Effects of pelvic floor bioelectrical stimulation on frozen embryo transfer patients with thin endometrium

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Abstract
This study was to observe the effects of pelvic floor bioelectrical stimulation on pregnancy outcome and serum estradiol (E2) and progesterone (P) levels in frozen embryo transfer patients with thin endometrium. 120 cases frozen embryo transfer patients with thin endometrium in our hospital from March 2016 to April 2017 were selected. These patients received artificial cycle replacement plan before embryo transfer. According to whether pelvic floor bioelectrical stimulation therapy was accepted, 120 cases of thin endometrial frozen embryo transfer were divided into control group and observation group. 50 cases of the control group received artificial cycle to prepare for embryo transplantation, while 70 cases of observation group received bioelectrical stimulation as intervention treatment on the basis of artificial cycle. The intima thickness, subintimal hemodynamic parameters, embryo implantation rate, and clinical pregnancy rate were compared between the two groups before and after treatment. There was no significant difference in endometrial thickness between the two groups on the 10th day of menstruation (P > 0.05). The thickness of endometrium and the index of intima growth in the observation group were significantly higher than those in the control group (P < 0.05). The pulsatility index, resistance index, and systolic and diastolic blood flow velocity ratio of subintimal blood flow in the observation group were significantly higher than those in the control group (P < 0.05). Before treatment, there was no significant difference in serum E2 and P between the two groups (P > 0.05). After treatment, the improvement of serum E2 and P in the observation group was significantly better than that of the control group (P < 0.05). The embryo implantation rate (25.7%) in the observation group was significantly higher than that in the control group (17.0%) (P < 0.05). The clinical pregnancy rate (47.1%) in the observation group was significantly higher than that in the control group (30%) (P < 0.05). In conclusion, the intervention of pelvic floor bioelectrical stimulation can improve the blood perfusion of thin endometrium, improve the endometrial receptivity, increase the implantation rate of frozen embryo transfer in patients with thin endometrium, and improve the level of serum estradiol and progesterone.

Keywords
endometrial receptivity, estradiol, frozen embryo transfer, pelvic floor bioelectrical stimulation, progesterone

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Introduction
Frozen embryo transfer (FET) technology has now become a routine treatment of in vitro fertilization (IVF) embryo transfer, which can not only reduce ovarian hyperstimulation syndrome and patient costs but also increase the cumulative pregnancy rate of IVF.1 At present, the definition of thin endometrium is usually referring to the endometrial
thickness < 7 mm in mid-luteal phase (6–10 days after ovulation). Research shows that the pregnancy rate decreases significantly when the intimal thickness < 7 mm. Treatment methods for thin endometrium are numerous, but there is still no universal and effective method. Endometrial receptivity affects the implantation of fertilized eggs, and poor endometrial growth is an important factor in the failure of embryo transfer. Suitable endometrial receptivity is a prerequisite for successful frozen embryo transplantation by FET technology. There is no uniform standard of judgment for thin endometrium caused by poor growth. It is generally believed that the thickness of normal endometrium is up to 8–14 mm in the late proliferation period. Thin endometrium will affect endometrial receptivity, and the embryo implantation rate and clinical pregnancy rate significantly decrease. At present, the stimulation treatment of thin endometrial cycle is whole-embryo freezing. FET cycles use either artificial-period hormone replacement therapy or ovulation induction to adjust endometrial receptivity, but the clinical pregnancy rate is unsatisfactory. Therefore, how to improve the thickness of thin endometrium and endometrial receptivity is the hot and difficult point of current reproductive medicine. Pelvic floor bioelectric stimulation refers to the electrodes placed in the perineum stimulate uterine smooth muscle contraction and relaxation through different frequency currents; then increasing blood circulation of pelvic floor, vagina, endometrium, and uterine muscle; increasing tissue nutrition; and improving endometrial receptivity. At present, it is unclear whether the pelvic floor bioelectrical stimulation can improve the clinical pregnancy rate of thin endometrium. This article retrospectively analyzed 120 cases of thin endometrial patients treated with FET to explore the application of pelvic floor bioelectrical stimulation intervention in FET cycle of thin endometrial patients, thus providing reference for clinical work.

**Materials and methods**

**General information**

A total of 120 thin endometrial patients with frozen embryo transplantation who underwent artificial cycle replacement in our hospital from March 2016 to April 2017 were selected as research subjects. Their ages ranged from 22 to 43 years and the mean age was 35.2 ± 1.7 years. Years of infertility arranged from 1 to 6 years and the average age was 5.1 ± 0.7 years. The numbers of high quality embryos transferred were 1–3 pieces, and the average was 2.1 ± 0.4 pieces.

**Inclusion criteria:** The main infertility factors were pelvic adhesions, fallopian tube obstruction, endometriosis, and male factors. Second, endometrial thickness was <7 mm during the past one or more superovulation-cycle human chorionic gonadotropin (HCG) days. Last but not least, all subjects should not have any other internal and surgical diseases. **Exclusion criteria:** patients with sparse ovulation and ovulation disorders; patients with reproductive tract organic disease; patients with uterine fibroids, adenomyosis, endometrial polyps, submucosal fibroids, and tuberculosis; patients with long-term use of drugs, including non-steroidal anti-inflammatory drugs; patients with premature ovarian failure, and ovarian cysts; and patients with endocrine dysfunction, such as hyperprolactinemia. In total, 120 thin endometrial patients with cryogenic embryo transfer were divided into control group and observation group, including 50 cases in the control group and 70 cases in the observation group. There were no significant differences in baseline characteristics between the two groups ($P > 0.05$), such as age, years of infertility, and number of high quality embryos transplantation. All patients signed informed consent form.

**Therapeutic methods**

In the control group, the endomembrane preparation was performed before embryo transfer with artificial cycle. The oral administration of progynova (estradiol valerate; Bayer, Germany) began on the second day of menstruation at 6 mg/day for 9 days. Ultrasound detection of endometrial thickness, and endometrial and subintimal blood perfusion was started on the 10th day of menstruation. When the LH peak appeared, and endometrial thickness was more than 8 mm, it met with the conditions of transplantation. The patients in observation group were given pelvic floor bioelectric stimulation on the basis of artificial cycle preparation for endometrium, which means that patients in observation group were also orally administrated of progynova began on the second day of menstruation at 6 mg/day for 9 days. PHENIXU SB4 pelvic nerve stimulation device
was used (Vivaltis, France; Figure 1). Pelvic floor bioelectrical stimulation intervention started at the 10th day of menstrual period after ultrasound detection of endometrial thickness, and endometrial and subintimal blood perfusion. The treatment probe was placed in the patient’s vagina, while the electrode plates A1+, A1−, A2+, A2−, B1+, B1−, B2+, B2− were paste to rectus abdominis, lateral oblique abdominis, and transverse abdomen, and the size of each electrode was 50 mm × 50 mm. The alternating current frequency was 40 Hz, and the pulse width was 250 μs. According to patient tolerance, current intensity was set at 10–50 mA. The treatment was performed for 30 min every other day. When the LH peak appeared, and endometrial thickness was more than 8 mm, it met with the conditions of transplantation. The embryos transferred in both groups were all high-quality embryos with 7–8 cells on the third day.

Observational indicators

The score of resuscitative embryo was based on the Edwards criteria. Frozen embryos were scored immediately after resuscitation to calculate the number of surviving blastomeres, and the second assessment was performed after cultured for 18 h. The embryos with more than six blastomeres, uniform size and fewer than 20% fragments were identified as high-quality embryos.

Intracavitary probe frequency of color Doppler ultrasound diagnostic apparatus (Xuzhou Dawei Electronics Co., Ltd., China) was 5–9 MHz. All patients were operated by the same person with the same settings. Detection indexes included endometrial thickness measured in the uterine longitudinal section, and intima and submucous hemodynamic parameters contained pulsatility index (PI), resistance index (RI), systolic and diastolic blood flow velocity ratio (S/D), which were measured by the Doppler frequency that was taken at the brightest point between dark zone and colored blood at the junction of endometrium sagittal section and myometrium.

On the 14th day after transplantation, blood HCG ≥ 5 U/L suggested biochemical pregnancy. Vaginal ultrasonography was performed on the 25th day after transplantation, and gestational sac and yolk sac suggested clinical pregnancy. Clinical pregnancy rate=(weeks of clinical pregnancy/weeks of transplantation) × 100%, and implantation rate=(implantation embryo number/total number of embryos transferred) × 100%

Serum E2 (estradiol) and P (progesterone) concentration was detected before and after treatment by chemiluminescence.

Statistical analysis

SPSS 21.0 software was used for statistical analysis. The results were expressed as mean ± standard deviation or rate (%). Intima thickness, intimal growth value, and serum E2 and P concentrations were compared by t-test. The comparison of PI, RI, S/D between endometrial and subintimal blood was performed by Mann–Whitney U test. χ² test was used in qualitative data. P < 0.05 was considered statistically significant.

Results

Comparison of endometrial thickness between two groups

There was no significant difference in endometrial thickness between the two groups on the 10th day of menstruation (P > 0.05). The thickness of endometrium and the intima growth value in the observation group were significantly higher than those in the control group on transplant day (P < 0.05) (Table 1).

Comparison of PI, RI, and S/D in endometrial and subintimal blood between two groups after treatment

The PI, RI, and S/D values of subintimal blood in observation group were significantly higher than those in control group (P < 0.05) (Table 2).
Comparison of serum E2 and P values between two groups before and after treatment

Before treatment, there was no significant difference in serum E2 and P between the two groups ($P > 0.05$). After treatment, the improvement of serum E2 and P in the observation group was significantly better than that of the control group ($P < 0.05$) (Table 3).

Comparison of pregnancy outcomes between two groups

The embryo implantation rate in the observation group (25.7%) was significantly higher than that in the control group (17.0%, $P < 0.05$). The clinical pregnancy rate in the observation group (47.1%) was significantly higher than that in the control group ($P < 0.05$) (Table 4).

Discussion

Two prerequisites for successful embryo implantation include well-developed embryos and well-tolerated endometrium. Human-assisted reproductive technology has greatly solved the infertility caused by various factors. However, poor receptivity of endometrium is still an important factor leading to implantation failure. Currently, the treatment of thin endometrium includes surgical treatment, hormone therapy, vasodilator drugs, intrauterine perfusion of granulocyte colony stimulating factor, and recent medical methods of stem cell regeneration. Endometrial preparation programs usually used in FET include the natural cycle, inducing ovulation cycle and hormone replacement cycles. Certain thickness of the endometrium is a prerequisite for the successful implantation of the embryo. Embryo implantation rate and clinical pregnancy rate were significantly reduced when endometrial thickness of ultrasound $< 8 \text{ mm}$ on HCG day or giving luteal support day. The results of this study showed that the intima thickness and growth value in thin endometrium patients underwent pelvic floor bioelectrical stimulation intervention were significantly higher than the control group.

Bionic pelvic floor biological stimulation, also known as pelvic nerve muscle stimulation, can stimulate uterine smooth muscle contraction and relaxation; increase blood supply of pelvic floor, vagina, endometrium, and uterine muscle; promote blood circulation; increase tissue nutrition; accelerate tissue repair and recovery of physiological function; improve the uterine microenvironment; promote the growth of the endometrium; and promote uterine receptivity by placing electrodes in the perineal to release different frequencies of current. PI, RI, and S/D values of endometrium can reflect the endometrial perfusion and the degree of blood flow, and good perfusion is conducive to the successful implantation of fertilized eggs. The results of this study showed that endometrial hemodynamic parameters including PI, RI, and S/D values of thin endometrium in patients improved significantly by

### Table 1. Comparison of endometrial thickness between two groups.

| Group            | N  | 10th day of menstruation (mm) | Transplant day (mm) | Intima growth value (mm) |
|------------------|----|-------------------------------|---------------------|--------------------------|
| Control group    | 50 | $5.49 \pm 0.61$               | $8.79 \pm 0.93$     | $2.95 \pm 0.48$          |
| Observational    | 70 | $5.52 \pm 0.73$               | $9.52 \pm 0.44$     | $3.46 \pm 0.55$          |
| $t$              |    | $0.51$                        | $4.83$              | $5.00$                   |
| $P$              |    | $>0.05$                       | $<0.05$             | $<0.05$                  |

### Table 2. Comparison of PI, RI, and S/D in endometrial and subintimal blood between two groups after treatment.

| Group            | N  | After treatment |
|------------------|----|-----------------|
| Control group    | 50 | $1.17 \pm 0.24$ |
| Observational    | 70 | $1.41 \pm 0.35$ |
| Mann-Whitney U test |    | 13.45            |
| $P$              |    | $<0.05$         |

PI: pulsatility index; RI: resistance index; S/D: systolic and diastolic blood flow velocity ratio.
pelvic floor bioelectrical stimulation, indicating that uterus endometrial blood perfusion and endometrial receptivity were improved, thereby increasing the embryo implantation rate and clinical pregnancy rate. The result of this study was consistent with previous studies at home and abroad.12

In this study, we also found that pelvic floor bioelectrical stimulation intervention can improve serum E2 and P values effectively and improve embryo implantation rate, and help to obtain a more satisfactory clinical pregnancy rate. Due to the small number of cases in this study, its effectiveness and safety should be verified by expanding clinical samples for further study. In short, pelvic floor bioelectrical stimulation intervention is still a new endomembrane preparation program for thin endometrial patients with FET. Thus, it is worth further clinical promotion.

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### Table 3. Comparison of serum E2 and P values between two groups before and after treatment.

| Group           | N  | E2 (pg/mL) Before treatment | E2 (pg/mL) After treatment | P (ng/mL) Before treatment | P (ng/mL) After treatment |
|-----------------|----|-----------------------------|---------------------------|---------------------------|---------------------------|
| Control group   | 50 | 376.4 ± 15.3                | 252.7 ± 13.4              | 17.3 ± 2.5                 | 8.9 ± 2.3                 |
| Observational group | 70 | 375.3 ± 16.2                | 243.5 ± 12.6              | 17.1 ± 2.8                 | 6.6 ± 2.1                 |
|     t           |    | 0.451                       | 5.624                     | 0.173                     | 6.729                     |
|     P           |    | >0.05                       | <0.05                     | >0.05                     | <0.05                     |

### Table 4. Comparison of pregnancy outcomes between two groups.

| Group           | N  | Embryo implantation rate | Clinical pregnancy rate |
|-----------------|----|--------------------------|-------------------------|
| Control group   | 50 | 17/100 (17.0)            | 15 (30.0)               |
| Observational group | 70 | 36/140 (25.7)            | 33 (47.1)               |
|     $\chi^2$    |    | 9.03                     | 10.14                   |
|     P           |    | <0.05                    | <0.05                   |
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