used to achieve pregnancy that includes oocyte extraction, in vitro fertilization, and embryo transfer [1]. As an integral part of all comprehensive infertility treatment options, IVF now offers a range of technological possibilities that theoretically allow us to control reproduction [2]. The success of IVF-ET depends on a capable blastocyst, a receptive endometrium, and successful crosstalk between the embryo and the mother [3]. So far, there is no clear definition or functional impairment of recurrent implantation failure (RIF) [4]. RIF refers to failure to achieve a clinical pregnancy after transfer of at least four good-quality embryos in a minimum of three fresh or frozen cycles in a woman under the age of 40 years [5]. The cause of RIF in most IVF-ET patients is multifaceted such as embryo quality and endometrial receptivity (ER) [6]. As a result, many infertile women turn to complementary and alternative medicine

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treatments [7], of which acupuncture appears to be beneficial for women who have failed IVF-ET [8].

Acupuncture treatment can improve the pregnancy rate of infertile women and reduce anxiety before pregnancy, while high-quality primary studies are still needed due to severe heterogeneity and methodological quality defects [9, 10]. A randomized clinical trial has observed that two-session acupuncture can result in a significant reduction in frequency of biochemical, clinical, and ongoing pregnancy rates in comparison to the one-session acupuncture [11], and 2–3 acupuncture sessions on or around the embryo transfer day are not enough to improve birth outcomes of IVF, but can significantly decrease IVF-related stress [12]. Gonadotropin-releasing hormone agonists (GnRHa) are used in the assisted reproductive cycle to reversely block pituitary function and prevent luteinizing hormone surges. Long-acting use of GnRHa can increase the clinical pregnancy rate in patients undergoing IVF-ET [13]. Significantly, GnRHa, in combination with other auxiliary treatments, has been considered to improve pregnancy outcomes in IVF-ET [14–16]. In conformity with this, this retrospective analysis was executed to assess the effect of acupuncture plus GnRHa on ER and pregnancy outcomes in patients undergoing IVF-ET who had experienced RIF in the hope of supporting the development of adjuvant therapy targeting acupuncture plus GnRHa to improve IVF-ET outcomes.

Materials and methods

Ethical statement

This study proceeded with the approval of the ethics committee of our hospital.

Research subjects

Patients who intended to undergo frozen-thawed embryo transfer after RIF of IVF-ET in the Reproductive Center of our hospital from November 2019 to June 2021 were selected for study subjects.

Inclusion criteria: (i) The patient and their spouse were aged between 25 and 40 years old; (ii) failure to achieve a clinical pregnancy after transfer of at least four good-quality embryos in a minimum of three fresh or frozen cycles; (iii) patients had high-quality embryos [17] in frozen storage; (iv) the hysteroscopy was normal; (v) patients were informed of the treatment and signed informed consent.

Exclusion criteria: (i) The test results for anti-sperm antibodies (AsAb), anticardiolipin antibodies (ACA), anti-endometrial antibodies (EmAb), and blocking antibodies were positive; (ii) chromosome abnormalities in both spouses; (iii) the serological screening results of TORCH, hepatitis B, hepatitis C, human immunodeficiency virus, and syphilis were positive, including gonorrhea, chlamydia, mycoplasma, and AIDS virus infection; (iv) patients suffered from uterine fibroids, uterine adhesions, endometriosis, adenomyosis, and other diseases; (v) patients with a history of adverse pregnancy and childbirth, immune system diseases and family history, hematological and thrombotic diseases, history of other systemic diseases, and female partner's unhealthy habits such as smoking and drinking; (vi) patients had received acupuncture and moxibustion treatment within 3 months before entering the study. After inclusion and exclusion criteria, 164 patients were ultimately enrolled in this study.

These 164 eligible participants were randomized into two groups, the study group and the control group, each comprised of 82 patients. Patients in the control group were treated with conventional hormone replacement therapy (HRT), and those in the study group were treated with acupuncture combined with GnRHa and HRT.

Treatments

Patients in the control group were treated with HRT. All patients received an estrogen regimen on the 2nd or 3rd day of menstruation, with oral administration of Progynova (Bayer, Berlin, Germany) at a starting dose of 4 mg/day [15]. After 5 days, the endometrial thickness (EMT) was examined by GE-E8 four-dimensional color Doppler ultrasound diagnostic instrument (USA), with a frequency of 5–9 MHz, equipped with RIC5-9 three-dimensional intracavity probe. The dosage of estradiol valerate tablets was adjusted based on the patient's endometrial growth. When estrogen was used for ≥ 11 days and EMT was ≥ 8 mm, oral administration with dydrogesterone tablets (Abbott Laboratories, Chicago, USA) at 20 mg/day plus intramuscular injection with progesterone (Zhejiang Xianju Pharmaceutical Co., Ltd., H33020828, 1 mL: 20 mg) at 40 mg/day was performed based on the maintenance dose of Progynova to achieve endometrial transformation. Embryo transfer (this study used conventional vitrification freezing method to freeze, thaw, and revive embryos) was performed 4 days after endometrial transformation (day 3). Serum human chorionic gonadotropin (hCG) was detected 14 days after embryo transfer, and if the result was negative, all hormones were stopped. Then, all pregnant women continued to receive HRT up to 12 weeks gestation [18].

The patients in the study group were treated with acupuncture combined with GnRHa and HRT. All patients were given an intramuscular injection with 3.75 mg Leuprorelin acetate microspheres sustained release for injection (long-acting preparation of GnRHa, Beijing Biote Pharmaceutical Co., Ltd., H20093809) on the 2nd or 3rd day of menstruation, and acupuncture began on the second day of GnRHa injection. After 30 days of GnRHa injection, vaginal

ultrasound was performed and hormone levels were examined to evaluate that patients reached the down-regulation standard. Then, HRT was adopted with the same procedures as mentioned in the control group [19].

Acupoint selection methods: Guanyuan (RN4), Qihai (RN6), Zhongji (RN3), bilateral Dahe (KI12), bilateral Shenshu (BL23), bilateral Zusanli (ST36), bilateral Sanyinjiao (SP6), bilateral Taixi (KI3), and bilateral Taichong (LR3) acupoints were selected. Acupuncture was conducted with the ENERGY brand disposable sterile acupuncture needles manufactured by Wuxi Jiajian Medical Instruments Co., Ltd.; the specifications were 0.30 × 25 mm, 0.30 × 40 mm, and 0.30 × 50 mm, with a diameter of 0.30 mm and length specifications of 1 inch, 1.5 inches, and 2 inches. Guanyuan, Dahe, Shenshu, Qihai, and Zusanli acupoints were acupunctured at 1–1.5 inches, and Zhongji, Sanyinjiao, Taixi, and Taichong acupoints at 0.5–1 inches. Acupuncture techniques: abdominal acupoints with twisting and reinforcing method, so that the needle sensation to the vulva, the remaining points using uniform reinforcing-reducing method, that is, after the needle into the gas uniformly reinforcing and reducing, so that the needle acupuncture points to produce a feeling of acidity, numbness, and heavy distension and to the patient could tolerate as appropriate. Acupuncture points were as follows: Guanyuan: in the lower abdomen in front of the midline, 3 inches below the umbilicus; Qihai: in the body in front of the midline, 1 inch and a half below the umbilicus; Zhongji: in the lower abdomen in front of the midline, 4 inches below the umbilicus; bilateral Dahe: in the lower abdomen, when the umbilicus in the lower 4 inches, the front of the midline next to the opening of 0.5 inches; bilateral Shenshu: in the lumbar region, the second lumbar vertebrae spine protrusions below the rear of the midline next to the opening of 1.5 inches; bilateral Zusanli: on the front lateral side of the calf, when the Dubi 3 inches below, from the anterior edge of the tibia a transverse finger (middle finger); bilateral Sanyinjiao: on the inner ankle of the foot 3 inches behind the medial edge of the tibia; bilateral Taixi: on the inner side of the foot, the inner ankle behind the heel bone tendon and the depression between the tendons; bilateral Taichong: on the dorsal side of the foot, the first two metatarsals before the union of the depression. The needle was twisted every 10 min and was left for 30 min. Acupuncture was performed twice a week. The treatment process of the two groups of patients is shown in Fig. 1.

Observation indices

General indicators included age, body mass index (BMI), infertility years, infertility types, ovulation-stimulating regimen, follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), number of eggs obtained, and number of available embryos.

Characteristics of frozen-thawed embryo transfer included the number of transferred embryos, number of transferred high-quality embryos (the standard for high-quality embryos: embryos were graded based on the Istanbul Consensus (Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011)) [20]. High-quality embryos at the cleavage stage consisted of class I (translucent cells of equal size, without particles in the cytoplasm and ≤ 5% fragments) and class II (cells with slightly uneven size, and there were particles in the cytoplasm and 6–20% fragments) embryos after thawing. Meanwhile, high-quality blastulas were those with an expansion grade ≥ IV and the trophoblast and inner cell mass did not contain C (IVAA, IVAB, IVBB, VAA, VAB, and VBB), post-thaw embryo survival rate, and EMT.

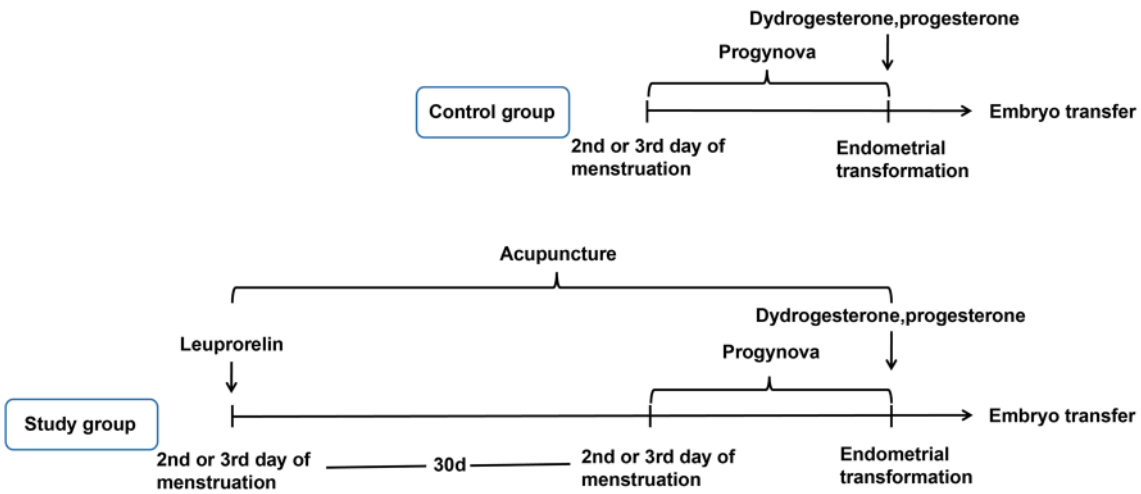

Fig. 1 Process flow charts for two groups of patients

All patients were examined by the same physician before endometrial preparation and on the day of endometrial transformation for endometrial morphological classification and submucosal uterine blood flow classification using a GE-E8 four-dimensional color Doppler ultrasound diagnostic device, and EMT was recorded 1 day before transfer. Endometrial morphological classification was classified into A, B, and C types according to the Gonen and Capser endometrial morphological classification criteria [21]: A type: triple-line pattern, the outer and middle layers show strong echo, the inner layer shows low echo, and the midline echo is obvious; B type: relatively uniform high echo, the same as the endometrial myometria image, and uterine midline echo is not obvious; C type: a homogeneous strong echo, no uterine midline echo. According to the echo characteristics, A type and B type are marked as linear, and C type is marked as non-linear. Typical A, B, and C type endometrial ultrasound images are shown in Fig. 2. Blood flow classification was performed using the Applebaum classification method [22]: type I: blood vessels passed through the low-echo area of the lateral intima, but did not enter the outer edge of the hyperechoic intima; type II: blood vessels pass through the outer edge of the hyperechoic intima; type III: blood vessels enter the intima.

Pregnancy outcome included clinical pregnancy rate (number of clinical pregnancies / number of transfer cycles × 100%), embryo implantation rate (number of pregnancy sacs determined by ultrasound / total number of embryos × 100%), and early abortion rate (number of early abortion cases / number of clinical pregnancies × 100%).

Statistical analysis

SPSS26.0 software (SPSS, Chicago, IL, USA) was applied to data processing. The measurement data of normal distribution were represented by mean ± standard deviation. Categorical variables were expressed as the number of cases. Independent-sample *t*-test was utilized for comparisons of measurement data, and χ^2 test or exact Fisher test for data analysis for enumeration data. $p < 0.05$ (double-tailed) was considered statistically significant.

Results

General information comparison

The general information for the study group and the control group included age, BMI, infertility years, infertility types, ovulation-stimulating regimen, basic FSH, basic LH value, basic E2 value, number of eggs obtained, and number of available embryos. Statistical analysis showed no significant difference in the above data between the two groups ($p > 0.05$; Table 1).

Comparison of parameters related to frozen embryo transfer

The parameters related to frozen embryo transfer in the study group and the control group were compared, including the number of transferred embryos, number of transferred high-quality embryos, post-thaw embryo survival rate, basic EMT, endometrial morphological classification, and submucosal uterine blood flow classification. Data analysis found no marked difference in the above indicators between the two groups ($p > 0.05$; Table 2).

Comparison of endometrial conditions

EMT, endometrial morphological classification, and submucosal uterine blood flow classification were compared in the study group and the control group (Table 3). No notable differences were seen in EMT, endometrial morphological classification, and submucosal uterine blood flow classification

Fig. 2 Typical A-, B-, and C-type endometrial ultrasound images. **A** A-type endometrial ultrasound image. **B** B-type endometrial ultrasound image. **C** C-type endometrial ultrasound image

Table 1 General information

Data	Control group ($n=82$)	Study group ($n=82$)	p
Age (years)	33.18 ± 2.58	32.99 ± 2.76	0.641
BMI (kg/m^2)	21.92 ± 2.05	21.43 ± 2.26	0.148
Infertility years	3.41 ± 1.36	3.15 ± 1.09	0.179
Infertility types			0.429
Primary	32	37	
Secondary	50	45	
Ovulation-stimulating regimen			0.613
Long-acting regimen	30	27	
Short-acting regimen	21	24	
Antagonists	29	26	
Luteal phase stimulating ovulation	2	5	
Basic FSH (IU/L)	6.72 ± 1.23	6.91 ± 1.30	0.338
Basic LH (IU/L)	5.76 ± 2.31	5.87 ± 2.70	0.715
Basic E2 (pmol/L)	192.34 ± 47.25	179.87 ± 52.36	0.111
Number of eggs obtained	15.49 ± 3.32	15.21 ± 3.74	0.613
Number of available embryos	3.21 ± 1.10	3.24 ± 1.29	0.873

BMI body mass index, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *E2* estradiol, *AFC* antral follicle count

Table 2 Characteristics of frozen-thawed embryo transfer

Item	Control group ($n=82$)	Study group ($n=82$)	p
Number of transferred embryos	1.82 ± 0.39	1.76 ± 0.43	0.351
Number of transferred high-quality embryos	1.30 ± 0.46	1.27 ± 0.45	0.674
Post-thaw embryo survival rate (%)	149/150 (99.33)	144/146 (98.63)	0.546
Endometrial thickness (mm)	4.88 ± 1.39	4.68 ± 1.23	0.331
Endometrial morphological classification			0.344
Linear (A + B type)	38	32	
Non-linear (C type)	44	50	
Submucosal uterine blood flow classification			0.408
Type I	44	34	
Type II	29	38	
Type III	9	10	

Table 3 Endometrial receptivity-related indicators

Item	Control group ($n=82$)		Study group ($n=82$)	
	Before treatment	After treatment	Before treatment	After treatment
Endometrial morphological classification				
Linear (A + B type)	38	39	32	56*
Non-linear (C type)	44	43	50	26*
Submucosal uterine blood flow classification				
Type I	44	44	34	24*
Type II	29	29	38	30
Type III	9	9	10	28*
Endometrial thickness (mm)	4.88 ± 1.39	9.74 ± 1.31	4.68 ± 1.23	10.24 ± 1.51*

Before treatment refers to before endometrial preparation. In evaluating endometrial morphological classification and submucosal uterine blood flow classification, after treatment refers to the day of endometrial transformation; in evaluating endometrial thickness, after treatment refers to 1 day before transfer. *$p < 0.05$ vs. control group

between the two groups before endometrial preparation ($p > 0.05$). EMT of the study group was higher than that of the control group 1 day before transfer ($p < 0.05$). There were more patients with linear endometrium (A + B type) in the study group on the day of endometrial transformation and fewer patients with non-linear endometrium (C type) than in the control group ($p < 0.05$). The number of patients with type I submucosal uterine blood flow in the study group was decreased and the number of patients with type III was increased compared with the control group on the day of endometrial transformation ($p < 0.05$).

Pregnancy outcomes

Pregnancy outcomes including clinical pregnancy rate, embryo implantation rate, and early abortion rate were evaluated (Table 4). The clinical pregnancy rate and embryo implantation rate of the study group were higher than those of the control group ($p < 0.05$). The early abortion rate of the study group was lower than that of the control group, showing no significant difference ($p > 0.05$).

Adverse reactions

Both groups did not experience any adverse reactions during the treatment process.

Relationship of endometrial morphological classification and submucosal uterine blood flow classification with clinical pregnancy.

In the patient cohort, the clinical pregnancy rate of patients with linear endometrium (A + B type) was higher than that of patients with non-linear endometrium (C type) ($p < 0.05$). The clinical pregnancy rate of patients with linear endometrium (A + B type) was higher than that of patients with non-linear endometrium (C type) in the control groups ($p < 0.05$). In the study group, the clinical pregnancy rate of patients with linear endometrium (A + B type) was higher than that of patients with non-linear endometrium (C type), but the difference was not statistically significant ($p > 0.05$). The clinical pregnancy rate of different subintimal blood flow types was in the order of type III> II> I from high to low ($p < 0.05$). The clinical pregnancy rate of submucosal uterine blood flow type III patients was the highest in the study and control groups, followed by type II and type I patients ($p < 0.05$). Table 5 and Table 6 show the detailed results.

Table 5 Comparison of clinical pregnancy rates of different endometrial morphological classifications and submucosal uterine blood flow classifications in total patients

Item	Clinical pregnancy rate (%)	*p*
Endometrial morphological classification		< 0.001
Linear (A + B type)	53/95 (55.79)	
Non-linear (C type)	10/69 (14.49)	
Submucosal uterine blood flow classification		< 0.001
Type I	9/68 (13.24)	
Type II	25/59 (42.37)	
Type III	29/37 (78.38)	

Discussion

Successful implantation of an embryo into the receptive endometrium is essential to establishing a viable pregnancy, and RIF is a real challenge in assisted reproduction that exacerbates the physical trauma of infertile women undergoing IVF-ET [23]. Poor ER is the main factor leading to RIF [24]. Therefore, it is a great challenge to improve endometrial receptivity (ER) and thereafter pregnancy rates and pregnancy outcomes in women with RIF of IVF-ET. Long-acting GnRHa has attracted clinical attention in IVF/intracytoplasmic sperm injection and embryo transfer [25, 26]. In some clinical practices, GnRHa has been applied to improve IVF-ET outcome [13, 27]. However, its effect is limited, rendering relative fertilization rate and live birth rate compared with other treatment options [28]. Therefore, GnRHa combined with other treatment regimens has entered clinical practice [14, 29, 30].

This study collected data from patients with RIF of IVF-ET and found no significant difference in general data and characteristics of frozen-thawed embryo transfer. To evaluate ER in patients with RIF of IVF-ET, endometrial thickness (EMT), endometrial morphology, and subendometrial blood flow classification were analyzed. The collected data indicated that either HRT or combined therapy with acupuncture and GnRHa thickened the endometrium, and more notably, EMT was higher after combined therapy with acupuncture and GnRHa compared with after HTR. The significance of EMT has been considered discreetly due to its detrimental effect on pregnancy outcomes [31, 32]. It has been described that acupuncture and its combined therapy increase EMT

Table 4 Indicators related to pregnancy outcomes

Pregnancy outcomes	Control group ($n = 82$)	Study group ($n = 82$)	*p*
Clinical pregnancy rate (%)	24/82 (29.26)	39/82 (47.56)	0.016
Embryo implantation rate (%)	30/149 (20.13)	51/144 (35.42)	0.004
Early abortion rate (%)	4/24 (16.67)	2/39 (5.13)	0.130

Table 6 Clinical pregnancy rates of different endometrial morphological classifications and submucosal uterine blood flow classifications in the control and the study groups

Group	Endometrial morphological classification		*p*	Submucosal uterine blood flow classification			*p*
	Linear (A + B type)	Non-linear (C type)		Type I	Type II	Type III	
Control group ($n = 82$)	16/39 (44.19)	8/43 (17.65)	0.026	5/44 (11.36)	12/29 (41.38)	7/9 (77.78)	<0.001
Study group ($n = 82$)	30/56 (67.16)	9/26 (33.33)	0.110	6/24 (25.00)	15/30 (50.00)	18/28 (64.28)	0.017

and effectuate to treat female infertility [33]. Moreover, acupuncture in combination with a traditional Chinese medicine formula, erbuzhuyu decoction, can increase EMT and improve ER in a superovulation animal model [34].

In addition to that, this study found more patients with linear endometrium after combined therapy with acupuncture and GnRHa compared to HRT. Consistent with this finding, a previous report has validated the improvement effect of acupuncture on ER as evidenced by endometrial morphology in polycystic ovary syndrome (PCOS) [35]. Also, a combined regimen with acupuncture and endovascular therapy can increase EMT and the proportion of patients with pattern A endometrium and improves embryo implantation rate and clinical pregnancy in patients with thin endometrium [36]. In women undergoing frozen-thawed embryo transfer, the endometrial triple-line pattern is present more often in those with transcutaneous electrical acupuncture point stimulation [37]. Meanwhile, this study found fewer patients with type I submucosal uterine blood flow and more patients with type III submucosal uterine blood flow after combined therapy with acupuncture and GnRHa. Consistently, electroacupuncture has been shown to decrease the uterine blood flow [38, 39].

To assess the pregnancy outcome of patients with RIF of IVF-ET, clinical pregnancy rate, embryo implantation rate, and early abortion rate were measured, showing that combined therapy with acupuncture and GnRHa achieved a higher clinical pregnancy rate and embryo implantation rate compared with HRT, and a lower early abortion rate, which was not significantly different. A randomized controlled trial has tested that 3 times of acupuncture before and after embryo transfer can improve the proportion of pattern A endometrium and pregnancy rate in patients with PCOS undergoing IVF-ET [40]. Furthermore, in women with a history of IVF failure, acupuncture might have benefits for pregnancy outcomes. However, studies with better methodologies and larger scales are warranted to verify these findings due to the poor reporting and methodological flaws [41]. It should be noted that endometrial morphology and subendometrial blood flow type are associated with pregnant outcomes, indicating that ER improvement is beneficial to improve frozen-thawed embryo transfer after RIF of IVF-ET, which is consistent with other reports [42, 43].

In summary, our study highlights that acupuncture combined with GnRHa can improve the ER of patients with RIF of IVF-ET, thereby increasing clinical pregnancy rates and improving pregnancy outcomes. However, the sample size was not calculated in this study, and the results of the study may have biases. Meanwhile, we should further design the groups as (1) acupuncture + GnRH agonists, (2) acupuncture alone, (3) GnRH agonist alone, and (4) conventional hormonal treatment to better present our findings.

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Data Availability The original contributions presented in the study are included in the article/Supplementary Material, furtherinquiries can be directed to the corresponding author.

Declarations

Conflict of interest The authors declare no competing interests.

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