Original Article

Factors Associated with Effectiveness of Treatment and Reproductive Outcomes in Patients with Thin Endometrium Undergoing Estrogen Treatment

Si-Miao Liu, Yuan-Zheng Zhou, Han-Bi Wang, Zheng-Yi Sun, Jing-Ran Zhen, Keng Shen, Cheng-Yan Deng, Jing-He Lang

Department of Obstetrics and Gynecology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100730, China

Abstract

Background: Thin endometrium is associated with poor reproductive outcomes; estrogen treatment can increase endometrial thickness (EMT). The aim of this retrospective cohort study was to investigate the factors influencing the effectiveness of estrogen treatment and reproductive outcomes after the treatment in patients with thin endometrium.

Methods: Relevant clinical data of 101 patients with thin endometrium who had undergone estrogen treatment were collected. Possible factors influencing the effectiveness of treatment were analyzed retrospectively by logistic regression analysis. Eighty-seven infertile women without thin endometrium who had undergone assisted reproduction served as controls. The cases and controls were matched for age, assisted reproduction method, and number of embryos transferred. Reproductive outcomes of study and control groups were compared using Student's *t*-test and the Chi-square test.

Results: At the end of estrogen treatment, EMT was ≥8 mm in 93/101 patients (92.1%). Effectiveness of treatment was significantly associated with maximal pretreatment EMT ($P = 0.017$) and treatment duration ($P = 0.004$). The outcomes of assisted reproduction were similar in patients whose treatment was successful in increasing EMT to ≥8 mm and the control group. The rate of clinical pregnancy in patients was associated with the number of good-quality embryos transferred in both fresh ($P = 0.005$) and frozen-thawed ($P = 0.000$) embryo transfer cycles.

Conclusions: Thinner EMT before estrogen treatment requires longer treatment duration and predicts poorer treatment outcomes. The effectiveness of treatment depends on the duration of estrogen administration. Assisted reproductive outcomes of patients whose treatment is successful (i.e., achieves an EMT ≥8 mm) are similar to those of controls. The quality of embryos transferred is an important predictor of assisted reproductive outcomes in patients treated successfully with exogenous estrogen.

Key words: Assisted Reproduction; Endometrial Thickness; Estrogen; Thin Endometrium

Introduction

Thin endometrium is often associated with poor reproductive outcomes of assisted reproductive cycles.[1-3] According to a meta-analysis in 2014, the pregnancy rate is significantly lower in women with thin endometrium undergoing *in vitro* fertilization-embryo transfer (IVF-ET) cycles.[4] In fresh or frozen-thawed embryo transfer (FET) cycles, thin endometrium often results in the cancelation of ET and cryopreservation of all the embryos. Therefore, thin endometrium in infertile women requires treatment. Methods used to treat thin endometrium include hysteroscopic adhesiolysis, estrogen treatment, vasoactive measures, intrauterine infusion of granulocyte colony-stimulating factor, and regenerative medicine.[5-9] Of these, exogenous estrogen treatment is the most commonly used and convenient method for increasing endometrial thickness (EMT) and improving reproductive outcomes; however, assessment of the effectiveness of treatment and protocols for estrogen

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Address for correspondence: Dr. Cheng-Yan Deng, Department of Obstetrics and Gynecology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100730, China
E-Mail: chydmd@hotmail.com

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administration vary among published studies.[10,11] Few studies have evaluated the factors that may influence the effectiveness of estrogen treatment on thin endometrium and the subsequent reproductive outcomes. In addition, alternative methods should be considered for patients who are insensitive to estrogen treatment. We believe that identifying the factors that influence the effectiveness of estrogen treatment and subsequent reproductive outcomes would be helpful for formulating individual treatment strategies and improving both effectiveness of treatment and reproductive outcomes.

To identify such factors and evaluate their influence on the effectiveness of estrogen treatment and reproductive outcomes, we performed a retrospective cohort study of 101 patients who had undergone estrogen treatment for thin endometrium.

Methods

In our center, thin endometrium is diagnosed when the maximal EMT is <8 mm, dominant follicles are 18 mm in diameter in ovulatory cycles, or estrogen has been used for endometrial preparation for 20 days and serum estradiol (E_2) concentrations maintained above 200 pg/ml in FET cycles. Infertile women who met these criteria for diagnosing thin endometrium and had undergone estrogen treatment in the Assisted Reproductive Center, Peking Union Medical College Hospital (Beijing, China) between January 2004 and December 2014 were included in this study. Otherwise eligible patients with incomplete clinical data were excluded. The maximal EMT was measured by transvaginal ultrasonography. The clinical records of these patients were analyzed retrospectively and the data were completely anonymous. All the study patients had undergone abdominal, pelvic and breast ultrasonic examination, and routine blood tests plus assessment of the following: Lipid profile, liver function, coagulation function, and blood human chorionic gonadotropin concentrations before commencing estrogen treatment to exclude contraindications to estrogen administration. This study was completely anonymous, obviating the need for informed consent, and was approved by the Ethics Committee of our hospital.

Estrogen treatment, in the form of 15–18 mg of estradiol valerate (Progynova; Bayer, Leverkusen, Germany) per day, was given orally starting on the 2nd day of each menstrual cycle. Thereafter, the dose of estrogen was adjusted to keep serum E_2 concentrations above 600 pg/ml. If serum E_2 concentrations were not maintained above 600 pg/ml with oral administration, 2–4 mg/d of intravaginal 17β-E_2 (estradiol tablets in Femoston®, Abbott Biologicals B.V, Olst, the Netherlands) was added. The serum E_2 was kept below 5000 pg/ml during treatment to minimize the occurrence of severe adverse effects associated with extremely high serum estrogen concentrations. Serum E_2 concentrations and EMT were evaluated every 15–20 days during the treatment, the goal being to increase the EMT to ≥8 mm. Estrogen treatment ended when the EMT reached 8 mm or the adverse effects were obvious. To protect the endometrium from pathological proliferation during treatment, the continuous use of estrogen was interrupted by intramuscular injection of progesterone for 5 days every 3 months, which was followed by withdrawal bleeding. Because the duration of estrogen treatment was usually much longer than that of spontaneous menstrual cycles and exposure to high estrogen concentrations for a long time may alter endometrial receptivity, assisted reproduction was not performed during estrogen treatment. Rather, when the EMT had reached 8 mm, progesterone was injected intramuscularly for 5 days and assisted reproductive cycles started on the 2nd day of the subsequent withdrawal bleeding. Monitoring for possible adverse effects of estrogen use was achieved by performing ultrasonic examination of breasts and reproductive tract and blood lipid and coagulation function tests regularly during treatment and the 2-year follow-up period after treatment finished. Any adverse effects associated with estrogen use and their management was recorded.

Assessed reproductive outcomes after estrogen treatment included rates of clinical pregnancy, live delivery, and miscarriage. Patients' clinical characteristics and factors that may have influenced treatment or reproductive outcomes were retrospectively investigated. The reproductive outcomes of patients with thin endometrium who were treated successfully (i.e., achieved an EMT ≥8 mm during treatment) were compared with those of women with normal EMT undergoing assisted reproduction as controls. Cases and controls were matched by age (±1 year), assisted reproduction method, and number of embryos transferred. The year when the assisted reproductive technology was performed was matched between cases and controls, in case the success rates were different in different years.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), version 17.0 (SPSS, Chicago, IL, USA). Continuous data are expressed as mean ± standard deviation (SD). Categorical data are presented as count and percentage. Continuous variables were compared by Student's *t*-test or the Mann–Whitney *U*-test. The Chi-square test or Fisher's exact test were used to compare categorical variables. A logistic regression model was adopted to adjust confounding variables and to identify risk factors. A $P < 0.05$ was considered to indicate statistical significance. Odds ratios (*OR*s) are expressed with 95% confidence intervals (*CI*s).

Results

A total of 104 infertile women with thin endometrium underwent exogenous estrogen treatment were enrolled in this study. Three of these patients were excluded because of incomplete clinical data. Relevant characteristics of the remaining 101 patients with thin endometrium are shown in Table 1. The potential cause of thin endometrium was

identified in 73 patients (72.3%), no cause being identified in the other 28 patients (27.7%).

The maximal EMT prior to estrogen treatment ranged from 2.9 mm to 7.8 mm. During the high-dose estrogen treatment, the maximal EMT increased significantly in all patients compared with that of before treatment ($P = 0.000$, $t = 16.82$, df = 100, 95% *CI*: 2.37, 3.01) [Table 2]. EMT ≥8 mm was achieved during treatment in 93 patients (92.1%). The EMT was significantly thinner before treatment in the patients whose EMT did not reach 8 mm during treatment than those in whom treatment was successful (4.69 ± 1.16 mm vs. 6.61 ± 1.00 mm, $P = 0.000$, $t = -5.18$, df = 99, 95% *CI*: −2.66, −1.19). In addition, the increase in EMT was less significant in patients whose treatment failed than in those who reached the treatment goal (1.16 ± 0.81 mm vs. 2.82 ± 1.59 mm, $P = 0.005$, $t = -2.90$, df = 99, 95% *CI*: −2.79, −0.53).

The results of univariate and multivariate logistic analysis of factors that may have influenced effectiveness and outcomes of treatment showed that maximal EMT prior to treatment and duration of treatment were significantly associated with the effectiveness of treatment ($P = 0.017$, *OR* = 6.142, 95% *CI*: 1.383, 27.277 and $P = 0.004$, *OR* = 0.969, 95% *CI*: 0.949, 0.990, respectively) whereas age, body mass index, basal follicle-stimulating hormone, serum E_2 concentration during treatment, and cause of thin endometrium were not significantly associated. Correlation analysis showed that patients with thinner initial EMT required a significantly longer duration of treatment ($P = 0.002$). Figure 1 shows the relationship between treatment duration and cumulative success rate.

Table 1: Characteristics of patients with thin endometrium (*n* = 101)

Variables	Results
Age (years)	33.67 ± 3.76
BMI (kg/m^2)	21.53 ± 2.41
Basal FSH (mU/ml)	6.39 ± 1.86
Indication for ART, *n* (%)	
Female factor	57 (56.4)
Endometriosis	2 (2.0)
Tubal factor	42 (41.6)
Anovulation	13 (12.9)
Male factor	15 (14.9)
Combined factor	21 (20.8)
Unexplained	8 (7.9)
Possible etiology of thin endometrium, *n* (%)	
Intrauterine operation	63 (62.4)
Inflammatory factor	7 (6.9)
Combined factors	3 (3.0)
Unexplained	28 (27.7)

Values are showed as mean ± SD or *n* (%). BMI: Body mass index; FSH: Follicle-stimulating hormone; ART: Assisted reproductive technology; SD: Standard deviation.

Table 2: Effectiveness of estrogen treatment on thin endometrium (*n* = 101)

Variables	Results
Maximal EMT prior to treatment (mm)	6.46 ± 1.13
Maximal EMT during treatment (mm)	9.15 ± 1.50
Serum estradiol concentration during treatment (pg/ml)	1948.50 ± 894.95
Duration of treatment (days)	95.64 ± 60.88

Values are showed as mean ± SD. EMT: Endometrial thickness; SD: Standard deviation.

Of the 93 patients who achieved the treatment goal, 87 underwent assisted reproduction in our center. The clinical characteristics of the 87 patients and control group are shown in Table 3. The 3 patients who underwent intrauterine insemination did not achieve pregnancy. The pregnancy outcomes of the other 84 patients are compared with those of the control group in Table 4. Although the maximal EMT in the assisted reproductive cycles was significantly thinner in patients with thin endometrium than in controls, there was no statistically significant difference in reproductive outcomes between patients with thin endometrium whose EMT reached 8 mm during treatment and the control group. In the FET cycles, the doses of estradiol valerate (E_2V) were higher in patients comparing with controls (17.58 ± 1.04 mg vs. 13.66 ± 2.65 mg, $t = 10.525$, df = 114, $P = 0.000$), while the duration for endometrium preparation was similar between them (16.48 ± 2.08 days vs. 16.83 ± 2.12 days, $t = -0.884$, df = 114, $P = 0.378$). Two of the 8 patients whose EMT did not reach 8 mm during treatment underwent FET, but none achieved pregnancies.

Logistic analysis of those who underwent ET revealed that in patients whose EMT reached 8 mm during the treatment, clinical pregnancies were associated with the number of good-quality embryos transferred in both FET cycles ($P = 0.000$, *OR* = 9.633, 95% *CI*: 3.221, 28.806) and fresh IVF-ET/intracytoplasmic sperm injection (ICSI) cycles ($P = 0.005$, *OR* = 18.385, 95% *CI*: 2.416, 139.897).

The most common adverse effect associated with estrogen treatment was mild hypertriglyceridemia, which occurred in 7 patients, their triglyceride concentrations ranging from 2.28 mmol/L to 4.75 mmol/L. Estrogen treatment was ceased on detection of hypertriglyceridemia and blood lipid tests repeated regularly. Triglyceride concentrations returned to

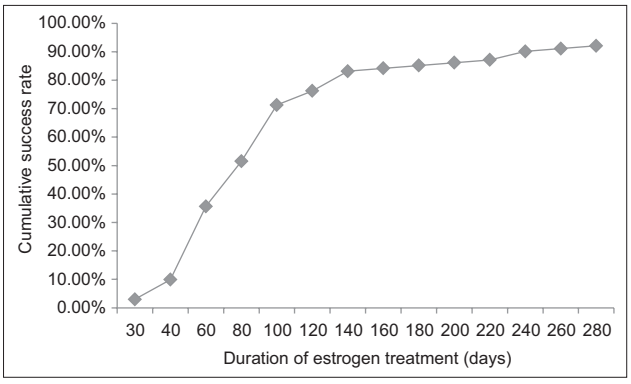

Figure 1: Relationship between duration of estrogen treatment and cumulative success rate.

Table 3: Clinical characteristics of patients with thin endometrium whose EMT reached 8 mm during estrogen treatment and controls without thin endometrium

Characteristics	Cases (n = 87)	Control (n = 87)	P	t (df)
Age, years	33.62 ± 3.57	33.62 ± 3.79	1.000	0.000 (86)
Basal FSH, mU/ml	6.36 ± 1.90	6.49 ± 2.14	0.694	−0.395 (86)
BMI, kg/m²	21.34 ± 2.48	21.91 ± 2.59	0.193	−1.314 (86)
Etiology of infertility			0.901	
Female factor	49 (56.3)	48 (55.2)		
Male factor	13 (14.9)	15 (17.2)		
Combined factor	19 (21.8)	20 (23.0)		
Unexplained	6 (6.9)	4 (4.6)		
ART protocols			1.000	
Fresh IVF/ICSI-ET,	26 (29.9)	26 (29.9)		
FET	58 (66.7)	58 (66.7)		
IUI	3 (3.4)	3 (3.4)		
Number of embryos transferred	2.23 ± 0.50	2.15 ± 0.45	0.109	1.62 (83)
Number of good-quality embryos transferred	0.99 ± 0.74	1.00 ± 0.81	0.912	−0.11 (83)

Values are showed as mean ± SD or n (%); P values are for t-test or Chi-square test. FSH: Follicle-stimulating hormone; BMI: Body mass index; EMT: Endometrial thickness; IVF-ET: *In vitro* fertilization-embryo transfer; ICSI: Intracytoplasmic sperm injection; FET: Frozen-thawed embryo transfer; IUI: Intrauterine insemination, ART: Assisted reproductive technology; SD: Standard deviation.

Table 4: Reproductive outcomes of patients with thin endometrium whose EMT reached 8 mm during estrogen treatment and controls undergoing fresh or FET

Variables	Cases (n = 84)	Control (n = 84)	χ^2	P
Implantation rate, n/N (%)	59/187 (31.6)	64/181 (35.4)	0.599	0.439
Fresh IVF-ET/ICSI	19/75 (25.3)	20/62 (32.3)	0.799	0.371
FET	40/112 (35.7)	44/119 (37.0)	0.040	0.842
Clinical pregnant rate, n/N (%)	47/84 (56.0)	51/84 (60.7)	0.392	0.531
Fresh IVF-ET/ICSI	13/26 (50.0)	14/26 (53.8)	0.077	0.781
FET	34/58 (58.6)	37/58 (63.8)	0.327	0.568
Live birth rate, n/N (%)	33/84 (39.3)	36/84 (42.9)	0.221	0.638
Fresh IVF/ICSI-ET	9/26 (34.6)	10/26 (38.5)	0.083	0.773
FET	24/58 (41.4)	26/58 (44.8)	0.141	0.708

EMT: Endometrial thickness; implantation rate was calculated as the number of intrauterine gestational sacs observed divided by the number of transferred embryos; IVF-ET: *In vitro* fertilization-embryo transfer; ICSI: Intracytoplasmic sperm injection; FET: Frozen-thawed embryo transfer.

normal with dietary management and increased exercise in five of these patients; the other two were treated with antilipemic agents. Triglyceride concentrations were normal in all the patients during follow-up. Scanty vaginal bleeding occurred in 1 patient on the 16th day of estrogen treatment; her estrogen administration was stopped immediately and 40 mg/d of progesterone given intramuscularly for 5 days, inducing a normal amount and duration of withdrawal bleeding. Pelvic ultrasonic examination revealed no abnormalities. This patient's subsequent treatment cycles were uneventful; her treatment was successful and she achieved a pregnancy. Nausea occurred in some patients and was alleviated by replacing some of the oral estradiol valerate (E_2V) with intravaginal 17β-E_2. No severe adverse effects such as cancer or deep venous thrombosis occurred during treatment or follow-up.

Discussion

To the best of our knowledge, this is the first study of factors influencing the effectiveness of estrogen treatment in Chinese women with thin endometrium. The initial question that motivated our study was "What factors influence the effectiveness of estrogen therapy and the subsequent reproductive outcomes in patients with thin endometrium?" We found the effectiveness of estrogen treatment was satisfactory and stable in most patients. Prior to receive estrogen treatment, some of the patients had already undergone artificial endometrial preparation for FET with up to 15–18 mg of E_2V per day for 20 days; however, their EMT had not reached 8 mm. We, therefore, deduced a longer duration of estrogen administration was required to repair their thin endometrium and increase the EMT. In addition, according to Xu *et al*., proliferation of endometrial stem/progenitor cells, which may contribute to the repair of the endometrium, is stimulated only by higher than physiological estrogen concentrations.[12] In our study, the shortest time any patient took to achieve the treatment goal was 30 days, which supported our contention. However, 90% of the patients achieved the target EMT of ≥8 mm within a cumulative 240 days of estrogen administration that was regularly interrupted by progesterone injections; a longer duration conferred negligible additional benefit. Although most patients received the same dosage of estrogen, their serum E_2 concentrations varied significantly for various metabolic reasons. What is more, serum E_2 concentrations were associated neither with treatment outcomes nor degree of increase in EMT, which suggests that an adequate duration of estrogen treatment is more important for increasing the EMT than extremely high serum concentrations of E_2. We found that the effectiveness of treatment was associated with its duration and the maximal EMT prior to treatment. Thinner endometrium was less sensitive to estrogen treatment, which suggests that the endometrium has been more seriously injured in these patients. Consequently, thinner endometrium required longer treatment duration. Within 140 days of cumulative estrogen administration, 84/101 of patients (83.17%) had achieved the treatment goal of ≥8 mm; the cumulative success rate increased more slowly for longer treatment durations [Figure 1]. Our findings suggest that the pre-estrogen treatment EMT could be used as a predictor of both the required treatment duration and treatment outcomes. In patients with extremely thin endometrium, alternative methods such as surrogacy, granulocyte colony-stimulating factor treatment,[13] or regenerative medicine[14] should be considered because of the likelihood of poor outcomes and long duration of estrogen treatment.

The reproductive outcomes of the patients who were treated successfully were similar to those of the control group. However, during reproductive cycles, the maximal EMT was significantly lower in the study patients than in the control group, suggesting that there is still a difference in endometrial status between patients and controls even after estrogen treatment. This also suggests that provided the EMT reaches 8 mm by the end of the treatment, uterine receptivity in subsequent reproductive cycles will be adequate. The reproductive outcomes of patients with thin endometrium were associated with the quality of embryos transferred after successful treatment; thus, the quality of embryos transferred may serve as a predictor of reproductive outcome after estrogen treatment. Previous studies have reported that reproductive outcomes are associated with the maximal EMT during reproductive cycles;[4] however, in our study, the patients' EMT during reproductive cycles was not associated with achievement of pregnancy. However, our analysis had some limitations because this study was retrospective. In our study, only 11 of the patients who had achieved the treatment goal of EMT ≥8 mm had a maximal EMT during reproductive cycles of less than 8 mm, four of them having an EMT <7 mm; thus, there may be some bias in the analysis.

Because the long-term use of estrogen without added progesterone is associated with increased risk of some types of tumor such as endometrial cancer, progesterone should be used intermittently. Besides, extremely high concentrations of estrogen may cause deep venous thrombosis. The serum E_2 should be monitored during treatment and estrogen-related adverse effects should be detected and treated promptly. In our study, no serious complications related to the use of estrogen were observed during or after treatment, confirming the safety of such treatment. In addition, the serum E_2 level is usually much higher during pregnancy in most women and it is tolerable. However, more large-scale studies may be needed to conclusively establish the safety of such treatment.

In conclusion, in our study, estrogen treatment that results in serum E_2 concentrations higher than the peak values in spontaneous ovulatory cycles was effective in increasing the thickness of thin endometrium. The effectiveness of treatment depended on the duration of estrogen administration. The thinner the EMT prior to estrogen treatment, the longer the treatment duration is required and more likely to achieve a poorer treatment outcome. Extremely, thin endometrium was insensitive to estrogen treatment; alternative approaches should be considered in these patients. Assisted reproductive outcomes of the patients who were treated successfully were similar to those of controls, even though the EMT of the former was still thinner. Embryos should be cryopreserved when thin endometrium is detected in IVF/ICSI cycles and FET performed after the patients have been successfully treated. The quality of embryos transferred is an important predictor of assisted reproductive outcomes in patients treated successfully with exogenous estrogen.

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Conflicts of interest

There are no conflicts of interest.

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