Clinical observation of endometrial prescription combined with TAM on FET of thin endometrial patients with kidney deficiency and blood stasis

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Abstract

Objective: To explore the best endometrial preparation scheme of freeze-thawed embryo transfer (FET) for patients with thin endometrium with kidney deficiency and blood stasis by comparing the clinical outcomes of three endometrial preparation schemes of estradiol valerate tablets Hormone Replacement (HRT), Tamoxifen (TAM) and Endometrial Prescription combined with TAM (EP-TAM group). Method: Retrospective analysis of 318 patients with thin endometrium with kidney deficiency and blood stasis who underwent FET in Change People’s Hospital of Shandong Province, Shandong Maternal and Child Health Hospital, and Shenzhen Hospital of Integrated Traditional Chinese and Western Medicine from September 2018 to March 2023, including 101 patients in HRT group (estradiol valerate tablets ); 108 patients in TAM group; 109 patients in EP-TAM group. The cycle cancellation rate, intimal thickness, clinical pregnancy rate, embryo implantation rate, early abortion rate and ectopic pregnancy rate were compared among the three groups. Result: Cycle cancellation rates of TAM group and EP-TAM group were significantly lower than HRT group (8.3%, 9.2% vs 17.8%, P < 0.05). The intimal thickness of TAM group ([8.11 ± 0.36] mm) and EP-TAM group ([8.21 ± 0.40] mm) was significantly higher than HRT group ([7.11 ± 0.43] mm) (P < 0.05). The clinical pregnancy rate and implantation rate of TAM group and EP-TAM group were higher than HRT group, the early abortion rate and ectopic pregnancy rate were lower than HRT group, and EP-TAM group was more significant (P < 0.05). Conclusion: Patients with thin endometrium underwent FET can try Endometrium Prescription combined with TAM (EP-TAM) to prepare the endometrium.

Keywords: endometrium prescription; estradiol valerate tablets; tamoxifen; hormone replacement; thin endometrium; freeze-thawed embryo transfer

Citation

Background
Since the first successful transfer of frozen-thawed embryos in 1983, with the improvement of embryo cryopreservation techniques [1], the use of Frozen-thawed Embryo Transfer (FET) has progressively increased [2], and whole embryo freezing followed by elective resuscitation transfer is effective in preventing ovarian hyperstimulation syndrome [3, 4]. Embryo quality, endometrial tolerance, and optimal embryo-maternal endometrial interaction are the main factors affecting the success of frozen-thawed embryo resuscitation transfer, and individualized endometrial preparation protocols are important influencing factors for successful embryo implantation [5]. In clinical practice, when the endometrial thickness is < 7 mm, it is generally considered unfavorable for embryo implantation and is referred to as thin endometrium [6]. This group of patients tends to have lower embryo implantation and clinical pregnancy rates [7, 8], commonly used endometrial preparation regimens include hormone replacement cycles (HRT), natural cycles, stimulation cycles, and down-regulation combined with hormone replacement cycles, but pregnancy rates are unsatisfactory regardless of endometrial preparation regimen [9]. Therefore, increasing the thickness of the thin endometrium, improving endometrial tolerance, and improving clinical outcomes are the key issues in fertility centers today.

The endometrial formula is an empirical formula created by the research team, which has the effect of tonifying the kidney, filling in essence, nourishing blood and activating blood circulation to regulate menstruation, and past clinical experience suggests that the endometrial formula is able to improve the intrauterine environment and enhance the endometrial receptivity. Tamoxifen has estrogen-like effects on the endometrium and can be used to promote ovulation and endometrial proliferation. Manual cycles have become a common protocol for clinicians performing frozen-thawed embryo resuscitation transfers because of their ease of administration and manageability. The purpose of this paper is to explore the best preparation protocol by retrospectively analyzing the clinical outcomes of freeze-thaw embryo transfer in thin endometrium as a combination of all three as an improvement of thin endometrium.

Materials and methods
Study population
Retrospective analysis of 318 patients with thin endometrium of kidney deficiency and blood stasis type who performed FET in Changle People's Hospital of Shandong Province, Maternal and Child Health Hospital of Shandong Province, and Shenzhen Hospital of Integrated Traditional Chinese and Western Medicine from September 2018 to March 2023 were divided into three groups. All the patients signed informed consent form. The collection of these clinical samples was conducted in accordance with medical ethics guidelines and was approved by the Medical Ethics Committee of Shenzhen Hospital of Integrated Traditional Chinese and Western Medicine (Approval No. SZXYJHYLYWH-2018-0901).

Inclusion criteria. Age ≤ 38 years, HCG trigger day and previous FET endometrial transformation day endometrial thickness < 7mm on ovulation retrieval cycle, and at least one quality embryo for this transfer. Patients with thin endometrium of kidney deficiency and blood stasis type as identified by at least 2 associate senior Chinese medicine practitioners.

Grouping. The three groups were: HRT group (estradiol valerate tablets ≥ 101 cases; Tamoxifen (TAM) group: 108 cases; Endometrial prescription combined with tamoxifen group (EP-TAM group): 109 cases. The infertility factors in each group were mainly tubal factors in the female partner and oligospermia and weak spermatozoa in the male partner.

Exclusion criteria. Patients with other factors affecting embryo implantation, such as uterine malformation, hydrocele, endometriosis, adenomyosis, uterine adhesions, and recurrent uterine effusion were not included in the study.

Preparation protocol for the HRT group
Starting from the 3rd day of menstruation, Estradiol valerate tablets (1mg/tablet, Bayer, Germany) was given 3 tablets in the morning and 3 tablets in the evening, on the 8th-11th day, ultrasound was used to monitor the endometrial lining, if the thickness of endometrial lining was ≤ 7mm, Tranexamic acid was given 1mg/d to be placed in the vagina, and the time of estrogen use was 14d–17d, if the thickness of endometrial lining was < 7mm, it was communicated with the patient that the current cycle could be abandoned. If the patient asked for implantation, estradiol valerate tablets were unchanged, and Dydrogesterone (10mg/tablet, Abbott, Netherlands) was given 1 tablet in the morning and 1 tablet in the evening, and Progesterone Sustained-release Vaginal Gel (90mg/strike, Merck Serono, Germany) was placed in the vagina. After 3 days later, one to two D3 cleavage embryos were transplanted.

Preparation protocol for TAM group
Oral tamoxifen citrate (10mg/tablet, Yangtze River Pharmaceutical) 20mg/d was started on the 3rd day of menstruation for 5 consecutive days, estradiol valerate tablets of 1 mg/d was given throughout the whole course until 14 days after the implantation of the measurement of blood β-HCG. The monitoring of follicular growth was started on the 8th day of menstruation, and when the dominant follicle was ≥ 18mm in diameter, HCG 6000 units were administered to induce ovulation, and the thickness of the uterine endometrial lining was measured on the day of ovulation. The cancelation criteria was the same as that of HRT group, after 3 days later, one to two D3 cleavage embryos were transplanted. Dydrogesterone 20 mg bid, Progesterone Sustained-release Vaginal Gel 1 branch qd, and HCG 2000 units qod were started on the day of ovulation for 3–4 doses. If no follicles develop or more than 3 follicles develop then the cycle is abandoned.

Preparation of endometrial prescription combined with TAM group (EP-TAM group)
After menstruation, take endometrial Prescription (no-decoction granules in bag, Jiangyin Pharmaceutical): Angelicae Sinensis Radix 6g, Paonioae Radix Rubra 6 g, Paonioae Radix Alba 6 g, Cuscutae Semen 12 g, Rehmannia Radix 6 g, Leonuri Fructus 6 g, Rhei Radix 6 g, Placenta Hominis 3 g, Dipsaci Radix 6 g, Cyathulae Radix 6 g, Cynanchi Breviorae Radix 10 g, Crataegi Fructus 6 g, Cistanche Deserticola 6 g, Dioscoreae Rhizoma 6 g, Cinnamomi Ramulus 3 g. Take one dose daily with boiled water after meals. Other medications and ovulation promotion procedures of TAM in this group were the same as those in the TAM group.

Evaluation of pregnancy
Blood human chorionic gonadotropin (HCG) levels were assessed 14 days post-transplantation. Individuals testing positive underwent transvaginal ultrasound examination 30 days post-transplantation. Detection of a gestational sac during ultrasound constituted clinical pregnancy, with biochemical pregnancies excluded from the pregnancy count. The clinical indicators were calculated:

- Cycle cancellation rate = (number of cycles canceled for transplantation/total number of cycles) × 100%;
- Clinical pregnancy rate = (number of clinical pregnancy cycles/number of transferred cycles) × 100%;
- Early miscarriage rate = (number of early miscarriages/number of clinical pregnancies) × 100%;
- Ectopic pregnancy rate = (number of ectopic pregnancies/number of clinical pregnancies) × 100%.

General information
There was no statistical difference in the average age of patients, BMI, infertility years, bFSH, bLH, IM, infertility type, number of transferred embryos between the three groups and infertility cause (P > 0.05) (Table 1, Table 2).
Table 1 Comparison of general data in HRT group, TAM group, and EP-TAM group (%)

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Age (year)</th>
<th>BMI/ (kg/m²)</th>
<th>Infertility years</th>
<th>bFSH/ (U/L)</th>
<th>bLH/ (U/L)</th>
<th>bE2/ (pg/ml/L)</th>
<th>AMH/ (ng/mL)</th>
<th>Infertility type</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRT group</td>
<td>101</td>
<td>34.2 ± 3.41</td>
<td>23.60 ± 3.35</td>
<td>3.35 ± 2.81</td>
<td>8.15 ± 4.05</td>
<td>5.17 ± 2.76</td>
<td>391 ± 3.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAM group</td>
<td>108</td>
<td>33.7 ± 3.87</td>
<td>24.95 ± 3.29</td>
<td>8.01 ± 2.77</td>
<td>5.05 ± 4.21</td>
<td>197.3 ± 5.21</td>
<td>413 ± 3.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EP-TAM group</td>
<td>109</td>
<td>34.5 ± 3.55</td>
<td>23.76 ± 3.38</td>
<td>8.21 ± 2.64</td>
<td>5.54 ± 4.78</td>
<td>199.5 ± 2.43</td>
<td>386 ± 3.43</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Compared with the three general data, P > 0.05, no significant difference.

Table 2 Comparison of causes of infertility in HRT group, TAM group, and EP-TAM group (%)

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Infertility cause</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>The fallopian tube factors</td>
</tr>
<tr>
<td>HRT group</td>
<td>101</td>
<td>21 (20.8%)</td>
</tr>
<tr>
<td>TAM group</td>
<td>108</td>
<td>23 (21.3%)</td>
</tr>
<tr>
<td>EP-TAM group</td>
<td>109</td>
<td>24 (22.0%)</td>
</tr>
<tr>
<td>P</td>
<td>318</td>
<td>1.003</td>
</tr>
</tbody>
</table>

Note: The causes of infertility in the three groups were P > 0.05.

Statistical analysis
SPSS 25.0 software was used for processing and analysis, means were expressed as mean ± standard deviation (X ± S). Data were tested by chi-square test and non-parametric test was used for measuring data due to the lack of normal distribution, and the difference was considered to be statistically significant at P < 0.05.

Results
Comparison of cycle cancellation rate, endothelial thickness and percentage of embryo grade
The cycle cancellation rate of TAM group and endothelial side combined TAM group (EP-TAM group) was significantly lower than that of HRT group, which was statistically significant (P < 0.05). The endothelium on the day of progesterone conversion of HRT group was significantly thinner than that of TAM group and EP-TAM group, which was statistically significant (P < 0.05). There was no statistically significant difference in the percentage of grade I and II embryos transferred in the three groups (P > 0.05) (Table 3).

Clinical outcomes
The pregnancy and implantation rates were statistically significantly higher in the endothelial side combined with TAM group (EP-TAM group) than in the HRT group (P < 0.05). The clinical pregnancy rate and embryo implantation rate of the TAM group were slightly higher than those of the HRT group, but there was no statistical difference (P > 0.05). The rate of early miscarriage was higher in the HRT group than in the TAM group and the endometrial side combined with TAM group. The rate of ectopic pregnancy was higher in the HRT group than in the TAM group and the endometrial side combined with TAM group, which was statistically significant (P < 0.05) (Table 4).

Discussion
Although endometrial tolerance has been studied in a number of ways, it is still practically common for clinicians to use endometrial thickness to infer endometrial tolerance. It is commonly believed that an excessively thin endometrium (< 7 mm) leads to failure of embryo implantation [10]. When endometrial thickness in the luteal phase is less than 7 mm, the so-called “thin endometrium”, both physicians and patients are faced with a difficult and uncertain choice of treatment. Exogenous estrogen supplementation, sildenafil citrate, low-dose aspirin, and intrauterine instillation of granulocyte colony-stimulating factor (GCSF) are the mainstream of treatment for thin endometrium [11], and the results of these therapies are unsatisfactory. Among them, the treatment plan of sildenafil and low-dose aspirin was proposed based on the hemodynamic characteristics of thin endometrium, and its efficacy is currently controversial among clinical scholars, so it has not been widely promoted [12]. Granulocyte colonization factor has the effect of promoting cell proliferation and differentiation, although it can increase the thickness of endometrium as confirmed by animal experiments, there are still different opinions on the clinical effect, although some scholars in China believe that intrauterine instillation of granulocyte colonization factor has a tendency to improve the FET outcome of patients with thin endometrium, but in general the selection of patients for intrauterine instillation, the timing of the instillation, the type and dose of the drug instilled and even the number of installations may affect the efficacy of this technique as well as its clinical dissemination, and further studies are needed to confirm this [13]. The study of stem cell therapy for thin endometrium is still in the exploratory stage [14], and therefore the search for a new therapeutic option has become an important issue for clinical practitioners.

Successful pregnancy outcomes in frozen-thawed embryo transfer (FET) cycles critically depend on the efficacy of endometrial preparation protocols. Currently, available methods encompass hormone replacement cycles, natural cycles, stimulation cycles, and down-regulation combined with hormone replacement cycles. Among these, hormone replacement cycles stand out, involving the administration of exogenous estrogen and progesterone to facilitate endometrial growth, maturation, and transformation. Renowned for their stability, operational convenience, reduced need for frequent patient follow-up visits, and widespread adoption in clinical practice, hormone replacement cycles were selected as the control group in this study. The aim is to discern a more optimal treatment regimen specifically targeting thin endometrium.
There is no record of thin endometrium in Chinese medicine, but according to its clinical manifestations, it should belong to the category of “infertility” and “menstrual disease”. Integrating Chinese and Western medicine in the treatment of infertility is the specialty and advantage of the hospital where the research team is located. Summarizing the long-term clinical experience, it is found that most of the patients with thin endometrium are suffering from kidney deficiency and blood stasis. Domestic scholars also believe that the pathogenesis of this type of patient is based on renal deficiency and Chong Ren disorders, with Qi (Qi) is regarded as the fundamental essence of the universe, the elemental force from which all things originate and derive their existence. Within the human body, qi is believed to be the essential substance that sustains life’s vital functions. Blood, coursing through the veins as a nourishing crimson fluid, is likewise recognized as a foundational element in the human body, crucial for maintaining life’s activities.) and blood stasis as its symptoms [15]. Therefore, treatment should start with tonifying the Kidneys and activating Blood. In the formula, *Placenta Hominis* and *Cuscutae Semen* are the royal herbs, which can tonify the kidney and essence, nourish blood and benefit Qi; *Cistanche Deserticola* can tonify the kidney and help yin; *Dioscorea Rhizoma* and *Angelicae Sinensis Radix* can tonify the blood and regulate menstruation and promote blood circulation, which will benefit Qi and nourish Qi and benefit Yin. *Rehmanniae Radix Praeparata and Paeoniae Radix Alba* nourish Blood and astringing Yin. *Cuscutae Semen* and *Crataegi Fructus* benefit Qi and activate Blood to regulate menstruation; *Rehmanniae Radix* and *Paeoniae Radix Rubra* clearing heat and cooling blood, promoting blood circulation and dissipating blood stasis, nourishing Yin and promoting the production of body fluid. *Gyathula Officinalis* promotes blood circulation and menstruation, nourishes the liver and kidney, and draws fire downward. At the same time, for infertility patients who are difficult to relieve their emotions, and often have concomitant evidence of qi stagnation, so it is added with *Cypet Rhizoma* to relieve the liver and depression, and regulate the manager’s qi, so that there is a laxative in the remedy of the formula, which is complementary, not stagnant, and nourishing without being greasy. Modern pharmacology suggests that kidney tonic herbs have estrogen-like effects, which can overcome the estrogen deficiency caused by ovulation promotion, improve the intrauterine environment, and increase endometrial tolerance. Kidney tonifying and blood activating herbs are commonly used and effective in improving thin endometrium. Wu Juanjuan et al. found that meta-analysis of kidney tonifying and blood activating herbs in the treatment of thin endometrium showed that these herbs, whether used alone or in combination with western medicines, could improve the overall effective rate of patients with thin endometrium [16]. TAM is an estrogen receptor modulator with estrogen-like effects on the endometrium and promotes endometrial proliferation. Its mechanism of action is twofold: (1) Estrogen pathway: TAM can activate estrogen receptors, regulate the signaling pathway of estrogen response genes, activate steroidogenic factors, promote endometrial gland proliferation and repair, and, at the same time, improve the synthesis and metabolism of estrogen to increase the thickness of the endometrium [17]. (2) Non-estrogen pathway: TAM up-regulates the expression of vascular endothelial growth factor, insulin-like growth factor-1, and other cytokines, which promotes endometrial microvesSEL formation, improves endometrial microenvironment, and promotes endometrial development [18]. Therefore, TAM can be one of the alternatives for preparing the endometrium in patients with thin endometrium. In the present study, it was found that both the TAM group and the endometrial formula combined with TAM group were better than the conventional HRT group for the enhancement of endometrial thickness [19], which might be related to the estrogen level in patients with thin endometrium. This study also found that the embryo implantation rate of patients in both groups using TAM was superior to that of the HRT group, similar to the findings of Liu [20]. In this study, 18 cycle cancellations in the HRT group were due to endometrial thickness less than 7 mm on the day of endometrial transformation, and the patients requested to cancel the cycle after communication, which was a significantly higher cancellation rate than in the other two groups (P < 0.05). In the TAM group (9) and EP-TAM group (10) cycle cancellations were due to failure of ovulation or multiple follicular development to prevent the risk of OHSS. The data of this study showed that the rates of early miscarriage and ectopic pregnancy in both groups using TAM were lower than that of the HRT group by about 10%, and there was a statistical difference in the rate of ectopic pregnancy in both groups when compared to the HRT group (P < 0.05). Some studies have found a significant increase in the rate of ectopic pregnancy and spontaneous abortion with an endometrium less than 8 mm [17, 21, 22], which supports the results of the data in this paper, and it can be concluded that one of the high-risk factors for ectopic pregnancies and early miscarriages is an excessively thin endometrium. In this study, the endometrium of the two groups using TAM thickened significantly, the clinical pregnancy rate and implantation rate increased to a certain extent, and the ectopic pregnancy rate and spontaneous abortion rate decreased, and there was a significant difference in the clinical pregnancy rate and implantation rate of taking endometrial formula on the basis of TAM ovulation promotion compared with that of the HRT group, which was probably related to the fact that the endometrial formula could overcome estrogen insufficiency induced by ovulation promotion, improve the
intrauterine environment, and increase the tolerance of the endometrium.

In conclusion, compared with HRT preparation of endometrium, TAM pro-ovulatory cycles and endometrial formula combined with TAM pro-ovulatory cycles can significantly thicken thin endometrium, reduce high cycle cancellation rate, ectopic pregnancy rate and early miscarriage rate due to insufficient thickness, and there is also a tendency to increase the pregnancy and implantation rate, and the effect of adding endometrial formula is more obvious. In this study, due to the small number of FET cycles in patients with thin endometrium, there is still a need to expand the sample size for further controlled studies. Therefore, for clinical patients with thin endometrium, FET cycles can be tried with the endometrial formula combined with TAM to promote ovulation to prepare the endometrium.

References


