Endometrial thickness changes after progesterone administration do not affect the pregnancy outcomes of frozen-thawed euploid blastocyst transfer: a retrospective cohort study

Ziqi Jin, M.M., Hao Shi, Ph.D., Manman Lu, Ph.D., Zhiqin Bu, Ph.D., Mingzhu Huo, M.M., and Yile Zhang, M.D.

Reproductive Medical Center, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, People's Republic of China

Objective: To evaluate whether the change in endometrial thickness from progesterone administration day to transfer day is related to pregnancy outcomes in single frozen-thawed euploid blastocyst transfer cycles.
Design: Observational cohort study.
Setting: Single reproductive medical center.
Patient(s): All patients were transferred with a single biopsied euploid blastocyst, and their endometrium was prepared with hormone replacement therapy (HRT).
Intervention(s): The endometrial thickness on the day of blastocyst transfer and progesterone administration was measured by transvaginal ultrasound, and the difference between them and the change ratio were calculated.
Main Outcome Measure(s): Clinical pregnancy rates and live birth rates.
Result(s): Endometrial ultrasound images of 508 euploid blastocyst transfer cycles using HRT were evaluated by transvaginal ultrasound. Overall, pregnancy outcomes were comparable in different groups of endometrial thickness changes. The results of multiple logistic regression showed that the clinical pregnancy rate and live birth rate did not significantly increase with the increase in endometrial thickness change ratios (per 10%) in the fully adjusted model as a continuous variable. In the adjustment model as a categorical variable, there was no statistical difference in pregnancy outcomes among the groups with changes in endometrial thickness. Interaction analysis showed that after adjusting for confounders, there was no statistically significant interaction between the endometrial thickness change ratio and pregnancy outcomes in all subgroups.
Conclusion(s): In the euploid blastocyst transfer cycle of preparing the endometrium with HRT, the endometrial thickness change ratio after progesterone administration was not related to pregnancy outcomes. (Fertil Steril® 2021;116:1502-12. ©2021 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Endometrial thickness change ratio, euploid blastocyst transfer, pregnancy outcomes, hormone replacement therapy, transvaginal ultrasound

Endometrial receptivity refers to the state of the uterus as it relates to allowing blastocysts with synchronous development adhesion and implantation under the action of ovarian steroid hormones in a specific period: midsecretory phase (usually in the 20th–24th day of the menstrual cycle) (1). This period is called the "window of implantation", which determines the appropriate time for transfer (2). The implantation of normal embryos into the receptive endometrium is the key to pregnancy. In recent years, with the continuous development of preimplantation genetic testing (PGT) technology and the optimization of embryo laboratory technology, it has become possible to obtain euploid

Received June 11, 2021; revised July 22, 2021; accepted August 5, 2021; published online September 15, 2021.
Z.J. has nothing to disclose. H.S. has nothing to disclose. M.L. has nothing to disclose. Z.B. has nothing to disclose. M.H. has nothing to disclose. Y. Z. has nothing to disclose.
Supported by the National Natural Science Foundation of China, China (grant 31970799).
Reprint requests: Yile Zhang, M.D., Reproductive Medical Center, the First Affiliated Hospital of Zhengzhou University, No.1 of Jianshe East Road, Zhengzhou 450052, Henan Province, People's Republic of China (E-mail: luna020996@126.com).

Fertility and Sterility® Vol. 116, No. 6, December 2021 0015-0282

https://doi.org/10.1016/j.fertnstert.2021.08.008

embryos (3). Although embryo quality has greatly improved, endometrial receptivity disorders still lead to approximately two-thirds of implantation failures, which has become a prominent problem affecting the success of pregnancy (4). Therefore, it is particularly important to accurately identify the window of implantation of the endometrium.

Previous studies suggested that transvaginal ultrasound examination of endometrial thickness can evaluate the development of the endometrium more accurately than invasive biopsy techniques, and it has become a routine examination method for evaluating the endometrium and an index for predicting endometrial receptivity (5, 6). However, no consensus has been reached on the relationship between endometrial thickness and pregnancy outcomes. Some scholars believe that when the endometrial thickness is <7 mm or 8 mm before ovulation, the cycle should be canceled because of the low clinical pregnancy rate and the high miscarriage rate (7–9). Some studies have shown that the endometrial thickness at the end of proliferation ranges from 9 to 14 mm to achieve the best implantation rate and clinical pregnancy rate (10). However, some studies have confirmed that endometrial thickness has nothing to do with clinical outcomes and cannot predict pregnancy outcomes (11–15). The timing of endometrial monitoring in these studies was different, and most of the studies focused on preovulation (human chorionic gonadotropin trigger day) in fresh cycles or at the end of the proliferation period in frozen-thawed embryo transfer (FET) cycles, which made comparisons between studies difficult.

Compared with the endometrium before ovulation and at the end of proliferation, the endometrium on the day of embryo transfer is usually considered to be in the window of implantation, which represents endometrial receptivity. Therefore, it is necessary to evaluate the endometrium on the day of transfer. On the other hand, in the FET cycles, the endometrium changes because of a series of secretion changes induced by progesterone after progesterone administration. Therefore, we believe that it is more meaningful to study the changes in endometrial thickness after progesterone administration than to study only the absolute value of endometrial thickness during a certain period of the cycle for predicting pregnancy outcomes. At present, there are few studies on the effect of endometrial thickness changes on pregnancy outcome after progesterone administration, and their conclusions are contradictory (16–20).

The purpose of this study was to explore the influence of endometrial thickness changes on pregnancy outcomes from the progesterone administration day to the blastocyst transfer day in FET cycles. Our study included women who were treated with preimplantation genetic diagnosis and selected biopsied euploid blastocysts for transfer to eliminate the abnormal ploidy of embryos and to ensure the quality of embryos. Considering the great differences between different endometrial preparation protocols, we chose the same hormone replacement therapy (HRT) cycle using exogenous estrogen and progesterone to reduce the factors that may affect the endometrium in each cycle and to ensure consistency in the study.

MATERIALS AND METHODS

Study Design and Population

Because chromosome translocation and monogenic diseases are the main diseases treated by preimplantation genetic diagnosis in our center, this retrospective cohort study included women who received PGT for chromosomal structural rearrangements or PGT for monogenic/single gene defects and conducted their first single FET, for which the endometrium was prepared by HRT in the Reproductive Medical Center of the First Affiliated Hospital of Zhengzhou University between January 2014 and December 2019. All patients were followed up for at least 1 year. The research data were extracted from the Clinical Reproductive Medicine Management System/Electronic Medical Record Cohort Database of our reproductive medicine center. This study was approved by the institutional review board and ethics committee of the First Affiliated Hospital of Zhengzhou University. Informed consent was waived because of the retrospective nature of the study.

Briefly, this study recruited 602 patients with monogenic disease or chromosome translocation who received the first single FET for which HRT was used to prepare the endometrium between January 2014 and December 2019. Patients were excluded if any of the following criteria were present: gonadotropin-releasing hormone agonist was used in advance for down-regulation because of a thinner endometrium in controlled ovarian stimulation cycles; routine hysteroscopy before preimplantation genetic diagnosis treatment showed the presence of uterine pathology; endometriosis; a thinner endometrial thickness (<7 mm on progesterone administration day); or missing endometrial data. The obtained resulting cohort included 508 participants for the final analysis.

Ovarian Stimulation and Laboratory Protocols

In fresh cycles, all patients used the same controlled ovarian stimulation protocol for ovulation induction: the midluteal, short-acting, gonadotropin-releasing hormone agonist long protocol. Intracytoplasmic sperm injection was performed 4–6 hours after oocyte retrieval. The formed embryos were cultured to the blastocyst stage in Vitrolife (Goteborg, Sweden) sequential medium. The details of the protocol, the operating method of intracytoplasmic sperm injection, and the culture process have been published elsewhere (21). On the fifth or sixth morning after the oocyte retrieval, experienced embryologists scored the blastocysts according to Gardner and Schoolcraft's system and selected blastocysts of 3BC grade and above. Additionally, 3–5 trophoblast cells were biopsied by the laser method. Whole genome amplification of the trophoblast cells was performed by multiple displacement amplification according to the standard protocol provided by the QIAGEN REPLI-g Single Cell kit, for which the specific steps were described in our previous study (22). The biopsied blastocysts were vitrified and preserved in liquid nitrogen.

Endometrial Preparation and Endometrial Thickness Assessment

All patients were treated with HRT for endometrial preparation. Estradiol (2 mg [Progynova]; Bayer, Leverkusenbaer, Germany) was taken twice a day from the third day of the menstrual cycle. The oral dose was adjusted every 4 days according to endometrial thickness. After 12–14 days, the endometrium was monitored by transvaginal ultrasound, and the serum progesterone level was measured. When the thickness of the endometrium reached $\geq$7 mm and the level of serum progesterone was $<$1 ng/mL, 60 mg of intramuscular progesterone and 10 mg (changed to 20 mg after 2 days) of oral dydrogesterone were added to the protocol. Blastocyst transfer was performed on the sixth day of progesterone administration (the first day of progesterone administration was diarized as D1, and blastocyst transfer was performed on D6). From the transfer day, 90-mg vaginal progesterone gel (Crinone 8%; Merck Serono, Switzerland), 20-mg oral dydrogesterone (Duphaston; Abbott, Netherlands), and 6-mg estradiol valerate were provided daily. Transvaginal ultrasound was performed 5 weeks after blastocyst transfer to clinically confirm the pregnancy.

On the day of progesterone administration and blastocyst transfer, 2 ultrasound technicians used the Voluson S8 and ALOKA (α-6) color Doppler transvaginal ultrasound diagnostic instruments to monitor the endometrium. The ultrasound technicians presented the best images of longitudinal cross-sections of the endometrium through the center of the uterus, including the cervical canal. After freezing the images, the electronic caliper built into the software was used to measure the endometrial thickness from the endometrium-myometrium junction on one side of the endometrial cavity to that on the other side of the endometrial cavity and to input the data into the Electronic Data Capture system (23). Finally, the images were collected and permanently saved using the VisionPACS image archiving and transmission system.

To minimize the potential bias of the measurement results, 2 of the investigators (H.S. and M.L.) who were blinded to the pregnancy outcome independently confirmed the selected images and verified the measurement of endometrial thickness before the research was performed to ensure the accuracy of the measurement. If there were any discrepancies between the verified results, another investigator (Y.Z.) was invited to make the final decision.

According to the difference in endometrial thickness between the blastocyst transfer day and the progesterone administration day, the patients were divided in 3 groups: the endometrial thickness decreased group, increased group, and no change group. The change ratio of endometrial thickness was defined as the difference between the endometrial thickness on the blastocyst transfer day and the progesterone administration day divided by the endometrial thickness on the progesterone administration day.

Definition of Clinical Outcomes

Clinical outcomes were defined through the consensus reached by the American Society for Reproductive Medicine in 2017 (24). Clinical pregnancy was defined as 1 or more gestational sacs detected by ultrasound. Live birth was defined as the delivery of at least 1 live baby after 22 weeks of gestation. Miscarriage was defined as a spontaneous abortion before 22 weeks of intrauterine gestation.

Statistical Analysis

We described the baseline characteristics of the patients. For continuous variables, the data were represented by the mean $\pm$ standard deviation (normal distribution) or the median (interquartile range) (skewed distribution); Student's t test or analysis of variance, Wilcoxon's rank-sum test, and Kruskal-Wallis test were used appropriately. For categorical variables, the data were expressed as frequencies or percentages, and the chi-square test was used for comparison.

Univariate logistic analysis was used to evaluate the effect of various variables on pregnancy outcomes. Multivariate logistic regression analysis used the unadjusted and multivariable-adjusted models to calculate the crude odds ratios (ORs) and adjusted ORs of 95% confidence intervals (CI). The relationship between the change ratio of endometrial thickness and pregnancy outcomes was analyzed. If the covariates were significantly related to clinical outcomes, affected the estimated value of pregnancy outcome by $\geq$10% by endometrial thickness change ratio, or based on recently published studies and clinical experience, they were included in the final model as potential confounding factors. In the adjusted model I, the adjusted variables included female age, body mass index (BMI), infertility duration, and infertility type. In the adjusted model II, the adjusted variables included all covariables in model I plus gravidity, parity, and basal serum follicle-stimulating hormone. In the adjusted model III, the adjusted variables included all covariables in model II plus morphology score, day of embryo development at transfer, and genetic category. The trend test used a linear regression method, and the median of 3 endometrial thickness change groups (decreased group, no change group, and increased group) were inputted into the models as continuous variables.

Subgroup analyses were conducted by female age, BMI, genetic type, morphology score, and the day of embryo development at transfer using stratified logistic regression models with adjustment for confounders. The interaction between subgroups was determined by the likelihood ratio test.

Considering that the slight difference between the measurement values of endometrial thickness on the day of progesterone administration and the day of transfer could be because of person-to-person variation or the measurement error, we conducted sensitivity analyses. Regrouped according to the endometrial thickness change ratio, the decreased group was redefined as the endometrial thickness on the transfer day $\geq$5% less than that on the progesterone administration day, and the increased group was defined as an increase of $\geq$5% in the endometrial thickness. The unchanged group was defined as the endometrial thickness change of within 5%. We also conducted multivariate logistic regression analysis under multiple adjustment strategies to analyze whether the results were robust or not. Because the percentage of missing data was small ($<$6%), we believed it

would not cause statistical bias or decrease the statistical power; thus, no imputation was performed.

All statistical analyses were conducted with the statistical software package R, version 3.4.3 (The R Foundation, Vienna, Austria). The statistical significance was evaluated by a 2-sided significance level of 0.05. The data collection method of this study is shown in Figure 1.

RESULTS

Characteristics of the Study Cohort

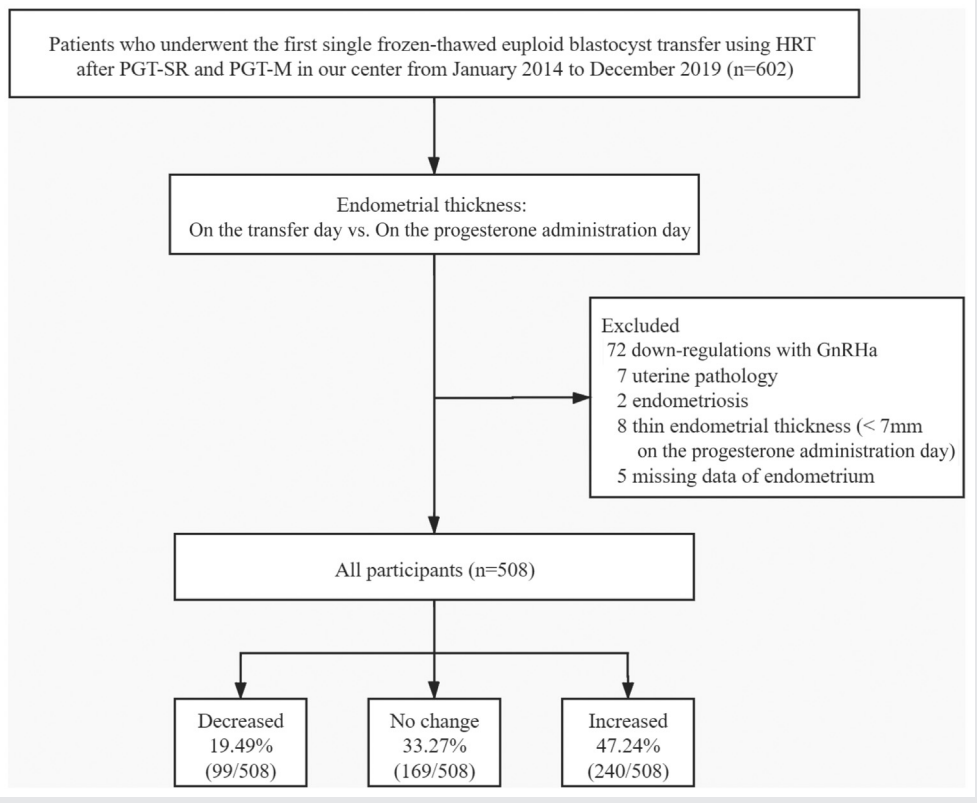
In this study, a total of 602 women who had received HRT to prepare the endometrium and had undergone the first single FET were recruited. Among them, 72 cases of down-regulation by gonadotropin-releasing hormone agonist because of the thin endometrium in the previous controlled ovarian stimulation cycle, 7 cases of uterine pathology, 2 cases of endometriosis, 8 cases of thinner endometrium (endometrial thickness of <7 mm on the day of progesterone administration), and 5 cases without endometrial data were excluded. Finally, this study included 508 patients with eligible HRT cycles (Fig. 1). In all FET cycles, 19.49% (99/508) of the patients had decreased endometrial thickness, 47.24% (240/508) of the patients had increased endometrial thickness, and the remaining 33.27% (169/508) of the patients had no change in the endometrial thickness on the blastocyst transfer day. Finally, among 508 participants, 284 patients achieved clinical pregnancy, and 244 patients achieved live birth.

Comparison of Differences Between Groups

The baseline characteristics of all participants divided into 3 groups (decreased, no change, and increased) according to the changes in the endometrial thickness after progesterone administration are shown in Table 1. Among these women, the endometrial decreased group had a shorter infertility duration and a higher proportion of secondary infertility and parity. However, the 3 groups were comparable in other baseline characteristics, such as female age, BMI, basal hormone level, and pregnancy outcomes.

In the total population, the average endometrial thickness was 9.31 ± 1.51 mm on the day of progesterone administration and 9.80 ± 1.79 mm on the day of FET. In the increased group, the thickness of the endometrium increased from 9.03 ± 1.27 mm, which was the thinnest on the progesterone administration day, to 10.58 ± 1.68 mm, which was the thickest on the transfer day. In contrast, the thickness of the

FIGURE 1



Flowchart of patients. GnRHa = gonadotropin-releasing hormone agonist; HRT = hormone replacement therapy; PGT-M = preimplantation genetic testing for monogenic/single gene defects; PGT-SR = preimplantation genetic testing for chromosomal structural rearrangements.

Jin. Endometrium change ratio of euploid FET. Fertil Steril 2021.

TABLE 1

Baseline characteristics and pregnancy outcomes of patients.

Characteristics	Total	Decreased	No change	Increased	*P* value
N	508	99	169	240	
Female age at oocyte retrieval (y)	28.54 ± 3.61	28.58 ± 3.63	28.78 ± 3.51	28.37 ± 3.66	.53
BMI (kg/m^2)	22.78 ± 3.03	22.55 ± 2.65	22.71 ± 3.14	22.93 ± 3.10	.54
Infertility duration (y)	2.00 (1.00–3.00)	1.00 (1.00–3.00)	2.00 (1.00–3.00)	2.00 (1.00–4.00)	.04
Infertility type (%)					.01
Primary	174 (34.25%)	22 (22.22%)	59 (34.91%)	93 (38.75%)	
Secondary	334 (65.75%)	77 (77.78%)	110 (65.09%)	147 (61.25%)	
Gravidity	1.00 (0.00–2.00)	1.00 (1.00–2.00)	1.00 (0.00–2.00)	1.00 (0.00–2.00)	.28
Parity, mean ± SD (Range)	0.23 ± 0.51 (0.00–3.00)	0.31 ± 0.60 0.00 (0.00–2.00)	0.17 ± 0.47 0.00 (0.00–3.00)	0.23 ± 0.50 0.00 (0.00–3.00)	.06
No. of miscarriages	1.00 (0.00–2.00)	1.00 (0.00–2.00)	1.00 (0.00–2.00)	1.00 (0.00–2.00)	.21
Basal serum FSH (mIU/mL)	6.30 ± 1.66	6.28 ± 1.50	6.25 ± 1.76	6.34 ± 1.65	.85
Basal serum E2 (pg/mL)	35.34 (24.99–46.62)	33.55 (26.34–43.21)	34.89 (23.63–46.06)	36.23 (25.58–47.76)	.23
AMH (ng/mL)	4.17 (2.78–6.04)	3.87 (2.66–5.52)	4.30 (2.70–6.01)	4.21 (2.81–6.41)	.78
No. of retrieved oocytes	19.50 (14.00–26.00)	18.00 (14.00–23.50)	19.00 (14.00–26.00)	20.00 (14.00–27.00)	.39
Genetic category (%)					.26
Reciprocal translocation	301 (59.37%)	53 (54.08%)	109 (64.50%)	139 (57.92%)	
Robertsonian translocation	128 (25.25%)	26 (26.53%)	42 (24.85%)	60 (25.00%)	
Single gene disorders	78 (15.38%)	19 (19.39%)	18 (10.65%)	41 (17.08%)	
Female age at blastocyst transfer (y)	28.88 ± 3.61	28.96 ± 3.64	29.13 ± 3.62	28.66 ± 3.60	.42
Endometrial thickness on the day of progesterone administration (mm)	9.31 ± 1.51	10.03 ± 1.76	9.29 ± 1.55	9.03 ± 1.27	< .001
Triple-line endometrial pattern on the day of progesterone administration (%)					.64
A	492 (96.85%)	96 (96.97%)	162 (95.86%)	234 (97.50%)	
B	16 (3.15%)	3 (3.03%)	7 (4.14%)	6 (2.50%)	
Endometrial thickness at transfer (mm)	9.80 ± 1.79	8.77 ± 1.57	9.29 ± 1.55	10.58 ± 1.68	< .001
Embryo expansion grade at transfer	3.40 ± 0.89	3.36 ± 0.80	3.41 ± 0.92	3.41 ± 0.90	.88
Embryo inner cell mass grade at transfer (%)					.22
A	28 (5.51%)	8 (8.08%)	8 (4.73%)	12 (5.00%)	
B	479 (94.29%)	90 (90.91%)	161 (95.27%)	228 (95.00%)	
C	1 (0.20%)	1 (1.01%)	0 (0.00%)	0 (0.00%)	
Embryo trophectoderm grade at transfer (%)					.86
A	4 (0.79%)	0 (0.00%)	2 (1.18%)	2 (0.83%)	
B	305 (60.04%)	61 (61.62%)	99 (58.58%)	145 (60.42%)	
C	199 (39.17%)	38 (38.38%)	68 (40.24%)	93 (38.75%)	
Morphology score (%)					.66
<4 BC	360 (70.87%)	73 (73.74%)	116 (68.64%)	171 (71.25%)	
≥4 BC	148 (29.13%)	26 (26.26%)	53 (31.36%)	69 (28.75%)	
Day of embryo development at transfer (%)					.85
Day 5	373 (73.43%)	72 (72.73%)	122 (72.19%)	179 (74.58%)	
Day 6	135 (26.57%)	27 (27.27%)	47 (27.81%)	61 (25.42%)	
Clinical pregnancy rate (%)	284 (55.91%)	55 (55.56%)	93 (55.03%)	136 (56.67%)	.95
Miscarriage rate (%)	40 (14.08%)	8 (14.55%)	11 (11.83%)	21 (15.44%)	.74
Live birth rate (%)	244 (48.03%)	47 (47.47%)	82 (48.52%)	115 (47.92%)	.99

Note: AMH = antimüllerian hormone; BMI = body mass index; E2 = estradiol; FSH = follicle-stimulating hormone.

Jin. Endometrium change ratio of euploid FET. Fertil Steril 2021.

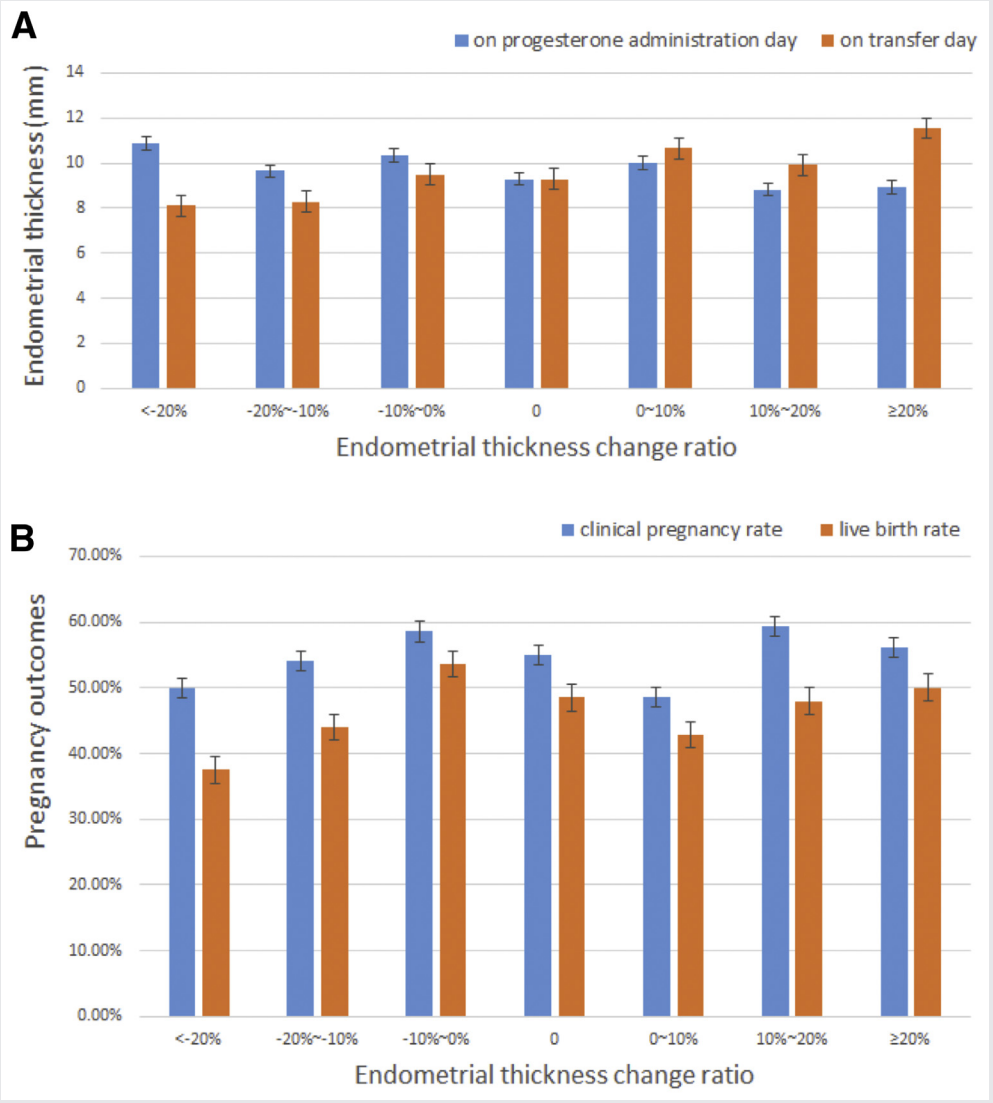
endometrium for the patients in the decreased group decreased from the 10.03 ± 1.76 mm (thickest) on the progesterone administration day to the 8.77 ± 1.57 mm (thinnest) on the transfer day. According to the endometrial thickness change ratio of every 10% as a unit, patients were subdivided into subgroups to observe endometrial thickness in 2 periods, which revealed the same trend (Fig. 2A). With the increase in the endometrial thickness change ratio, the endometrial thickness on the day of progesterone administration decreased gradually, whereas the endometrial thickness on the day of transfer gradually increased.

Regarding pregnancy outcomes, there was no significant difference in the clinical pregnancy rate, miscarriage rate, or live birth rate among the 3 groups. The patients were subdivided into subgroups according to the endometrial thickness change rate of 10%. The clinical pregnancy rate or live birth rate of each group was found to fluctuate around the same level, and there was no significant difference in clinical outcome among the subgroups (Fig. 2B).

Relationship Between Endometrial Thickness Change Ratio and Pregnancy Outcomes

Univariate logistic analysis (Supplemental Table 1, available online) was used to evaluate the effect of each variable on pregnancy outcomes. Generally, basal serum follicle-

FIGURE 2



Characteristics of different endometrial thickness change ratio groups. (**A**) Endometrial thickness on the day of progesterone administration and the day of blastocyst transfer in different endometrial thickness change ratio groups. (**B**) Association between the endometrial thickness change ratio and the clinical pregnancy rate and live birth rate.

Jin. Endometrium change ratio of euploid FET. Fertil Steril 2021.

stimulating hormone was positively correlated with the clinical pregnancy rate and live birth rate, whereas the day of embryo development at transfer was negatively correlated with pregnancy outcomes. However, the absolute value of endometrial thickness on the progesterone administration day and the transfer day or the endometrial thickness change rate after progesterone administration did not significantly affect pregnancy outcomes.

Multivariate logistic regression models (Table 2) were used to evaluate the relationship between the endometrial thickness change ratio and pregnancy outcomes. For the clinical pregnancy rate, when the endometrial thickness change ratio was used as a continuous variable (per 10% change), either in the unadjusted model (OR, 1.02; 95% CI, 0.90–1.15; $P=.81$) or in partially adjusted models or in the fully adjusted model adjusted for female age, BMI, infertility duration, infertility type, gravidity, parity, basal serum follicle-stimulating hormone, morphology score, day of embryo development at transfer, and genetic category (adjusted OR, 1.01; 95% CI, 0.89–1.15; $P=.85$), we found no significant relationship between the change rate of endometrial thickness and the clinical pregnancy rate. When categorically divided into 3 groups according to the change in endometrial thickness and taking the no change group as reference, no matter whether in the unadjusted model (P for trend $=.80$), partially adjusted models, or the fully adjusted model (P for trend $=.92$), we found no significant difference in the clinical pregnancy rate between the decreased group or the increased group with the no change group. Similarly, for the live birth rate, whether the change rate of endometrial thickness was a continuous variable or divided into 3 groups as categorical variables, no significant relationship was found between the endometrial thickness change ratio and live birth rate in either the unadjusted model or the adjusted models.

Subgroup Analyses

To understand whether the relationship between the endometrial thickness change ratio and pregnancy outcomes is stable in different subgroups, we conducted a stratified analysis and an interactive analysis (Supplemental Table 2, available online). The data showed that there was no significant correlation between the endometrial thickness change rate and the clinical pregnancy rate or the live birth rate in any of the subgroups: age, BMI, genetic category, morphology score, and the day of embryo development at transfer. Furthermore, there was no statistically significant interaction in any of the subgroups ($P>.05$ for all comparisons).

Sensitivity Analyses

Considering the slight differences between man-made measurements, the endometrial thickness change group was redefined according to whether the endometrial thickness change rate was >5% after progesterone administration. Among them, the decreased group (endometrial thickness decreased by ≥5%) accounted for 18.90% (96/508), the increased group (endometrial thickness increased by ≥5%) accounted for 46.26 (235/508), and the unchanged group (endometrial thickness changed within 5%) accounted for 34.84% (177/508). The results of multiple regression analysis also showed that no matter whether the endometrial thickness change ratio was a continuous variable or a redefined categorical variable, there was no significant relationship between the change rate of endometrial thickness and the clinical pregnancy rate or the live birth rate in either the unadjusted model or the adjusted models (Supplemental Table 3, available online).

DISCUSSION

In this retrospective cohort study with a sample size of 508 cases, we confirmed that for patients who received the first single FET in endometrium prepared with HRT, there was no relationship between endometrial thickness changes and pregnancy outcomes from the progesterone administration day to the blastocyst transfer day. With increasing endometrial thickness, the clinical pregnancy rate and live birth rate did not change significantly. Second, the miscarriage rate among the groups was also comparable.

At present, there are few articles on the influence of endometrial thickness changes on pregnancy outcomes after progesterone administration in FET cycles, and the conclusions are not all the same. The earliest study by Haas et al. (16) also included women who used HRT for endometrial preparation in 2019. Haas et al. (16) believed that endometrial compaction (defined as endometrial thickness decrease by ≥10%) after progesterone administration led to higher ongoing pregnancy rates. Subsequently, Zilberberg et al. (17) from the same center used euploid blastocysts for transfer, and also suggested that endometrial compaction was related to higher ongoing pregnancy rates. Despite the same endometrial preparation methods and the same timing for evaluating the endometrium, the compaction rates in these 2 studies were 30.6% and 29.3%, respectively, much higher than the 19.49% (99/508) in our data; this difference can possibly be explained because in the studies of Haas et al. (16) and Zilberberg et al. (17), the endometrium was evaluated by transabdominal ultrasound on the day of blastocyst transfer. However, in our study, the endometrium was evaluated by transvaginal ultrasound, which is considered to be more accurate than transabdominal ultrasound in measuring the thickness of the endometrium; this may explain the difference between the research results.

Bu et al. (19) and Ye et al. (20) studied the natural and HRT cycles, respectively, considering the different preparation protocols of the endometrium. The study by Bu et al. (19) showed that regardless of which endometrial preparation protocol was used, endometrial increases after progesterone administration had a higher clinical pregnancy rate. Our study was a follow-up study from the same center. Although our compaction rate of 19.49% was similar to the 19.6% compaction rate of Bu et al. (19), we did not conclude that an increase in endometrial thickness was beneficial to pregnancy outcomes because we only included euploid blastocysts after biopsies in HRT cycles for FET; however, Bu et al. (19) included high-quality blastocysts that had not been biopsied; thus, the influence of embryo quality on their

TABLE 2

Association between endometrial thickness change ratio and pregnancy outcomes in different models.

	Crude model[a]		Adjusted model I[b]		Adjusted model II[c]		Adjusted model III[d]	
Pregnancy outcomes	**OR (95% CI)**	***P* value**	**OR (95% CI)**	***P* value**	**OR (95% CI)**	***P* value**	**OR (95% CI)**	***P* value**
Clinical pregnancy rate								
Endometrial thickness change ratio (per 10%)	1.02 (0.90–1.15)	.81	1.01 (0.89–1.15)	.85	1.01 (0.89–1.15)	.83	1.01 (0.89–1.15)	.85
Grouping of endometrial thickness change ratio								
=0 (no change)	Reference		Reference		Reference		Reference	
<0 (decreased)	1.02 (0.62–1.68)	.93	1.03 (0.62–1.71)	.90	1.02 (0.61–1.71)	.93	1.02 (0.60–1.71)	.95
>0 (increased)	1.07 (0.72–1.59)	.74	1.05 (0.71–1.57)	.80	1.04 (0.69–1.56)	.86	1.03 (0.69–1.56)	.88
P for trend	.80		.89		.93		.92	
Live birth rate								
Endometrial thickness change ratio (per 10%)	1.03 (0.91–1.16)	.64	1.03 (0.91–1.17)	.63	1.03 (0.91–1.17)	.59	1.03 (0.91–1.17)	.64
Grouping of endometrial thickness change ratio								
=0 (no change)	Reference		Reference		Reference		Reference	
<0 (decreased)	0.96 (0.58–1.58)	.87	0.96 (0.58–1.59)	.88	0.96 (0.57–1.60)	.86	0.92 (0.54–1.54)	.74
>0 (increased)	0.98 (0.66–1.45)	.90	0.97 (0.65–1.44)	.88	0.96 (0.64–1.44)	.85	0.94 (0.62–1.41)	.76
P for trend	.98		.99		.97		.99	

Note: CI = confidence interval; OR = odds ratio.
[a] No adjustments for other covariates.
[b] Adjusted for female age, body mass index, infertility duration, and infertility type.
[c] Adjusted for all covariables in model I plus gravidity, parity, and basal serum follicle-stimulating hormone.
[d] Adjusted for all covariables in model I plus morphology score, day of embryo development at transfer, and genetic category.

Jin. Endometrium change ratio of euploid FET. Fertil Steril 2021.

results cannot be ruled out. Ye et al. (20) found that the change in endometrial thickness after progesterone administration had no significant effect on the clinical pregnancy rate and live birth rate in FET cycles, which was the same as our conclusion. However, it was interesting that they used the embryo on the third day without biopsy. Further research is needed to explore whether the results will change when frozen-thawed euploid embryo transfer is used. More recently, Riestenberg et al. (18) conducted a study on single euploid blastocyst transfer including 259 HRT cycles. Their results showed that the change rate of the endometrium was not related to the live birth rate or the spontaneous abortion rate, which was consistent with our results, although their evaluation of the timing of endometrial transfer was performed the day before the blastocyst transfer, which is different from our evaluation on the transfer day. However, when evaluating the endometrium, the duration of progesterone exposure was similar; thus, we believed that it would not have a significant impact on the results.

From a biological point of view, there should be a series of corresponding changes in the endometrial morphology, thickness, glands, and blood vessels due to changes in progesterone after progesterone administration (25). Our data showed that after progesterone administration, the change in endometrial thickness did not represent or predict endometrial receptivity, which led us to think whether it is necessary to repeatedly evaluate the endometrial thickness before transfer. However, a previous study has shown that continued endometrial proliferation in the luteal phase was related to progesterone receptor deficiency or progesterone resistance, which indicated poor receptivity (16). The molecular mechanisms of all these phenomena, as well as contradictory and limited conclusions, need to be explored more deeply in the future.

The advantage of this study is that we used biopsied blastocysts for transfer, which ensured embryo quality by eliminating the effect of embryo ploidy on the outcomes, and focused on the endometrium. Second, to our knowledge, our study has the largest sample size among similar studies, which ensures the stability and reliability of our results. Third, we collected more variables and quantified the relationship between endometrial thickness changes and pregnancy outcomes by presenting several multiple regression models that were adjusted for confounders. Fourth, the operating procedure was standardized, all patients used the same midluteal, short-acting, gonadotropin-releasing hormone agonist long protocol for controlled ovarian stimulation and had a unified endometrial preparation protocol, and all endometrial evaluations were performed by transvaginal ultrasound. This standardization enabled us to minimize the factors that may affect the endometrium, thus ensuring the reliability of the results. Fifth, our research comes from real clinical data. While ensuring clinical applicability, the nature of the retrospective study avoided observational bias.

However, this study also had limitations. First, although the endometrium of each patient was evaluated by a uniformly trained and experienced sonographer using the ultrasound instrument in our center, variability in endometrial thickness measurement was still inevitable. Because these potential biases were evenly distributed among all patients, we believed that this would not affect our main results. Second, because of the retrospective nature of the study, we were unable to fully collect and control all possible confounding factors. However, we used a reasonable study design that balanced the factors affecting the endometrium among all subjects as far as possible and used appropriate statistical methods such as multivariate regression analysis models under multiple adjustment strategies, subgroup analysis, and sensitivity analysis to ensure the reliability of the results. Third, in this study, we included only single FET cycles using HRT for endometrial preparation. The relationship between endometrial thickness change and pregnancy outcomes in different types of transfer cycles needs to be further studied in the future.

In conclusion, we explored the relationship between the change rate of endometrial thickness after progesterone administration and clinical outcomes in the single euploid FET cycle in which the endometrium was prepared by HRT. The results showed that the endometrial thickness of most patients remained unchanged or increased after progesterone administration. However, with the increase in endometrial thickness, the clinical pregnancy rate and live birth rate did not change significantly. In the future, we still need a large sample size of randomized clinical trials to confirm our results and determine whether it is necessary to re-evaluate endometrial thickness before transfer.

Acknowledgments: The authors thank the professional manuscript services of American Journal Experts.

REFERENCES

1. Bergh PA, Navot D. The impact of embryonic development and endometrial maturity on the timing of implantation. Fertil Steril 1992;58:537–42.
2. Dey SK, Lim H, Das SK, Reese J, Paria BC, Daikoku T, et al. Molecular cues to implantation. Endocr Rev 2004;25:341–73.
3. Kuliev A, Rechitsky S. Preimplantation genetic testing: current challenges and future prospects. Expert Rev Mol Diagn 2017;17:1071–88.
4. von Grothusen C, Lalitkumar S, Boggavarapu NR, Gemzell-Danielsson K, Lalitkumar PG. Recent advances in understanding endometrial receptivity: molecular basis and clinical applications. Am J Reprod Immunol 2014;72:148–57.
5. Shapiro H, Cowell C, Casper RF. The use of vaginal ultrasound for monitoring endometrial preparation in a donor oocyte program. Fertil Steril 1993;59:1055–8.
6. Zhang X, Chen CH, Confino E, Barnes R, Milad M, Kazer RR. Increased endometrial thickness is associated with improved treatment outcome for selected patients undergoing in vitro fertilization-embryo transfer. Fertil Steril 2005;83:336–40.
7. Yuan X, Saravelos SH, Wang Q, Xu Y, Li TC, Zhou C. Endometrial thickness as a predictor of pregnancy outcomes in 10787 fresh IVF-ICSI cycles. Reprod Biomed Online 2016;33:197–205.
8. Kasius A, Smit JG, Torrance HL, Eijkemans MJ, Mol BW, Opmeer BC, et al. Endometrial thickness and pregnancy rates after IVF: a systematic review and meta-analysis. Hum Reprod Update 2014;20:530–41.
9. Liu KE, Hartman M, Hartman A, Luo ZC, Mahutte N. The impact of a thin endometrial lining on fresh and frozen-thaw IVF outcomes: an analysis of over 40 000 embryo transfers. Hum Reprod 2018;33:1883–8.

10. El-Toukhy T, Coomarasamy A, Khairy M, Sunkara K, Seed P, Khalaf Y, et al. The relationship between endometrial thickness and outcome of medicated frozen embryo replacement cycles. Fertil Steril 2008;89:832–9.
11. Zhang T, Li Z, Ren X, Huang B, Zhu G, Yang W, et al. Endometrial thickness as a predictor of the reproductive outcomes in fresh and frozen embryo transfer cycles: a retrospective cohort study of 1512 IVF cycles with morphologically good-quality blastocyst. Medicine (Baltimore) 2018;97:e9689.
12. Kovacs P, Matyas S, Boda K, Kaali SG. The effect of endometrial thickness on IVF/ICSI outcome. Hum Reprod 2003;18:2337–41.
13. Barker MA, Boehnlein LM, Kovacs P, Lindheim SR. Follicular and luteal phase endometrial thickness and echogenic pattern and pregnancy outcome in oocyte donation cycles. J Assist Reprod Genet 2009;26:243–9.
14. Laasch C, Puscheck E. Cumulative embryo score, not endometrial thickness, is best for pregnancy prediction in IVF. J Assist Reprod Genet 2004;21:47–50.
15. Griesinger G, Trevisan S, Cometti B. Endometrial thickness on the day of embryo transfer is a poor predictor of IVF treatment outcome. Hum Reprod Open 2018;2018:hox031.
16. Haas J, Smith R, Zilberberg E, Nayot D, Meriano J, Barzilay E, et al. Endometrial compaction (decreased thickness) in response to progesterone results in optimal pregnancy outcome in frozen-thawed embryo transfers. Fertil Steril 2019;112:503–9.e1.
17. Zilberberg E, Smith R, Nayot D, Haas J, Meriano J, Barzilay E, et al. Endometrial compaction before frozen euploid embryo transfer improves ongoing pregnancy rates. Fertil Steril 2020;113:990–5.
18. Riestenberg C, Quinn M, Akopians A, Danzer H, Surrey M, Ghadir S, et al. Endometrial compaction does not predict live birth rate in single euploid frozen embryo transfer cycles. J Assist Reprod Genet 2021;38:407–12.
19. Bu Z, Yang X, Song L, Kang B, Sun Y. The impact of endometrial thickness change after progesterone administration on pregnancy outcome in patients transferred with single frozen-thawed blastocyst. Reprod Biol Endocrinol 2019;17:99.
20. Ye J, Zhang J, Gao H, Zhu Y, Wang Y, Cai R, et al. Effect of endometrial thickness change in response to progesterone administration on pregnancy outcomes in frozen-thawed embryo transfer: analysis of 4465 cycles. Front Endocrinol (Lausanne) 2020;11:546232.
21. Li G, Wu Y, Niu W, Xu J, Hu L, Shi H, et al. Analysis of the number of euploid embryos in preimplantation genetic testing cycles with early-follicular phase long-acting gonadotropin-releasing hormone agonist long protocol. Front Endocrinol (Lausanne) 2020;11:424.
22. Shi D, Xu J, Niu W, Liu Y, Shi H, Yao G, et al. Live births following preimplantation genetic testing for dynamic mutation diseases by karyomapping: a report of three cases. J Assist Reprod Genet 2020;37:539–48.
23. Dickey RP, Olar TT, Taylor SN, Curole DN, Matulich EM. Relationship of endometrial thickness and pattern to fecundity in ovulation induction cycles: effect of clomiphene citrate alone and with human menopausal gonadotropin. Fertil Steril 1993;59:756–60.
24. Zegers-Hochschild F, Adamson GD, Dyer S, Racowsky C, de Mouzon J, Sokol R, et al. The International Glossary on Infertility and Fertility Care, 2017. Fertil Steril 2017;108:393–406.
25. Munro MG. Uterine polyps, adenomyosis, leiomyomas, and endometrial receptivity. Fertil Steril 2019;111:629–40.

Cambios en el grosor endometrial luego de la administración de progesterona no afecta los resultados de embarazo de la transferencia congelado-descongelado del blastocito euploide: un estudio de cohorte retrospectivo.

Objetivo: Evaluar si el cambio en el grosor endometrial entre el día de administración de progesterona y el día de transferencia está relacionado con los resultados de embarazo de los ciclos de transferencia congelado-descongelado de blastocitos euploides únicos.

Diseño: estudio cohorte observacional.

Entorno: centro de medicina reproductiva único.

Paciente(s): Todas las pacientes fueron transferidas con un blastocito euploide biopsiado único, y su endometrio fue preparado con terapia de reemplazo hormonal (HRT).

Intervención(es): El grosor endometrial en el día de transferencia del blastocito y la administración de progesterona fueron medidos por ecografía transvaginal, y la diferencia entre ellos y la proporción de cambio fueron calculadas.

Medidas de resultado principal(es): índices de embarazo clínico y nacidos vivos.

Resultado(s): Imágenes de ecografía endometrial de 508 ciclos de transferencia de blastocito euploide usando HRT fueron evaluados por ecografía transvaginal. En general, los resultados de embarazo fueron comparables entre los diferentes grupos de cambio de grosor endometrial. Los resultados de la regresión múltiple logística demostraron que los índices de embarazo clínico y nacidos vivos no aumentaron significativamente con el aumento de la proporción de cambio del grosor endometrial (por 10%) en el modelo ajustado completo como una variable continua. En el modelo ajustado como una variable categórica, no hubo diferencias estadísticas en los resultados de embarazo entre los grupos de cambio de grosor endometrial. El análisis de interacción demostró que luego de ajustar por variables de confusión, no hubo interacción estadísticamente significativa entre la proporción de cambio de grosor endometrial y los resultados de embarazo entre todos los subgrupos.

Conclusión(es): En los ciclos de transferencias de blastocitos euploides preparando el endometrio con HRT, la proporción de cambio del grosor endometrial luego de la administración de progesterona no fue relacionado con los resultados de embarazo.