The efficacy of tamoxifen in the treatment of thin endometrium in frozen-thawed embryo transfer cycle

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Research

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Abstract

Background: Thin endometrium is known to adversely affect reproductive performance. There is no agreement about a consensus treatment on thin endometrium. Tamoxifen(TAM) has a positive effect on endometrium when used as ovulation induction agent. Little information is available regarding its use in patients with thin endometrium during frozen-thaw embryo transfer (FET) cycles. This study was designed to evaluate the effectiveness of TAM on women with thin endometrium in frozen-thaw embryo transfer cycles.

Methods: A total of 345 thin endometrium women were retrospectively analyzed during their FET cycles. Among them 190 received TAM protocol (TAM 20 mg per day for 5 days) and 155 hormone replacement therapy (HRT) protocol (estradiol valerate 6 mg/d for 14 to 21days). Endometrial thickness and pregnancy outcome were compared between the two groups.

Result(s): The endometrial thickness in TAM group was significantly higher compared with HRT group. The clinical pregnancy rate, implantation rate, ongoing pregnancy rate and live birth rate were significantly higher in TAM group than HRT group.

Conclusion(s): In patients of recurrent thin endometrium, tamoxifen treatment in endometrium preparation may be a successful alternative approach

Background

Endometrial thickness is one of the key parameters of endometrial receptivity (1) and consequently an important factor for successful embryo implantation. There has been no commonly accepted thickness below which successful implantation would be preclude, but it has been recognized by many investigators that endometrial thickness ≥7mm on the day of luteinizing hormone(LH) surge or human chorionic gonadotrophin(HCG) administration is preferred for embryo transfer(2-5). Thin endometrium (≤7 mm) which affects about 2.4% patients in in vitro fertilization(IVF) cycles (6) often results in cancelation of embryo transfer and cryopreservation of all the embryos.

Various strategies have been attempted to improve endometrial growth in these patients, including estrogen supplementation, vasoactive measures such as vaginal sildenafil, low-dose aspirin, pentoxifylline, or tocopherol, intrauterine infusion of granulocyte colony-stimulating factor, and regenerative medicine[7-11]. Among them, exogenous estrogen treatment is the most commonly used method[12]. Many factors may influence the effectiveness of estrogen treatment and the subsequent reproductive outcomes[12,13,14]. A high dose and a longer duration of estrogen administration may be effective for some of these patients[12]. However, supraphysiologic estrogen levels resulted from this treatment before FET may have a negative impact on embryo-endometrium asynchrony and consequently reduce implantation rates[15,16,17,18]. Moreover, a high rate of breakthrough bleeding (19), increased risk of some types of tumor such as endometrial cancer(20), deep venous thrombosis(21) and decreased downstream consequences on the quality of placentation and perinatal outcome result

from supraphysiologic estrogen levels affect its use in endometrial preparation for FET(22). In addition, some patients were insensitive to estrogen treatment. Alternative methods should be considered for endometrium preparation in these women during their FET cycles.

Tamoxifen (TAM) is a selective estrogen receptor modulator (SERM) with chemical properties similar to clomiphene citrate(CC)，used in clinic as a highly effective agent for the prevention and treatment of breast cancer，23，. TAM has also been used in ovulation induction，24-26，. In contrast to CC, whose anti-estrogenic effects at the level of the endometrium sometimes result in thin endometrium(27), ovulation induction with tamoxifen improves endometrial thickness in women with a history of thin endometrium (28). However, little information is available regarding its use during FET cycles in that kind of patients. The aim of this study is to evaluate the efficacy of TAM in improving endometrial thickness, optimizing clinical outcome in frozen-thaw embryo transfer cycles. We propose that the result of our study may contribute to the treatment of infertility patients with thin endometrium.

Methods

Study design

This retrospective study was performed during the period from January 2016 till August 2017 at the Reproductive Medical Center of Anhui Provincial Hospital. The study was approved by the institutional ethics committee of Anhui Provincial Hospital.

A total of 345 frozen-thaw embryo transfer cycles were included for this analysis. In our center, thin endometrium is diagnosed when the maximal endometrial thickness(EMT) is ＜7 mm, dominant follicles are 18 mm in diameter in ovulatory cycles, or after 12 to 16 days of estradiol (E2) replacement(4–6 mg, Progynova; Bayer Schering Pharma, Roubaix, France). Eligible patients had previously demonstrated a thin endometrium during their 1-2 previous cycles at our center. All women underwent diagnostic hysteroscopy prior to this FET cycle, and intrauterine synechiae were absent in all women. Thus, no participants had uterine or endometrial abnormalities except for a thin endometrium at the time of the FET cycles. Also, patients with repeated implantation failures were excluded. All the patients were between 20- 40 years old.

At the discretion of physicians and/or patients' preference, endometrium was prepared with TAM or HRT as described below. Patients in TAM group were fully counseled regarding the novel use of TAM. Women with multiple types of FETs following the same fresh IVF/ICSI cycle were excluded from the analysis. This study was completely anonymous, obviating the need for informed consent. Otherwise eligible patients with incomplete clinical data were excluded.

The FET cycle

In the group of TAM, 20 mg per day was giving from day 5 of the menstrual cycle for 5 days. Vaginal ultrasound examinations were performed on day 10 of the cycle to monitor the number and size of

developing follicles and endometrial thickness. Ovulation was induced with 10,000 I.U. Hcg when the leading follicle reached 18–22 mm and the endometrium thickness reached 7 mm. FET was performed four days later. If no dominant follicle developed, human menopausal gonadotropin was given from day 10 up to Hcg injection. In the group of hormone replacement treatment cycle, oral estradiol valerate (progynova,Schering, German) was taken 6 mg/d from menstrual cycle day 2-3. An ultrasound assessment was done 12 to 14 days later to assess endometrium thickness. Progesterone 40 mg/d which would be changed to 60 mg/d 2 days later, was given to transform the endometrium, provided the endometrial thickness exceeded 7 mm. If the endometrium thickness is not adequate, endometrial preparation continued with step-up dose of E2 or adding vaginal estradiol (Femoston, Solvay pharmaceuticals B.V.) 1-2 mg/d till the endometrium thickness reaching 7 mm. Cycles were canceled in patients whose endometrial thickness remained <7 mm after 21 days of continuous estradiol administration. FET was performed 4 days later.

Embryo vitrification and thawing

After fresh embryo transfer, surplus Day 3 or blastocyst embryos underwent vitrification. For Day 3 embryos, our laboratory procedure of vitrification and warming was the same as the method used for human oocytes as reported by Tong et al. previously (29). For blastocysts, a glass micro-needle was used to collapse the blastocyst before vitrification. The following steps were the same as for the Day 3 embryos. Embryo quality was graded as ‘good’, ‘reasonable’,‘moderate’ or ‘poor’ according to the number of cells, degree of fragmentation and renewed development of the embryo. This standard was based on the ESHRE Istanbul consensus on embryo assessment (30). A score was given to each embryo from 3 (good) to 1(moderate).

Definition of pregnancy

A serum β-hCG assay 11-14 days after ET. Clinical pregnancy was defined as the presence of a gestational sac on transvaginal ultrasound.

Clinical miscarriage was defined if the pregnancy terminated before 12 weeks of gestational age. Implantation rate was defined as the number of intrauterine sacs divided by the number of embryos transferred.

Ongoing pregnancy was defined as gestations that reached 20 weeks or more. Live birth was defined as give birth to an infant ≥24 weeks' gestation.

Statistical analysis

All analyses have been performed using IBM Spss statistics 21.For continuous variables, Student’s t-test and Mann–Whitney test were used for data with homogeneous variance and heterogeneous variance respectively. The x^2 test was used for categorical variables. The variable with greater clinical importance and larger variance was selected for multivariate assessment. Logistic regression analyses were

conducted to identify independent correlates between each possible confounding factor, especially protocols for endometrium preparation and pregnancy outcome after adjusting for other confounders that were identified in our univariate analysis. A p-value <0.05 was considered statistically significant.

Results

We analyzed FET cycles of 345women from our unit and 160 of these women (46.37%) achieved clinical pregnancy. Women who achieved a clinical pregnancy were younger and had a shorter duration of infertility. Also, they achieved more oocytes and had a thicker endometrium than their no clinical pregnancy counterparts. There were no significant differences in the other characteristics such as BMI (body mass index), type of infertility, diagnosis of infertility，duration of endometrium preparation, embryo score. The demographic and clinical characteristics of these cycles are summarized in Table I.

190 women received TAM and 155 received HRT. A summary of important aspects of the treatment outcome is presented in Table II. There were no significant differences in age, duration of infertility, BMI, type of infertility, the major indication for IVF, duration of stimulation and the number of oocytes retrieved. Statistically significant differences between the two groups were noted in terms of duration of endometrium preparation, embryos transferred, endometrial thickness and its increase in the research cycle compared with the previous cycle as well as embryo score. The clinical pregnancy rate, implantation rate, ongoing pregnancy rate and live birth rate were significantly higher in TAM group than HRT group.

Table III summarizes the statistics from the logistic regression analyses: The HRT group was used as a reference. Age, endometrial thickness, embryo score had a significant effect on clinical pregnancy rate, ongoing pregnancy rate and live birth rate. But for duration of infertility and No. of oocytes, the effect was not significant. After adjustment for the other variates, the ORs for CPR(clinical pregnancy rate), OPR(ongoing pregnancy rate) and LBR(live birth rate) in TAM group were[2.225（1.330-3.723）] , [1.733（1.038-2.893）] and [1.909（1.135-3.211）] respectively, which were significantly higher than HRT group.

Discussion

The aim of our study was to evaluate the effect of TAM in patients with a thin endometrium in FET cycles. By univariate and multivariate analysis, our results found a superior pregnancy outcome following FET in a TAM ovarian stimulation cycle compared to HRT cycle in women with thin endometrium.

In our study we observed that the durations of endometrium preparation were 4 day shorter in the TAM FET group (10 days) than in the HRT FET group (14 days). This phenomenon is interesting but hard to explain. We hypothesize that ovarian stimulation with TAM may stimulate a faster development of the dominant follicle(s). More prospective studies are needed to explore its clinical relevance.

Our results showed that endometrium thickness was much more improved in TAM group than HRT group. TAM has been reported to improve endometrium thickness while induce ovulation. Kasey Reynolds

switched infertile women undergoing OI (ovulation induction) with CC who have an endometrial thickness of <7 mm to TAM for OI in a subsequent cycle and found that endometrial thickness improved (31) .Wang et al compared TAM and CC in ovarian stimulation cycle in combination with HMG (human menopausal gonadotrophin) and found that TAM treated patients had a significantly increased endometrial thickness (28).

The literature on the treatment of thin endometrium with TAM is rather sparse. Chen X et al used TAM in 3 women who showed a repeated unresponsive thin endometrium and resulted in endometrium expansion to at least 7.7mm and conception[32]. Soon after, another study by the same group showed TAM increased the endometrial thickness from 6.5 to 8.8mm in women with thin endometrium undergoing FET [33]. Ke H et al explore the effect of TAM in patients with a thin endometrium in FET cycle. Their study included 226 women who had an EMT of less than 7.5mm in their previous cycles. After the use of TAM, EMTs were significantly improved. When stratified by different previous endometrium preparation protocols, EMT increased from 6.11±0.98mm to 7.87±1.48 in NC group, from 6.24±1.01 to 8.22±1.67 in HRT group and from 6.34±1.03 to 8.05±1.58mm in OI group[34]. In line with the previous data, our result showed that the EMT in TAM group was9.011[5.9-16.4]mm which was significantly thicker than the EMT in HRT groups. In addition, TAM group showed more improvement of EMT in the research cycle compared with previous cycles than HRT group.

Some authors found that TAM exposure promotes endometrial cell proliferation through estrogen and non-estrogen pathway. In estrogen signaling pathway, TAM stimulate the GPER (GPR30 Estrogen Receptor), which in turn, activates the SF1 (Steroidogenic Factor 1), which is a transcription factor that induce aromatase expression in endometrial cells (35). In none-estrogen signaling pathway, a study found that TAM up-regulates the expression of Ki67 and IGF-1,markers of proliferation[36].

Our study focus on demonstrating the clinical effect of TAM in FET cycles either. Different from the previous study, our study compared the effect of TAM with the mostly commonly used protocol- HRT protocol rather than describe the reproductive outcome only so that this effect can be analyzed precisely. Not only the clinical pregnancy rate and the implantation rate but also the ongoing pregnancy rate and live birth rate of TAM group were significantly higher than HRT group. Logistic regression analysis showed that after adjusting for the confounders that were identified in our univariate analysis, protocols for endometrium preparation is independent factors correlate with clinical outcome. Although the first studies by Chen X et al only include 3 recurrent thin endometrium patients who successfully conceived with one miscarriage after tamoxifen treatment[32], their following studies consisting of 61 thin endometrium women showed that the clinical pregnancy rate, early miscarriage rate and implantation rate were 44.3[(27/61)]7.4[(2/27)]24.2[(32/132) respectively. The authors concluded that in patients of recurrent thin endometrium, tamoxifen treatment may be a successful alternative approach[33]. The study of Ke H et al showed that when stratified by different etiologies of thin endometrium, patients with PCOS obtained the highest rate of clinical pregnancy (60%) and live birth (55.56%) per transfer. The clinical pregnancy rate and live birth rate in patients with history of intrauterine adhesion were 6/18(33.33%) and (5/18, 27.78%) respectively, while for patients with history of uterine curettage, the

clinical pregnancy rate and live birth rate were (39/101, 38.61%) and (32/101,31.68%) respectively[34]. Furthermore, Wu reported lower spontaneous abortion rates in patients with luteal-phase dysfunction treated with TAM as compared with CC[37]. We speculate that the significance between the subgroups in our study might be attributed to an improved endometrial environment for embryo implantation, which resulted from the different endometrial preparation methods. TAM could not only increase mean endometrial thickness effectively but also improve luteal-phase dysfunction, as in the study by Wu et al and Wang et al[37].

Conclusions

The results of this study showed that beyond endometrial expansion, TAM protocol beneficially affect pregnancy outcome in women with thin endometrium undergoing FET.

Declarations

Abbreviations TAM :Tamoxifen FET: Frozen-thaw embryo transfer LH :luteinizing hormone HCG: human chorionic gonadotrophin IVF: in vitro fertilization SERM: selective estrogen receptor modulator CC :clomiphene citrate EMT: endometrial thickness E2:estradiol BMI: body mass index CPR: Clinical pregnancy rate OPR: Ongoing pregnancy rate LBR: Live birth rate

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Availability of data and materials

Data are not publicly shared; please contact the authors for data requests.

Authors' contributions

J.J. was involved in substantial contributions to conception and design, acquisition, analysis and interpretation of data, drafting and revising the article and final approval of the version to be published; L.H. was involved in contributions to analysis and interpretation of data and final approval of the version to be published; L.L. was involved in contributions to acquisition of data and final approval of the version to be published.

Ethics approval

This study was approved by the Ethics Committee of Anhui Provincial Hospital (Institutional Review Board).

Consent for publication

All patients have provided their consent for the data to be used for research and publications.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table I Patient characteristics by ART outcome.

	Pregnancy	No pregnancy	P
N	160	185	
Age (year)	30.84±4.11	32.57±4.26	0.000 [a]
Duration of infertility (year)	3.297（1-15）	4.17（1-20）	0.005 [b]
BMI	22.59±3.441	22.95±2.961	0.259 [a]
Primary infertility, no. (%)	66/160(0.41)	61/185(0.328)	0.087 [c]
Indication for IVF treatment, no. (%)			0.371 [c]
tubal factor	96/160(0.60)	113/160 (0.609)	
EMS	26/160 (0.17)	22/185 (0.118)	
Ovulation dysfunction	16/160(0.10)	20/185 (0.109)	
male factor	22/160 (0.13)	30/185(0.162)	
GnRH angonist long protocol, no. (%)	109/160(0.682)	135/185(0.730)	0.338 [c]
Duration of ovarian stimulation (day)	12.95（4-29）	13.08（5-31）	0.619 [b]
No. of oocytes	13.61±7.51	11.84±7.01	0.025 [a]
Duration of endometrium preparation	12.10±4.000	11.78±3.16	0.676 [a]
No. of embryos transfered	1.75（1-3）	1.81（1-3）	0.333 [b]
Embryo score	4.33（1-9）	4.08（1-7）	0.172 [b]
Endometrial thickness	8.69（6-16.4）	8.01（5.2-14）	0.000 [b]

a Two-sample t-test. Values are mean+SD. b Two-sample Mann–Whitney test. Values are median (minimum–maximum).c Pearson x2 test. Values are number (percentage).

Table Patient characteristics and clinical outcomes in the two groups

	TAM group	HRT group	P
N	190	155	
age	32.2±4.16	32.6±4.22	0.105 [a]
Duration of infertility (year)	3.43（1-13）	4.04（1-20）	0.109 [b]
BMI	22.3±3.0	23.2±3.5	0.184 [a]
Primary infertility, no. (%)	0.4105	0.3355	0.212 [c]
Indication for IVF treatment, no. (%)			0.830 [c]
tubal factor	0.7	0.72	
EMS	0.074	0.142	
Ovulation dysfunction	0.0842	0.077	
male factor	0.1421	0.058	
GnRH angonist long protocol, no. (%)	0.695	0.6645	
Duration of ovarian stimulation (day)	12.71（5-31）	13.2（5-29）	0.278 [b]
No. of oocytes	11.23（1-35）	10.68（1-35）	0.132 [b]
Duration of endometrium preparation	10.36（5-18）	13.66（8-32）	0.00 [b]
No. of embryos transfered	1.68（1-3）	1.85（1-3）	0.009 [b]
Endometrial thickness	9.011（5.9-16.4）	7.74（5.2-12）	0.00 [b]
Increase of endometrium(in the research cycle compared with the previous cycle)	3.05±2.07	1.86±1.33	0.00 [a]
Embryo score	3.89（1-6）	4.48（1-7）	0.007 [b]
Clinical pregnancy Rate, no. (%)	0.579(110/190)	0.355(55/155)	0.00 [c]
Implantation Rate, no. (%)	0.419(134/320)	0.218(62/285)	0.00 [c]
Ongoing Pregnancy Rate, no. (%)	0.5(95/190)	0.323(50/155)	0.002 [c]
Live birth rate, no. (%)	0.495（94/190）	0.284（44/155）	0.000 [c]

a Two-sample t-test. Values are mean+SD. b Two-sample Mann–Whitney test. Values are median (minimum–maximum).c Pearson x2 test. Values are number (percentage).

Table Multivariable analysis of variables for clinical pregnancy and ongoing pregnancy

	Unadjusted ORs	P-value	Adjusted ORs (95% CI)	P-value
ical pregnancy				
(year)	0.909（0.863-0.985）	0.000	0.940（0.886-0.996）	0.037
ation of infertility (year)	0.908（0.842-0.982）	0.016	0.937（0.861-1.021）	0.138
of oocytes	1.034（1.004-1.065）	0.026	0.993（0.960-1.027）	0.683
ometrial thickness	1.383（1.195-1.602）	0.000	1.247（1.066-1.458）	0.006
ryo score	1.025（0.908-1.157）	0.698	1.099（0.962-1.255）	0.166
tocols for endometrium preparation				
M=1	2.887（1.854-4.497）	0.000	2.225（1.330-3.723）	0.002
Γ=2			Reference	Reference
oing pregnancy				
(year)	0.915（0.868-0.965）	0.001	0.943（0.895-0.994）	0.028a
ation of infertility (year)	0.923（0.853-0.999）	0.048	0.950（0.881-1.025）	0.184a
of oocytes	1.034（1.004-1.065）	0.027	1.024（0.993-1.056）	0.133a
ometrial thickness	1.386（1.201-1.599）	0.00	1.209（1.054-1.388）	0.007a
ding of best embryo transferred	0.987（0.873-1.116）	0.839	1.108（0.985-1.246）	0.089a
tocols for endometrium preparation				
M=1	2.295（1.465-3.549）	0.000	1.733（1.038-2.893）	0.035b
Γ=2	Reference	Reference	Reference	Reference
e birth rate				
(year)	0.903（0.856-0.952）	0.000	0.933（0.879-0.989）	0.021
ation of infertility (year)	0.930（0.860-1.005）	0.065	0.967（0.888-1.053）	0.439
of oocytes	1.042（1.011-1.073）	0.007	1.005（0.972-1.040）	0.762
ometrial thickness	1.386（1.186-1.577）	0.000	1.240（1.065-1.443）	0.006
ding of best embryo transferred	1.005（0.890-1.136）	0.932	1.061（0.929-1.212）	0.382
tocols for endometrium preparation				
M=1	2.687（1.713-4.216）	0.000	1.909（1.135-3.211）	0.015
Γ=2	Reference	Reference	Reference	Reference

a P-value of each variable’s overall effects after adjusting for the other variables. b P-value between each variable’s subgroups and reference group.