



# Influence of Maternal Age on the Relationship Between Endometrial Thickness and Ongoing Pregnancy Rates in Frozen–Thawed Embryo Transfer Cycles: A Retrospective Analysis of 2,562 Cycles

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### Specialty section:

This article was submitted to  
Reproduction,  
a section of the journal  
Frontiers in Endocrinology

Received: 24 November 2021

Accepted: 07 March 2022

Published: 27 April 2022

### Citation:

Tian H, Zhang H, Qiu H,  
Yang X, La X and Cui L (2022)  
Influence of Maternal Age on the  
Relationship Between Endometrial  
Thickness and Ongoing Pregnancy  
Rates in Frozen–Thawed Embryo  
Transfer Cycles: A Retrospective  
Analysis of 2,562 Cycles.  
Front. Endocrinol. 13:821753.  
doi: 10.3389/fendo.2022.821753

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**Background:** In frozen–thawed embryo transfer (FET) cycles, endometrial thickness (EMT) has been used routinely as the main clinical monitoring index. However, the current findings are conflicting.

**Method:** This was a single-center retrospective study of 2,054 couples (2,562 cycles) who underwent FET (including cleavage stage embryos and blastocysts) between January 2017 and August 2020 in the reproductive centers of First Affiliated Hospital of Xinjiang Medical University. The primary outcome measure was the ongoing pregnancy rate (OPR); the secondary outcome was the clinical pregnancy rate.

**Results:** After stratified analysis and adjusting for confounders such as maternal age, duration of infertility, number of high-quality embryos transferred, endometrial preparation protocol, number of transfer cycles, and stages of embryo transferred, we found a curvilinear relationship between EMT and the OPR in women < 35 years of age. For women with EMT ≤ 8 mm, the OPR increased by 150% for cleavage stage embryo transfer for every 1 mm increase in the EMT; similarly, it increased by 97% for blastocyst stage FET. However, there was a linear relationship between EMT and OPR in women aged ≥ 35 years. When blastocysts were transferred, for every 1 mm increase in the EMT the OPR increased significantly by 12%. But OPR after frozen-thawed cleavage stage embryos transfer did not increase significantly with increased EMT

**Conclusions:** Our study showed that the OPR increased significantly with increased EMT between young women aged < 35 years with EMT ≤ 8 mm and older women who underwent transfer of blastocysts.

**Keywords:** endometrial thickness, FET, ongoing pregnancy rates, cleavage stage embryos, blastocysts

## INTRODUCTION

In recent years, assisted reproductive technology (ART) has been applied worldwide. Although fresh embryo transfer is a clinical routine, frozen-thawed embryo transfer (FET) is becoming increasingly popular, mainly because it can help prevent ovarian hyperstimulation syndrome (OHSS). Embryos can be better implanted in natural cycles to avoid the negative impact of ovarian hyperstimulation (COH) on endometrial receptivity. This could help optimize the cumulative live birth rate in a single cycle. Considering the relatively limited success rate with corresponding costs, it would be interesting to assess the factors affecting pregnancy outcomes in FET and improve pregnancy rates.

The use of transvaginal ultrasound for measuring endometrial thickness (EMT) in ART is well established. Recording the EMT has become part of standard monitoring in ART, mainly because ultrasonography is simple, noninvasive, reproducible, and is the ideal tool for this task (1, 2). EMT is the most common and important indicator used for endometrial receptivity and potentially can predict the success of ART (3–5). Therefore, it is used as an indicator for clinical routine judgment of whether endometrial development is sufficient to determine the timing of embryo transfer. However, there is considerable controversy regarding EMT in ART (6, 7). Two recent large meta-analyses on the relationship between EMT and pregnancy outcomes have shown inconsistent results. This is because of heterogeneity in study populations and because different cut-off points of EMT have been used in various studies. The independent significance of EMT remains to be further investigated in ART procedures. We investigated whether EMT would affect the ongoing pregnancy rate (OPR) when cleavage stage embryos or blastocysts were transferred by FET in different age groups. This should provide a reference for decision-making in ART clinics.

## MATERIALS AND METHODS

### Study Design

This was a single-center retrospective observational study with a protocol approved by the hospital's ethics committee (permit number k202103-05). All couples provided written informed consent before treatment, and data were entered prospectively into a computerized database. We included 2754 FET cycles (including cleavage stage embryos and blastocysts) between January 1, 2017 and August 31, 2020. The exclusion criteria were as follows (1): congenital uterine malformations, such as unicornuate, bicornuate, didelphys, or septate uteri as diagnosed by hysteroscopy or laparoscopy (2); patients with recurrent pregnancy loss; and (3) missing key data on the day of ovulation or FET. Finally, 2562 cycles in 2054 couples were included for analysis. The causes of infertility were divided into tubal factors, endometriosis, abnormal ovulation, male factors, and unexplained.

## Treatment Procedures

All participants were treated according to routine ART procedures, and controlled ovarian hyperstimulation (COH) and oocyte retrieval have been described widely in the literature. Recombinant follicle stimulating hormone (Gonal-F; Merck-Serono) and human menopausal gonadotropin were used for COH. Protocols were selected according to the patient's documented ovarian reserve. These included an early follicular phase long-acting gonadotropin-releasing hormone (GnRH) agonist long protocol, a GnRH antagonist protocol, mid-luteal-phase short-acting GnRH-agonist long protocol, natural protocol, mild stimulation protocol, and progestin-primed ovarian stimulation. The final maturation of oocytes was induced by 5,000–10,000 IU of human chorionic gonadotropin (hCG), and oocytes were retrieved transvaginally at 36 h. Embryos were evaluated for quality at the time of FET according to the Istanbul embryo assessment consensus (8). Laboratory procedures and conditions were unchanged during this period.

## Endometrial Preparation Protocol

FET was performed in patients with a normal uterine cavity guided by ultrasonography. If endometrial or pelvic abnormalities were suspected, such as intrauterine adhesions, endometrial polyps, submucosal fibroids, uterine fluid or Fallopian tube effusion, appropriate diagnosis and treatment by hysteroscopy or laparoscopy were required from 1 month to 6 months before FET. This was performed using natural cycle (NC) or hormone replacement therapy (HRT). The NC approach was used for patients with normal ovulation, and HRT was applied according to each patient's requirement, particularly for those with irregular menstrual cycles.

## Natural Cycle

Follicle sizes and the EMT were measured using transvaginal ultrasonography (TVU) every other day or daily from day 10 of the menstrual cycle. When the mean diameter of the dominant follicle was > 17 mm, the decision to trigger ovulation with 10,000 IU of hCG was made, based on serum hormone levels. The day of ovulation was defined as D0, and vaginal progesterone gel was administered until 10 weeks of gestation if the woman was pregnant. Cleavage stage embryos and blastocysts were transferred on days 3 and 5, respectively.

## Hormone Replacement Therapy

Serum basal hormone levels and uterine ultrasonography were checked on days 2 or 3 of the menstrual cycle. If the results were normal, oral estradiol valerate at 4 mg/day was started and increased to 6 mg/day on day 6. After 10 days of treatment, the endometrium was examined by TVU. If the EMT was  $\geq 8$  mm and there was no sign of a dominant follicle or ovulation, the same dose of estradiol was continued for 4 days, followed by ultrasonographic measurement of EMT on the same day. If the EMT was < 8 mm, the dose of estradiol was increased by vaginal or oral routes, and the EMT was monitored every other day. Estrogen therapy was not continued for more than 21 days for

intimal transformation. Progesterone administration was started on D0 with oral dydrogesterone 10 mg twice daily, and vaginal gel (Crinone) was applied at 90 mg per day. The use of estradiol was tapered off when the patient had a positive hCG test. The use of Crinone vaginal gel continued until a fetal heartbeat was detected by TVU, and dydrogesterone was stopped at pregnancy day 10.

## Gonadotropin-Releasing Hormone Agonist Combined with Hormone Replacement Therapy (GnRHa-HRT)

GnRHa (3.75 mg) was injected during the menstrual period, and the process was similar to the above HRT cycle after 1 month.

## Measurement of EMT

EMT was measured uniformly using TVU performed by three trained reproductive specialists with a standardized protocol. They each had more than 10 years of experience using the same protocol. On the day of ovulation or the day of progesterone administration, EMT was defined as the thickest echogenic area from one stratum basalis endometrial interface across the endometrial canal to the other stratum basalis interface (9). The measurement accuracy was 0.1 mm and was recorded in our database. Most patients will have multiple uterine contractions every minute, and these can cause changes in the EMT of up to 3–4 mm. To ensure the most accurate measurement, protocol was followed strictly, waiting for the wave to pass before measurement. In our center, routine TVU of the endometrium is performed at least twice before embryo transfer, and patients with thin endometrium will be subjected to increased frequency of detection and drug treatment are used i to achieve the ideal endometrial status. The ultrasound equipment and measurement technology in our center was of good quality to reduce errors in EMT measurement values.

## Embryo Transfer

FET was performed under abdominal ultrasound guidance with a Cook catheter according to standard operating procedures. According to the specifications of our center, one or two embryos (occasionally three) were transferred into the uterine cavity. All frozen embryos were thawed on the day of transfer.

## Outcome Measures

The primary outcome was the ongoing pregnancy rate (OPR), defined as a live fetus identified by ultrasound on day 12. The secondary outcome was the CPR, defined as an intrauterine gestational sac with a fetal heart rate detected by ultrasonography 2–3 weeks after a positive hCG pregnancy test.

## Statistical Methods

Continuous variables are expressed as the mean  $\pm$  standard deviation (SD). Categorical variables are expressed as the frequency or percentage. Differences among groups were compared using single-factor analysis of variance and chi-square tests. Variables in skewed distribution were tested using the Kruskal–Wallis method. A smoothing function was applied to the EMT and OPR data. The turning point was determined and

provided the maximum likelihood model. The confounders were controlled for in two different models. Model 1 was adjusted for the following potential confounders: maternal age, endometrial preparation protocol, duration of infertility, number of good quality embryos transferred. Model 2 was further adjusted for the following covariables associated with OPR: maternal age, number of FET cycles, stage of transferred embryos.

Data analyses were conducted on three levels (1): correlation between EMT and OPR (linear or non-linear) (**Figure 2**) (2); factors that might affect the correlation between EMT and OPR (3); correlation between EMT and OPR after adjustment for interfering factors or after stratified analysis. We applied a generalized additive model (GAM) to estimate the independent relationship of association between EMT and OPR (**Tables 3, 4**). A smoothing linear regression GAM model was adjusted using R software to test any association between EMT and OPR (10, 11). The results are displayed graphically. The statistical software package R (<http://www.R-project.org>, the R Foundation) and Empower (R) ([www.empowerstats.com](http://www.empowerstats.com), X&Y Solutions, Inc. Boston, MA, USA) were used for data analyses. Two-way P values  $< 0.05$  indicated statistical significance. Results are presented as the odds ratio (OR) and 95% confidence interval (CI).

## RESULTS

### Patient Baseline Data

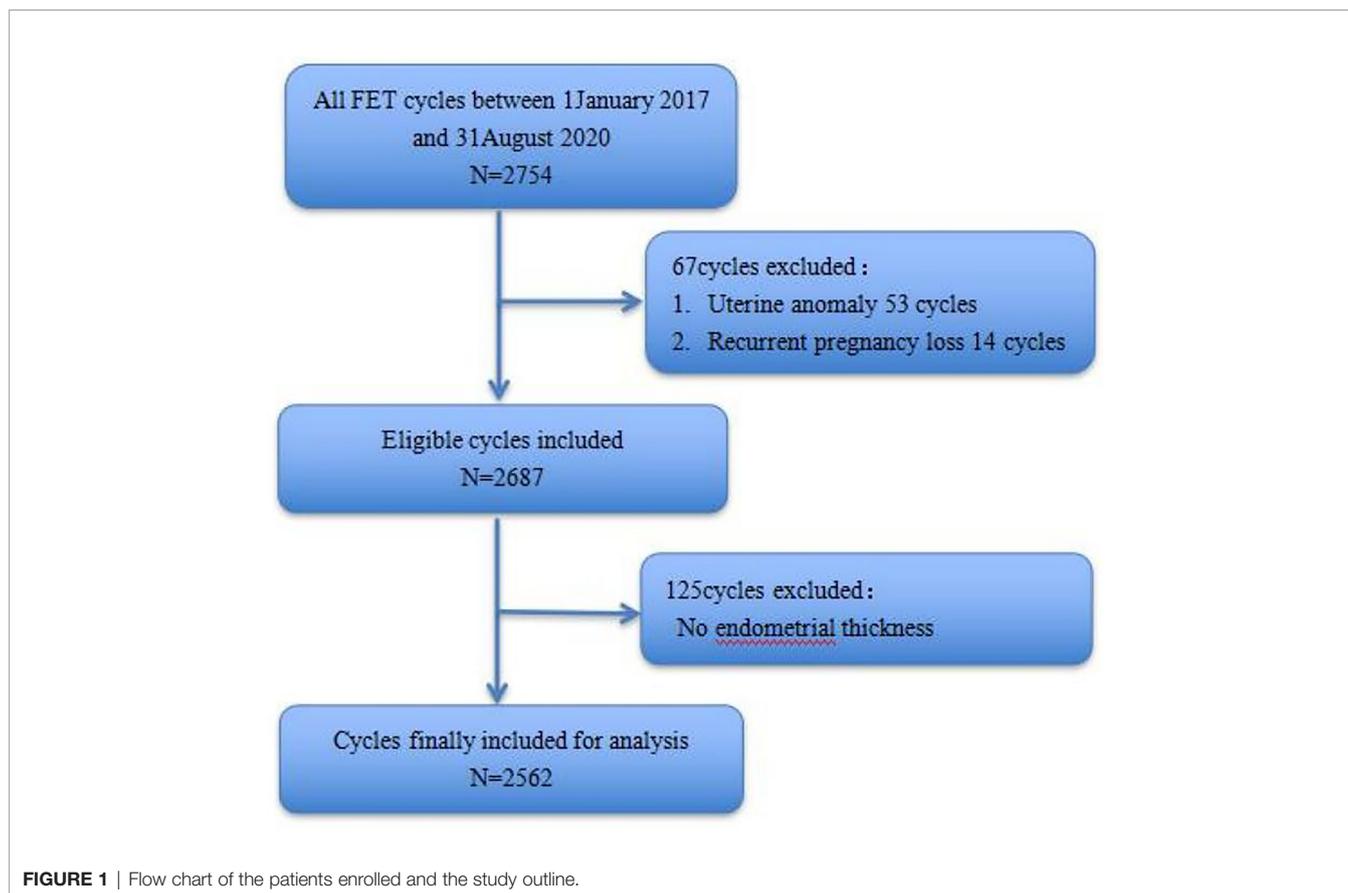
In all, 2754 FET cycles were recorded in our database and 192 cycles were excluded according to the criteria listed above (**Figure 1**). Finally, we included 2562 cycles in 2054 couples for analysis. For women aged  $< 35$  years, there were 1531 cycles (59.76% of the total), and for women aged  $\geq 35$  years, there were 1031 cycles (40.24%). They were grouped according to EMT with a turning point of 7.7 mm. For the group with  $EMT \leq 8$  mm, there were 522 cycles (20.37%), and for those with  $EMT > 8$  mm, there were 2040 cycles (79.63%). For these two groups, the CPRs were 49.62% (259/522) and 55.59% (1134/2040), and the OPRs were 40.8% (213/522) and 47.21% (963/2040), respectively (**Table 1**).

### Relationship Between EMT and Ongoing Pregnancy Rate

After adjusting for confounders such as age, duration of infertility, number of high-quality embryos transferred, endometrial preparation protocol, number of transferred cycles, and stage of embryo transfer, EMT was analyzed by curve fitting with the OPR according to age groups.

### Results for Women Aged $< 35$ Years

A nonlinear relationship was found between EMT and OPR in women aged  $< 35$  years. The turning point of the EMT was 7.7 mm (**Table 2**). For women with an EMTs  $< 7.7$  mm, the OPR increased significantly by 124% when the EMT increased by 1 mm, (OR 2.24; 95% CI 1.57–3.19;  $P < 0.0001$ ). Further sensitivity analysis was performed. Grouping by cleavage stage embryos or blastocysts and adjusting for potential confounders that may be associated with OPR by Models I and II in multiple



regression analysis showed that for  $EMT \leq 8$  mm, transfer of cleavage stage embryos and blastocysts and an increase in EMT of 1 mm, the OPR increased by 150% and 97%, respectively (OR 2.5; 95% CI 1.11–5.62;  $P = 0.026$ ; and OR 1.97 95% CI 1.29–3.00;  $P = 0.0016$ , respectively; **Table 3**).

When the EMT was  $> 7.7$  mm, there was a 0.06% decrease in OPR for every 1 mm increase in the EMT (**Table 2**), but EMT was not significantly correlated with the OPR (OR 0.94; 95% CI 0.87–1.01;  $P = 0.0904$ ).

### Results for Women Aged $\geq 35$ years

In women aged  $\geq 35$  years with a total of 1031 cycles (530 with blastocysts and 501 with cleavage-stage embryos), the EMT was correlated linearly with the OPR. When blastocysts were transferred, for every 1 mm increase in the EMT the OPR increased significantly by 12% (OR 1.12; 95% CI 1.01–1.24;  $P = 0.0336$ ). However, when cleavage stage embryos were transferred, changes in the EMT and OPR were not significantly correlated (OR 1.02; 95% CI 0.91–1.14;  $P = 0.7354$ ; **Table 4**).

## DISCUSSION

Our study is the first to reveal that EMT is associated with OPR in only some FET cycles. It explains the conflicting results of

previous studies regarding EMT as a marker of endometrial receptivity and as a predictor of the success of ART treatment.

The largest study (12) to date on EMT and pregnancy outcomes, including 20,114 FET cycles, suggested that the CPR and live birth rates decreased when endometrial thickness was less than 7 mm. The study included all women undergoing FET, but did not exclude the role of other confounding factors besides EMT that could have had an impact on pregnancy outcome.

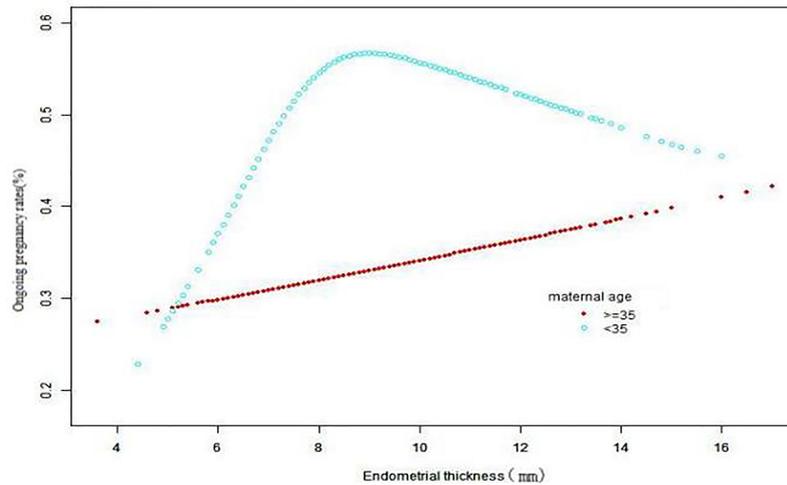
It is well known that the woman's age is a negative factor affecting pregnancy outcomes after FET. One study (13) found that the duration of infertility, EMT before frozen embryo transfer, and the number of embryos transferred were three independent predictors of live birth rate after cleavage stage frozen embryo transfer in young women. In addition, Vaegter et al. (14) showed that a woman's age was a predictor of live birth after ART. Maternal age was similarly shown to be a factor influencing OPR after FET in our study. Therefore, here the confounding factors including woman's age, height, duration of infertility, embryo transfer protocol, numbers of high-quality embryos transferred, and numbers of FET cycles were determined by screening and by adjusting the variables individually.

After adjusting for confounders, our study showed a curvilinear relationship between EMT and OPR in young women ( $< 35$  years old). The cut-off point of the EMT was

**TABLE 1** | Baseline characteristics of the participants.

| Endometrial thickness                   | < = 8         | >8            | Standardize diff.  | P-value |
|---|---------------|---------------|--------------------|---------|
| No. of participants                     | 522           | 2040          |                    |         |
| maternal age (years)                    | 34.61 ± 4.93  | 33.68 ± 4.73  | 0.19 (0.10, 0.29)  | <0.001  |
| paternal age (years)                    | 36.45 ± 5.51  | 35.70 ± 5.34  | 0.14 (0.04, 0.24)  | 0.004   |
| Duration of infertility (years)         | 3.86 ± 2.84   | 4.12 ± 2.70   | 0.09 (-0.00, 0.19) | 0.003   |
| gravidity                               | 1.15 ± 1.34   | 0.75 ± 1.09   | 0.32 (0.23, 0.42)  | <0.001  |
| parity                                  | 0.11 ± 0.33   | 0.10 ± 0.34   | 0.02 (-0.07, 0.12) | 0.624   |
| maternal BMI (kg/m <sup>2</sup> )       | 22.67 ± 2.98  | 22.96 ± 3.15  | 0.09 (-0.00, 0.19) | 0.060   |
| height of maternal (mm)                 | 161.60 ± 4.50 | 162.17 ± 5.07 | 0.12 (0.02, 0.22)  | 0.019   |
| weight of maternal (kg)                 | 59.28 ± 8.51  | 60.44 ± 9.06  | 0.13 (0.04, 0.23)  | 0.008   |
| height of paternal (mm)                 | 174.47 ± 5.36 | 174.26 ± 5.53 | 0.04 (-0.06, 0.14) | 0.437   |
| weight of paternal (kg)                 | 78.16 ± 11.77 | 78.42 ± 12.07 | 0.02 (-0.08, 0.12) | 0.663   |
| paternal BMI (kg/m <sup>2</sup> )       | 25.63 ± 3.36  | 25.76 ± 3.41  | 0.04 (-0.06, 0.14) | 0.426   |
| No. of good quality embryos transferred | 1.43 ± 0.59   | 1.36 ± 0.65   | 0.10 (0.01, 0.20)  | 0.093   |
| No. of embryos transferred              | 1.69 ± 0.48   | 1.69 ± 0.48   | 0.01 (-0.09, 0.10) | 0.887   |
| EMT (mm)                                | 7.21 ± 0.79   | 10.16 ± 1.43  | 2.55 (2.43, 2.67)  | <0.001  |
| No. of transferred cycles               | 1.43 ± 0.95   | 1.30 ± 0.72   | 0.15 (0.06, 0.25)  | <0.001  |
| endometrial preparation protocol        |               |               | 0.31 (0.22, 0.41)  | <0.001  |
| No. of natural cycle                    | 122 (23.46%)  | 335 (16.59%)  |                    |         |
| No. of HRT cycle                        | 63 (12.12%)   | 114 (5.65%)   |                    |         |
| No. of GnRHa-HRT cycle                  | 335 (64.42%)  | 1570 (77.76%) |                    |         |
| Type of infertility                     |               |               | 0.26 (0.16, 0.36)  | <0.001  |
| No. of primary infertility              | 230 (44.06%)  | 1161 (56.91%) |                    |         |
| No. of secondary infertility            | 292 (55.94%)  | 879 (43.09%)  |                    |         |
| tubal factor infertility                |               |               | 0.01 (-0.09, 0.10) | 0.883   |
| 0                                       | 220 (42.23%)  | 867 (42.58%)  |                    |         |
| 1                                       | 301 (57.77%)  | 1169 (57.42%) |                    |         |
| endometriosis                           |               |               | 0.08 (-0.01, 0.18) | 0.070   |
| 0                                       | 489 (93.86%)  | 1950 (95.73%) |                    |         |
| 1                                       | 32 (6.14%)    | 87 (4.27%)    |                    |         |
| anovulation                             |               |               | 0.10 (0.01, 0.20)  | 0.034   |
| 0                                       | 333 (63.92%)  | 1401 (68.78%) |                    |         |
| 1                                       | 188 (36.08%)  | 636 (31.22%)  |                    |         |
| male factor infertility                 |               |               | 0.02 (-0.07, 0.12) | 0.638   |
| 0                                       | 395 (75.82%)  | 1524 (74.82%) |                    |         |
| 1                                       | 126 (24.18%)  | 513 (25.18%)  |                    |         |
| unexplained                             |               |               | 0.10 (0.01, 0.20)  | 0.043   |
| 0                                       | 494 (94.82%)  | 1879 (92.24%) |                    |         |
| 1                                       | 27 (5.18%)    | 158 (7.76%)   |                    |         |
| Stimulation protocol                    |               |               | 0.16 (0.06, 0.25)  | 0.103   |
| Natural protocol                        | 0 (0.00%)     | 7 (0.34%)     |                    |         |
| EFL protocol                            | 282 (54.02%)  | 1222 (59.96%) |                    |         |
| GnRH antagonist                         | 79 (15.13%)   | 259 (12.71%)  |                    |         |
| MLSL protocol                           | 39 (7.47%)    | 127 (6.23%)   |                    |         |
| PPOS                                    | 113 (21.65%)  | 400 (19.63%)  |                    |         |
| mild stimulation protocol               | 9 (1.72%)     | 23 (1.13%)    |                    |         |
| Fertilization method                    |               |               | 0.10 (0.01, 0.20)  | 0.038   |
| IVF                                     | 379 (72.61%)  | 1385 (67.89%) |                    |         |
| ICSI                                    | 143 (27.39%)  | 655 (32.11%)  |                    |         |
| stage of embryo transferred             |               |               | 0.05 (-0.04, 0.15) | 0.283   |
| cleavage                                | 200 (38.31%)  | 730 (35.78%)  |                    |         |
| blastocyst                              | 322 (61.69%)  | 1310 (64.22%) |                    |         |
| Clinical pregnancy rates                |               |               | 0.12 (0.02, 0.22)  | 0.015   |
| 0                                       | 263 (50.38%)  | 906 (44.41%)  |                    |         |
| 1                                       | 259 (49.62%)  | 1134 (55.59%) |                    |         |
| maternal age                            |               |               | 0.16 (0.06, 0.25)  | 0.001   |
| >=35                                    | 242 (46.36%)  | 789 (38.68%)  |                    |         |
| <35                                     | 280 (53.64%)  | 1251 (61.32%) |                    |         |

EMT, endometrial thickness; BMI, body mass index; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; FET, frozen-thawed embryo transfer; EFL, early-follicular-phase long-acting GnRH-agonist long protocol; MLSL, mid-luteal-phase short-acting GnRH-agonist long protocol; PPOS, Progesterin-primed ovarian stimulation; HRT, hormone replacement therapy; GnRHa-HRT, gonadotropin-releasing hormone agonist combined with hormone replacement therapy. Values are presented as the mean ± SD or number and (%).



**FIGURE 2** | The association between EMT and OPR in two groups of women. A nonlinear relationship for women <math>< 35</math> years and a linear relationship for women <math>\ge 35</math> years were detected after adjusting for endometrial preparation protocol, duration of infertility, maternal height, number of good-quality embryos transferred, number of transferred cycles, and stage of embryo transferred.

7.7 mm; thus, when the EMT was <math>< 7.7</math> mm, the OPR increased by 124% when EMT increased by 1 mm. When the EMT was > 7.7 mm, there was no significant correlation between EMT and OPR.

In older women (age  $\ge 35$  years), we found a linear relationship between EMT and OPR. When frozen blastocysts

were transferred, EMT was positively and significantly correlated with OPR, which increased by 12% for every 1 mm increase in EMT; however, there was no significant positive relationship between EMT and OPR after frozen cleavage stage embryos were transferred. This is mainly because it is the euploidy of embryos rather than EMT that determines their OPR after frozen cleavage

**TABLE 2** | Threshold effect analysis of EMT and OPR using linear regression analysis in women <math>< 35</math> years of age.

| Inflection point of EMT | Effect size ( $\beta$ ) | 95%CI        | P value               |
|-------------------------|-------------------------|--------------|-----------------------|
| <math>< 7.7</math>      | 2.24                    | 1.57 to 3.19 | <math>< 0.0001</math> |
| <math>\ge 7.7</math>    | 0.94                    | 0.87 to 1.01 | 0.0904                |

Effect: OPR Cause: EMT.

Adjusted for maternal age, maternal height, duration of infertility, number of good-quality embryos transferred, endometrial preparation protocol, number of transferred cycles, stage of embryo transferred.

**TABLE 3** | Relationship between EMT and OPR in the two different models for women aged <math>< 35</math> years or with EMT  $\le 8$  mm.

| Stage Of Embryo Transferred | Non-adjusted                            | Adjust I                                | Adjust II                |
|-----------------------------|---|---|--------------------------|
| cleavage                    | 2.05 (1.00, 4.19) 0.0492                | 2.35 (1.07, 5.14) 0.0332                | 2.50 (1.11, 5.62) 0.0266 |
| blastocyst                  | 1.97 (1.33, 2.93) 0.0007                | 2.00 (1.32, 3.02) 0.0011                | 1.97 (1.29, 3.00) 0.0016 |
| Total                       | 1.99 (1.41, 2.81) <math>< 0.0001</math> | 2.06 (1.43, 2.95) <math>< 0.0001</math> | 2.05 (1.42, 2.95) 0.0001 |

$\beta$  value (95% CI) P value / OR (95%CI) P value. Non-adjusted model.

Model I was adjusted for maternal age; endometrial preparation for FET; duration of infertility; and the number of good-quality embryos transferred.

Model II was adjusted for maternal age; maternal height; duration of infertility; number of good-quality embryos transferred; endometrial preparation protocol; and number of FET cycles.

**TABLE 4** | Relationship between EMT and OPR in women  $\ge 35$  years of age in two different models.

| Stage of Embryo Transferred | Non-adjusted             | Adjust I                 | Adjust II                |
|-----------------------------|--------------------------|--------------------------|--------------------------|
| cleavage                    | 1.03 (0.93, 1.15) 0.5237 | 1.04 (0.93, 1.15) 0.5187 | 1.02 (0.91, 1.14) 0.7354 |
| blastocyst                  | 1.08 (0.99, 1.19) 0.0949 | 1.11 (1.00, 1.23) 0.0404 | 1.12 (1.01, 1.24) 0.0336 |
| Total                       | 1.06 (0.99, 1.14) 0.0947 | 1.07 (0.99, 1.15) 0.0795 | 1.06 (0.99, 1.14) 0.1018 |

stage embryos are used for FET in such women. Maternal age is the most important factor for the success of *in vitro* fertilization (15), and this is associated with embryo euploidy. Another study (16) also found that constant increases in chromosomal abnormalities with maternal age in frozen cleavage-stage ET. However, the chromosomal abnormality rates of the patients aged <35 years and those aged  $\geq 35$  years who underwent frozen blastocyst transfers were comparable. The inclusion of 13 RCT studies (17) found low quality evidence of live births and moderate quality evidence of clinical pregnancies suggesting that fresh blastocyst stage transfers have a higher rate than fresh cleavage stage transfers. A recent study (18) found that the EMT did not predict live birth in either fresh or FET cycles. A total of 287 FET cycles were included in the study, and the EMT values were 8.4 mm (range 7.4–9.7) and 9 mm (8, 19, 20) mm in women with and without live births, respectively ( $P = 0.38$ ). Only 11.8% (66/560) of the study population had an EMT < 7 mm. However, the number of patients with inadequate endometrium in that study was small and this might have caused bias. A total of 522 cycles (20.37%) with EMT  $\leq 8$  mm were included in our study (**Table 1**). It is important to note in order to reduce bias, sensitivity analyses were conducted compared with prior published studies. Our study showed that the OPR increased significantly with increased EMT between young women aged < 35 years with EMT  $\leq 8$  mm and older women who underwent transfer of blastocysts.

Multiple studies have shown that among women undergoing embryo transfer, the actual numbers of those with insufficient endometrium are underestimated, because clinicians or patients may be more reluctant to perform embryo transfer, and cancelled cycles were not included. This group of women should receive more attention in ART clinics because they will tend to have more cycles cancelled or require multiple cycles of treatment. This will prolong the time to achieve a live birth and will involve more economic burden. Our study will provide guidance for clinical practice, showing that specific populations with insufficient endometrial thickness will have significantly improved pregnancy outcomes if the EMT can be increased.

## Advantages and Limitations

Through sensitivity analysis, this study has revealed for the first time why there have been conflicting results between EMT and pregnancy outcomes in previous studies. Thus, in these FET cycles, EMT was significantly associated with the OPR only in young women with an EMT  $\leq 8$  mm and older women who underwent transfer of blastocysts. This study provides a reference for clinical transplantation strategies and better counseling and advice for patients.

This study was limited by its retrospective design, and the mechanisms underlying these associations need to be confirmed by prospective studies with larger samples. However, such prospective studies are likely to be difficult to implement in ART clinics because clinicians and patients are reluctant to perform FET when the endometrium is insufficient. This study was conducted in FET cycles, and it is unclear whether it is

applicable to fresh embryo transfer cycles. Moreover, the EMT on the day of FET was not recorded in this study, as transabdominal ultrasound measurements would have been required on the day of transplantation and these are not as accurate as TVU.

## Key Message

Through sensitivity analysis, this study has revealed for the first time why there have been conflicting results between EMT and pregnancy outcomes in previous studies. Thus, in these FET cycles, EMT was significantly associated with the OPR only in young women with an EMT  $\leq 8$  mm and older women who underwent transfer of blastocysts. This study provides a reference for clinical transplantation strategies and better counseling and advice for patients.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author/s.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of First Affiliated Hospital of Xinjiang Medical University. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

HT, XL, and LC were responsible for the concept and study design. HZ contributed to statistical analysis. LC and XL contributed to revising the article. HT, HZ, HQ, and XY contributed to data collection. HT drafted the manuscript. All authors contributed to the critical discussion of the data and final editing of the manuscript. All authors read and approved the final manuscript.

## FUNDING

This work was financially sponsored by Natural Science Foundation of Xinjiang (NO. 2021D01C296).

## ACKNOWLEDGMENTS

We thank the staff from the First Affiliated Hospital of Xinjiang Medical University and Xi'an Jiaotong University for their assistance. We thank all participants in this study.

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