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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

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Abstract

To evaluate the relationship between endometrial thickness during fresh in vitro fertilization (IVF) cycles and the clinical outcomes of subsequent frozen embryo transfer (FET) cycles.

FET cycles using at least one morphological good-quality blastocyst conducted between 2012 and 2013 at a university-based reproductive center were reviewed retrospectively. Endometrial ultrasonographic characteristics were recorded both on the oocyte retrieval day and on the day of progesterone supplementation in FET cycles. Clinical pregnancy rate, spontaneous abortion rate, and live birth rate were analyzed.

One thousand five hundred twelve FET cycles was included. The results showed that significant difference in endometrial thickness on day of oocyte retrieval ($P = .03$) was observed between the live birth group (n = 844) and no live birth group (n = 668), while no significant difference in FET endometrial thickness was found ($P = .261$) between the live birth group and no live birth group. For endometrial thickness on oocyte retrieval day, clinical pregnancy rate ranged from 50.0% among patients with an endometrial thickness of ≤6 mm to 84.2% among patients with an endometrial thickness of >16 mm, with live birth rate from 33.3% to 63.2%. Multiple logistic regression analysis of factors related to live birth indicated endometrial thickness on oocyte retrieval day was associated with improved live birth rate (OR was 1.069, 95% CI: 1.011–1.130, $P = .019$), while FET endometrial thickness did not contribute significantly to pregnancy outcomes following FET cycles. The ROC curves revealed the cut-off points of endometrial thickness on oocyte retrieval day was 8.75 mm for live birth.

Endometrial thickness during fresh IVF cycles was a better predictor of endometrial receptivity in subsequent FET cycles than FET cycle endometrial thickness. For those females with thin endometrium in fresh cycles, additional estradiol stimulation might be helpful for adequate endometrial development.

Abbreviations: BMI = body mass index, COH = controlled ovarian hyperstimulation, FET = frozen blastocyst embryo transfer, FSH = follicle-stimulating hormone, hCG = recombinant human chorionic gonadotropin, ICM = inner cell mass, ICSI = intracytoplasmic sperm injection, IVF = in vitro fertilization, PGD = preimplantation genetic diagnosis, ROC = receiver operating characteristic curve.

Keywords: endometrial thickness, estrogen supplementation, frozen embryo transfer, in vitro fertilization

1. Introduction

Great progress has taken place in assisted reproductive technology (ART) in the past a few decades, which contributes to improving pregnancy rates with the development of in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). At the same time, the identification of the optimal conditions for IVF and ICSI has long been the subject of intense investigation. The success of IVF cycles is mainly dependent on age, quality of the embryo, and endometrial receptivity.[1] In traditional protocol of controlled ovarian hyperstimulation (COH) and oocyte retrieval, 2 to 3 days following fertilization, the cleaving embryos will be transferred. With the rapid development of laboratory techniques and embryo culture media, the in vitro culture of embryos can succeed in reaching the blastocyst stage.[2] Many investigations have drew the correlation between blastocyst embryo transfer and higher success rates, which might be explained by an enhanced natural selection process with higher efficiency in selecting biologically superior embryos than morphological assessment of cleavage stage embryos.[3]

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Endometrial receptivity is widely regarded as a key factor in the success of IVF. Also, the evaluation of endometrial receptivity has been the focus of interest for many years due to its potential clinical importance. Generally, ultrasound examination has been carried out as routine method of endometrium evaluation in IVF cycles in most reproductive medicine agencies due to its convenient and noninvasive property. Several sonographic parameters have been developed in the identification of endometrial receptivity, including endometrial thickness, endometrial pattern, endometrial volume, and endometrial and subendometrial blood flow,[4–6] among which endometrial thickness and endometrial pattern have been widely accepted as prognostic indicators for endometrial receptivity. However, there is still no consensus on whether the endometrial sonographic characteristics can predict the pregnancy outcome.

As fresh IVF cycles often result in embryos which are supernumerary for a single IVF cycle, cryopreservation of embryos and frozen-thawed embryo transfer (FET) have become a significant part of IVF technique. However, while most investigations[7–11] have focused on the identification of the optimal conditions of FET, although suggesting a conflicting association between endometrial thickness and pregnancy outcomes following FET cycles, only a few studies explore the possible relationship between endometrial thickness during fresh cycles and pregnancy outcomes following sequent FET cycles. Also, as the key IVF outcome, live birth rate is seldom assessed.

Thus, in order to demonstrate a more accurate relationship between endometrial thickness during fresh cycles and clinical outcomes following sequent FET cycles including clinical pregnancy and live birth rates, we design this large sample size investigation using FET cycles with blastocysts of good morphological embryo quality only, which makes it easier to detect the influence of the endometrium.

2. Materials and methods

2.1. Patient recruitment and counseling

Since this study was retrospective and vaginal sonographic assessment during IVF process was done routinely in our center, Institutional Review Board approval for the study was not necessary. Briefly, all FET cycles using blastocysts between 2012 and 2013 at Reproduction Medicine Center of Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology (Wuhan, China) were reviewed. Exclusion criteria included the presence of a known endometrial polyp or uterine anomaly, oocyte donation cycles, and preimplantation genetic diagnosis (PGD) cycles. Except for these exclusions, all FET cycles during this period with transfer of at least one good-quality blastocyst-stage embryos were included in the analysis, regardless of diagnosis, reproductive history, stimulation protocol, FET protocol, or insemination method. Patients underwent no therapeutic intervention other than routine procedures. Embryo quality was analyzed according to the Istanbul consensus workshop on embryo assessment.[12] Only embryos transfers of at least one blastocyst expanding by day 5, and with at least fair-quality (grade B) ICM (inner cell mass) and (grade C) trophectoderm, were included in the study.

2.2. Treatment protocol

The methods of ovarian stimulation protocols, sperm preparation, IVF embryo culture, and FET protocols in our reproduction medicine center have been described elsewhere.[13–15] Briefly, ovarian stimulation protocols were chosen according to the maternal age, cause of infertility, ovarian response, and coexisting medical conditions. Recombinant human chorionic gonadotropin (hCG) (250 mg; Ovidrel; Serono; Geneva) was administered to trigger ovulation when 2 leading follicles reached a mean diameter of 18 mm. Transvaginal ultrasound-guided oocyte retrieval was conducted 36 after hCG administration. Fertilization of the oocytes took place either by IVF or ICSI, according to the sperm quality. Generally, fertilized oocytes were continuously cultured in G1 medium for 2 more days, after which resulting embryos were cultured in G2 medium for 3 days to achieve the blastocyst stage. According to relevant Chinese legislation, usually fewer than 2 best-quality embryos were transferred on day 3 after oocyte retrieval. Embryo cryopreservation by vitrification was carried out for the additional good-quality embryos or blastocysts.

FET protocols in our center are mainly divided into natural cycles after spontaneous ovulation and hormone replacement treatment cycles depending on the regularity of the menstruation. For the natural cycles, the assessment of endometrial thickness, follicle growth, and ovulation by transvaginal ultrasound examination and measurement of the serum progesterone levels were initiated from cycle days 10 to 12. Thawing and transferring of blastocysts was planned 3 days after ovulation. Intramuscular administration of progesterone for luteal support was started from 1 day after ovulation. For hormone replacement treatment cycles, administration of oral estradiol (Progynova; Bayer; Leverkusen) was initialed with 2 mg/day from cycle days 1 to 4, 4 mg/day from days 5 to 8, and 6 mg/day from days 9 to 12. Since day 13, transvaginal ultrasound examination was conducted to monitor the endometrial thickness and ovulation and the dose of estradiol was adjusted according to the endometrial thickness. When the endometrial thickness reached 8.0 mm or maximum, 40 mg intramuscular administration of progesterone was provided and maintained for the following 3 days. Thawing and transferring of blastocysts was performed on day 4 after 3 days of progesterone administration.

Clinical pregnancy was defined as the identification of a gestational sac with fetal heart activity on ultrasound examination 4 to 5 weeks after embryo transfer and clinical pregnancy rate is expressed per cycle. Spontaneous abortion was defined as spontaneous pregnancy loss after sonographic visualization of an intrauterine gestational sac and spontaneous abortion rate is expressed per clinical pregnancy cycle. Live birth rate was classified as those cycles resulting in the delivery of a viable infant after 24 weeks gestation and live birth rate is expressed per cycle.

2.3. Ultrasound measurement

Endometrial features assessed included endometrial thickness on day of oocyte retrieval in fresh cycles as well as endometrial thickness and pattern on the day of progesterone supplementation in FET cycles. Endometrial thickness was measured in the midsagittal plane of the uterus as the maximum distance between the 2 interfaces of endometrial–myometrial junction. Endometrial pattern were classified according to the morphology of the endometrium as: pattern A (triple-line type characterized by a hypoechoic endometrium with well-defined hyperechoic outer walls and a central echogenic line); pattern B (isoechoic endometrium with poorly defined outer walls and central echogenic line); pattern C (homogeneous hyperechoic endometrium).[16]

2.4. Statistical analysis

Maternal age, baseline serum follicle-stimulating hormone (FSH), body mass index (BMI), duration of infertility, endometrial thickness, and number of transferred blastocyst were compared between pregnancy and no pregnancy cycles as well as live birth and no live birth cycles by *t* test. The endometrial pattern was compared by Chi-square analysis. The relationships among endometrial thickness as well as pattern and subsequent clinical pregnancy, live birth were assessed by multiple logistic regression analysis, including other variables found to be related to treatment outcomes. Receiver operating characteristic (ROC) analysis was performed for the evaluation of discriminatory ability of maternal age and endometrial thickness both on day of oocyte retrieval and in FET cycles. A 2 tailed $P<.05$ was considered statistically significant. All analyses were performed using the Statistical Package for Social Sciences (SPSS version 21.0, IBM; New York).

3. Results

3.1. Baseline cycle characteristics

Table 1 describes FET cycle characteristics according to cycle outcome. Continuous data are presented as mean ± standard deviation and categorical data are presented as percentage (%). A total of 1512 FET cycles were investigated in this study. The overall clinical pregnancy rate was 66.7% and the spontaneous abortion rate was 16.4%, with an overall live birth rate of 55.8%. When subdividing cycles into pregnant ones (n = 1009) and no pregnant ones (n = 503), significant differences were observed between the 2 groups, which included maternal age both in FET cycles and on day of oocyte retrieval, duration of infertility, number of blastocysts transferred, and endometrial thickness both in FET cycles and on day of oocyte retrieval ($P<.05$). When cycles were subdivided according to live birth (n = 844) or not (n = 668), significant differences were observed including maternal age both in FET cycles and on day of oocyte retrieval, duration of infertility, number of blastocysts transferred, and endometrial thickness on day of oocyte retrieval ($P<.05$), while no statistically significant difference in endometrial thickness in FET cycles was found between the 2 groups ($P=.261$). Also, there was no statistically significant difference in endometrial patterns between pregnant and no pregnant groups or live birth and nonlive birth ones.

3.2. Maternal age

To have a better understanding of the maternal age's role in pregnancy outcome, clinical pregnancy rate, live birth rate, and spontaneous abortion rate were evaluated at each year of maternal age both on oocyte retrieval day and in FET cycles (Table 2) (Fig. 1A and B). For maternal age on oocyte retrieval day, with an increase from ≤22 to ≥38, clinical pregnancy rate ranged from 86.4% to 53.8%, live birth rate from 72.7% to 38.5%, and spontaneous abortion rate from 15.8% to 28.6%. For maternal age in FET cycles, with an increase from ≤22 to ≥38, clinical pregnancy rate ranged from 85.7% to 51.7%, live birth rate from 71.4% to 36.8%, and spontaneous abortion rate from 16.7% to 28.9%.

3.3. Endometrial thickness

Clinical pregnancy rate, live birth rate, and spontaneous abortion rate were evaluated at each millimeter of endometrial thickness to have a more accurate comprehension of the association between endometrial thickness and pregnancy outcome (Table 3) (Fig. 1C and D). For endometrial thickness on oocyte retrieval day, clinical pregnancy rate ranged from 50.0% among patients with an endometrial thickness of ≤6 mm to 84.2% among patients with an endometrial thickness of >16 mm, with live birth rate from 33.3%

Table 1

Patient and cycle characteristics by pregnancy outcome.

Variables	All cycles	Pregnant*	Nonpregnant	*P*-value	Live birth†	Nonlive birth	*P*-value
No. of cycles	1512.0	1009.0	503.0	—	844.0	668.0	—
Maternal age at FET, y	30.3 ± 4.2	29.8 ± 4.0	31.1 ± 4.4	<.001	29.7 ± 3.9	31.0 ± 4.4	<.001
Maternal age at oocytes retrieval, y	30.2 ± 4.1	29.8 ± 4.0	31.0 ± 4.3	<.001	29.7 ± 3.9	30.8 ± 4.3	<.001
BMI, kg/m^2	21.5 ± 5.8	21.5 ± 6.8	21.4 ± 2.9	.73	21.4 ± 7.3	21.5 ± 3.0	.69
Baseline FSH, IU/L	6.5 ± 1.8	6.5 ± 1.7	6.6 ± 1.9	.42	6.5 ± 1.7	6.5 ± 1.9	.59
Duration of infertility	4.5 ± 3.4	4.3 ± 3.2	5.0 ± 3.7	.001	4.3 ± 3.1	4.8 ± 3.6	.004
Infertility diagnosis, n (%)							
Tubal factor	708 (46.9%)	457 (45.3%)	251 (49.9%)	—	375 (44.4%)	333 (49.9%)	—
Ovulation disorder	52 (3.4%)	42 (4.2%)	10 (2.0%)	—	36 (4.3%)	16 (2.4%)	—
Pelvic and uterine factors	17 (1.1%)	9 (0.9%)	8 (1.6%)	—	6 (0.7%)	11 (1.6%)	—
Endometriosis	15 (1.0%)	12 (1.2%)	3 (0.6%)	—	10 (1.2%)	5 (0.7%)	—
Male factor	257 (17.0%)	188 (18.6%)	69 (13.7%)	—	163 (19.3%)	94 (14.1%)	—
Unexplained and other	52 (3.4%)	34 (3.4%)	18 (3.6%)	—	27 (3.2%)	25 (3.7%)	—
Multiple factors	411 (27.2%)	267 (26.5%)	144 (28.6%)	—	227 (26.9%)	184 (27.5%)	—
Endometrial thickness on day of oocytes retrieval, mm	10.7 ± 2.2	10.8 ± 2.2	10.4 ± 2.3	<.001	10.8 ± 2.2	10.5 ± 2.3	.003
Endometrial thickness in FET, mm	9.1 ± 1.6	9.1 ± 1.5	8.9 ± 1.6	.02	9.1 ± 1.5	9.0 ± 1.6	.26
Endometrial pattern in FET cycles, n (%)				.02			.07
Pattern A	901 (59.6%)	622 (61.6%)	279 (55.5%)	—	521 (61.7%)	380 (56.9%)	—
Pattern B	58 (3.8%)	34 (3.4%)	24 (4.7%)	—	29 (3.5%)	29 (4.3%)	—
Pattern C	553 (36.6%)	353 (35.0%)	200 (39.8%)	—	294 (34.8%)	259 (38.8%)	—
No. of transferred blastocyst	1.8 ± 0.4	1.8 ± 0.4	1.7 ± .5	<.001	1.9 ± 0.4	1.7 ± 0.5	<.001

BMI = body mass index, FET = frozen-thawed blastocyst transfer, FSH = follicle-stimulating hormone, NS = not significant.

* Patients who achieved a clinical pregnancy.

† Live birth who achieved live birth.

Table 2

Cycle outcomes according to maternal age.

Age, y	Cycles	Clinical pregnancy	Clinical pregnancy rate, %	Spontaneous abortion	Spontaneous abortion rate, %	Live birth	Live birth rate, %
≤22	21	18	85.7	3	16.7	15	71.4
23	31	22	71.0	3	13.6	19	61.3
24	46	32	69.6	3	9.4	29	63.0
25	94	69	73.4	16	23.2	53	56.4
26	97	73	75.3	8	11.0	65	67.0
27	119	88	73.9	11	12.5	77	64.7
28	129	92	71.3	15	16.3	77	59.7
29	162	108	66.7	17	15.7	91	56.2
30	142	102	71.8	17	16.7	85	59.9
31	121	78	64.5	13	16.7	65	53.7
32	125	86	68.8	10	11.6	76	60.8
33	100	59	59.0	6	10.2	53	53.0
34	86	55	64.0	12	21.8	43	50.0
35	65	39	60.0	8	20.5	31	47.7
36	46	27	58.7	7	25.9	20	43.5
37	41	16	39.0	3	18.8	13	31.7
≥38	87	45	51.7	13	28.9	32	36.8

to 63.2%. For endometrial thickness on starting day of progesterone supplementation in FET cycles, with an increase from ≤6 to >14 mm, clinical pregnancy rate ranged from 34.8% to 69.2%, with live birth rate from 26.1% to 46.2%. Spontaneous abortion rate varied with endometrial thickness, but showed no consistent increase with increasing endometrial thickness.

3.4. Binary logistic analysis and ROC curves of some variables on pregnancy outcomes

Binary logistic regression analysis was performed to evaluate the effects of maternal age in FET cycles, endometrial thickness both on oocyte retrieval day and on starting day of progesterone supplementation in FET cycles, endometrial patterns in FET cycles (pattern C was defined as the dummy variable with a OR of 1), duration of infertility and number of transferred blastocyst in FET cycles, on clinical pregnancy or live birth following FET (Table 4) (Fig. 2). The modified Hosmer–Lemshow goodness-of-fit Chi-square test statistics were 4.111 ($P=.847$) for the model of clinical pregnancy and 4.031 ($P=.854$) for the model of live birth, which suggested that the multivariable models were of good fit. The analysis indicated endometrial thickness on oocyte retrieval day and number of transferred blastocyst was associated with improved clinical pregnancy rate and live birth rate, while

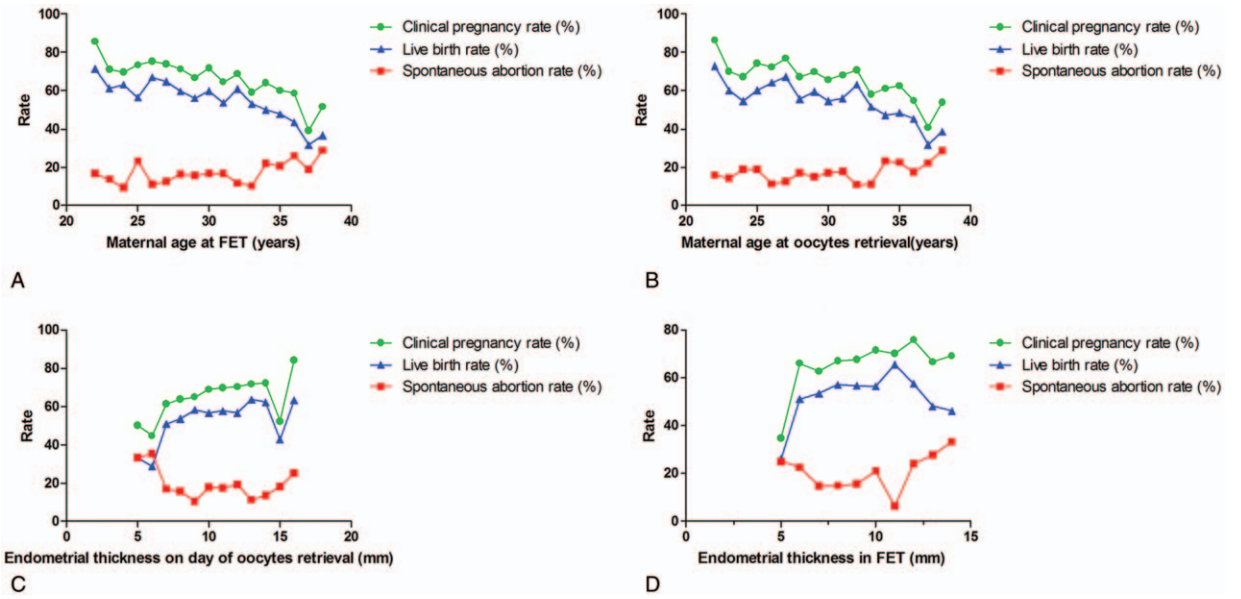

Figure 1. Relationship between endometrial thickness parameters (endometrial thickness on oocyte retrieval day and endometrial thickness on starting day of progesterone supplementation in frozen-thawed blastocyst embryo transfer (FET) cycles), maternal age (maternal age on oocyte retrieval day and during frozen-thawed blastocyst embryo transfer cycles) and pregnancy outcomes in 1512 frozen-thawed blastocyst embryo transfer cycles. (A) Maternal age during FET cycles and pregnancy outcomes. (B) Maternal age on oocyte retrieval day and pregnancy outcomes. (C) Endometrial thickness on oocyte retrieval day and pregnancy outcomes. (D) Endometrial thickness during FET cycles and pregnancy outcomes.

Table 3

Cycle outcomes according to endometrial thickness.

Endometrial thickness, mm	Cycles	Clinical pregnancy	Clinical pregnancy rate, %	Spontaneous abortion	Spontaneous abortion rate, %	Live birth	Live birth rate, %
Endometrial thickness on day of oocytes retrieval, mm							
≤6	24	12	50.0	4	33.3	8	33.3
>6 to ≤7	38	17	44.7	6	35.3	11	28.9
>7 to ≤8	106	65	61.3	11	16.9	54	50.9
>8 to ≤9	190	121	63.7	19	15.7	102	53.7
>9 to ≤10	266	173	65.0	18	10.4	155	58.3
>10 to ≤11	251	173	68.9	31	17.9	142	56.6
>11 to ≤12	245	171	69.8	30	17.5	141	57.6
>12 to ≤13	192	135	70.3	26	19.3	109	56.8
>13 to ≤14	99	71	71.7	8	11.3	63	63.6
>14 to ≤15	61	44	72.1	6	13.6	38	62.3
>15 to ≤16	21	11	52.4	2	18.2	9	42.9
>16	19	16	84.2	4	25.0	12	63.2
Endometrial thickness during FET, mm							
≤6	23	8	34.8	2	25.0	6	26.1
>6 to ≤7	47	31	66.0	7	22.6	24	51.1
>7 to ≤8	249	156	62.7	23	14.7	133	53.4
>8 to ≤9	589	395	67.1	59	14.9	336	57.0
>9 to ≤10	292	197	67.5	31	15.7	166	56.8
>10 to ≤11	172	123	71.5	26	21.1	97	56.4
>11 to ≤12	67	47	70.1	3	6.4	44	65.7
>12 to ≤13	33	25	75.8	6	24.0	19	57.6
>13 to ≤14	27	18	66.7	5	27.8	13	48.1
>14	13	9	69.2	3	33.3	6	46.2

FET = frozen blastocyst embryo transfer.

maternal age were negatively correlated with clinical pregnancy and live birth. Other variables involved including endometrial thickness in FET and endometrial pattern did not contribute significantly to pregnancy outcomes.

The ROC curves of endometrial thickness were constructed to analyze on maternal age during FET and endometrial thickness on both oocyte retrieval day and starting day of progesterone supplementation in FET for the evaluation of their predictive values for clinical pregnancy and live birth (Fig. 3). Because maternal age in FET was negatively related to clinical pregnancy and live birth, with the areas under the curve were 0.585 (1–0.415) (95% confidence interval (CI), 0.554–0.616) versus pregnancy and 0.579 (1–0.421) (95% CI, 0.550–0.608) versus live birth, respectively. The cut-off points of maternal age in FET was 32.5 (sensitivity of 34.9% and specificity of 77.3%) for live birth. The areas under the curve of endometrial thickness on oocyte retrieval day were 0.553 (95% CI, 0.522–0.584) versus pregnancy and 0.541 (95% CI, 0.512–0.570) versus live birth, respectively. The cut-off points of endometrial thickness on oocyte retrieval day was 8.75 (sensitivity of 84.6% and specificity of 22.8%) for live birth. Endometrial thickness on starting day of progesterone supplementation in FET had a cut-off for live birth of 8.25 mm (sensitivity of 73.2% and specificity of 31.6%), but the areas under the curve were 0.534 (95% CI, 0.504–0.565) versus pregnancy and 0.518 (95% CI, 0.488–0.547) versus live birth, making the cut-off a poor overall indicator of pregnancy outcomes following FET.

4. Discussion

Frozen-thawed embryo transfer is a procedure used for the storage and transfer of surplus embryos resulted from IVF/ICSI

Table 4

Binary logistic analysis of factors related to clinical pregnancy and live birth in FET cycles.

	Clinical pregnancy			Live birth		
	P	OR	95% CI for Exp (B)	*P*-value	OR	95% CI for Exp (B)
Maternal age at FET, y	<.001	0.944	0.916–0.973	<.001	0.943	0.916–0.970
Duration of infertility	.26	0.980	0.945–1.016	.66	0.992	0.958–1.028
Endometrial thickness on day of oocytes retrieval, mm	.02	1.068	1.007–1.133	.01	1.069	1.011–1.130
Endometrial thickness in FET, mm	.24	1.052	0.966–1.146	.94	0.997	0.921–1.080
No. of transferred blastocyst	<.001	2.536	1.961–3.278	<.001	2.472	1.912–3.196
Endometrial pattern in FET cycles						
Pattern A	.24	1.150	0.910–1.454	.30	1.123	0.899–1.402
Pattern B	.22	0.702	0.398–1.238	.41	0.792	0.455–1.380
Pattern C		1.000			1.000	

CI = confidence interval, FET = frozen blastocyst embryo transfer, OR = odds ratio.

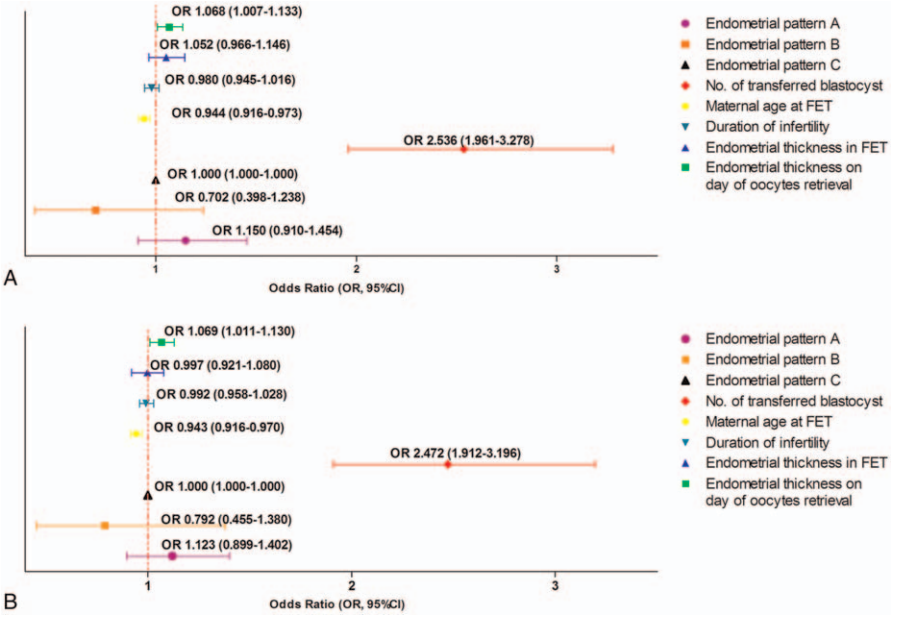

Figure 2. Multivariable logistic regression analysis showing independent predictors of pregnancy outcomes following frozen-thawed blastocyst embryo transfer cycles. (A) The clinical pregnancy following frozen-thawed blastocyst embryo transfer cycles. (B) The live birth following frozen-thawed blastocyst embryo transfer cycles.

cycles. There has been a progressive increase in FET cycles since the limitations on the number of embryos transferred in a single cycle and improvements of laboratory techniques in recent years, especially the wide application of vitrification.

Generally, maternal age has been accepted widely as a highly important negative predictor for successful IVF outcome, whilst good morphological embryo quality has positive correlation with IVF outcomes.[17,18] Moreover, endometrial receptivity is also an essential part of human reproduction and IVF outcomes. Endometrial receptivity is referred to the ability of endometrium to accept and accommodate a blastocyst, resulting in the process of implantation.[19] In most reproductive medicine agencies, endometrial thickness and endometrial pattern has been commonly used as reproducible and noninvasive method in the evaluation of endometrial receptivity. Generally, the role of endometrial thickness has been well documented in fresh IVF cycles, although with conflicting conclusions. Also, there is no consensus on the association between endometrial thickness and clinical outcome following FET cycles despite the relative abundance of studies. Furthermore, studies regarding the relationship between fresh cycle endometrial thickness and pregnancy outcomes of FET cycles are rarer. In this study, the selection of only cycles with transfer of blastocyst-stage embryos including at least one good-quality blastocyst limited the

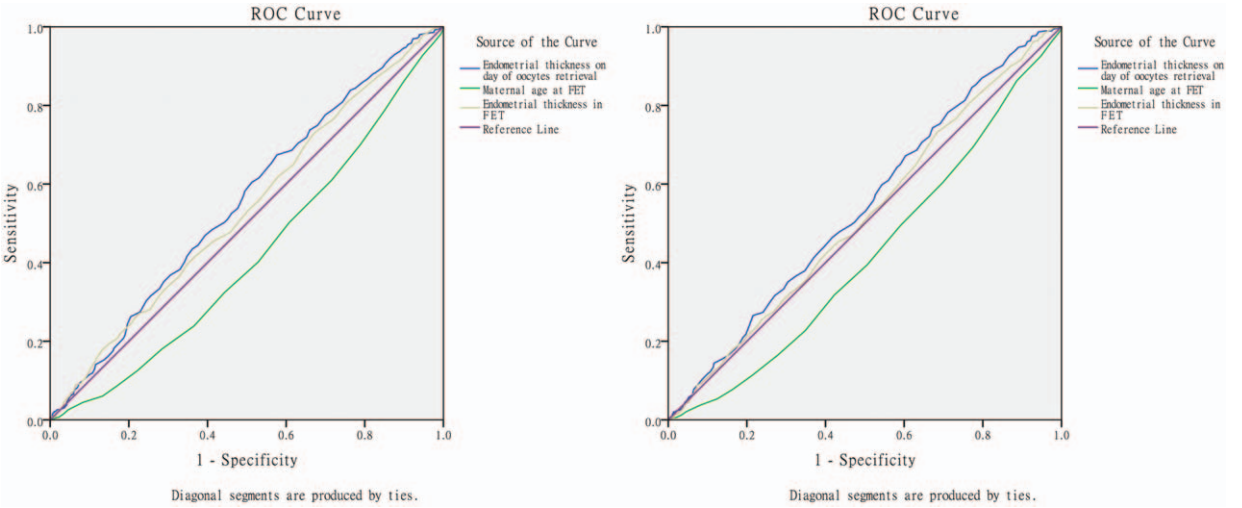

Figure 3. Receiver operator characteristic curve (ROC) of endometrial thickness parameters (endometrial thickness on oocyte retrieval day and endometrial thickness on starting day of progesterone supplementation in frozen-thawed blastocyst embryo transfer cycles) and maternal age during frozen-thawed blastocyst embryo transfer cycles. (A) The clinical pregnancy following frozen-thawed blastocyst embryo transfer cycles. (B) The live birth following frozen-thawed blastocyst embryo transfer cycles.

variability in embryo quality. Thus our inclusion criteria resulted in better homogeneity in embryo quality and the enhancement of the ability to detect the influence of endometrial thickness. Also, to the best of our knowledge, this study has the largest sample size in assessing the effect of endometrial thickness during fresh cycles on FET outcomes, with 1512 FET cycles with blastocyst transfer.

4.1. Comparison with other studies

The significance of endometrial thickness in IVF/ICSI cycles is conflicting. In fresh cycles, several studies found an positive association between endometrial thickness and IVF outcomes,[1,2,20–26] while others could not establish a significant correlation between endometrial thickness and pregnancy following IVF treatment.[27–29] However, it is generally accepted that an endometrial thickness below a minimum value of 6 to 8 mm showed negative predictive value for IVF outcomes,[1,2,24–26] and clinical pregnancy as well as live birth rates are significantly higher in patients with an endometrial thickness >9 to 10 mm.[1,2,20,22–25] Still, implantation can sometimes occur despite a thin endometrium.[30] On the other hand, many investigations[9–11,31–33] support endometrial thickness as a positive predictor of clinical outcomes following FET cycles, while others[8,34,35] observed no significant correlation between endometrial thickness and treatment outcome after FET cycles. Also, the endometrial thickness of 8 mm has been widely used as the threshold of endometrial receptivity in FET cycles.[7–9]

4.2. Key findings

After controlling for embryo quality by using good-quality blastocysts in FET cycles, our data indicated that endometrial thickness on the starting day of progesterone supplementation in FET cycles had no significant correlation with clinical pregnancy and live birth. However, the endometrial thickness on the day of oocyte retrieval did showed a significant association with clinical outcomes of FET cycles with a threshold of 8.75 mm according to ROC curve regarding to live birth.

Despite the well documentation of the association between endometrial thickness and pregnancy outcomes in fresh or in FET cycles, the studies on the relationship between fresh and FET cycles are rare. Jimenez et al[7] reported that previous fresh cycle endometrial thickness is related to subsequent FET cycle endometrial thickness, suggesting these females with a fresh cycle endometrial thickness of 11.5 mm or less might need additional estrogen supplementation for the achievement of adequate endometrial thickness in FET preparation. Early studies revealed that the clinical outcomes in FET cycles would still be inferior for female with failure of pregnancy in their fresh cycles.[36–38] Additionally, investigators[33,39–42] analyzed the reason for freezing and found that clinical results in FET cycles of females who conceived in fresh cycles or at risk of Ovarian Hyperstimulation Syndrome Syndrome were superior to group who failed to conceive in their fresh cycle, indicating the association of fresh cycle outcomes with FET cycle outcomes. Some studies concluded that embryo quality played a key role in this relationship.[36,42] However, other investigations[10] showed that this significant correlation between fresh cycle and FET cycle outcomes still existed even with the same number of good quality of embryos transferred, suggesting the predictive value of fresh endometrial thickness for FET endometrial receptivity.

Our data showed that it was the endometrial thickness during fresh cycles rather than in FET cycles proved to be a better predictor of endometrial receptivity in FET outcomes. In our center, the endometrial thickness of more than 8 mm has long been used as the marker of adequate endometrial preparation in FET cycles. However, the actual optimal endometrial thickness might be variable among individuals. Thus endometrial thickness in FET cycles is less effective than endometrial thickness in fresh cycles in the evaluation of endometrial receptivity and the prediction of FET outcomes. However, the mechanism explaining the association between thin endometrium and inverse IVF outcomes is still not clear. It has been speculated that the increasing basal layer endometrium oxygen concentrations in females with thin endometrium might be detrimental for embryo implantation.[43]

4.3. Clinical and research implications

Endometrial receptivity can be induced by exogenous administration of estradiol and progesterone in various protocols for endometrial preparation. Estradiol priming contributes to endometrial proliferation and induction of progesterone receptors. Then, the action of subsequent progesterone turns the estrogen-primed endometrium into a secretory structure for embryo survival and implantation.[44] Therefore, for those females with thinner endometrium in fresh cycles, it would be useful to prolong the duration of estradiol stimulation for optimal endometrial receptivity.

4.4. Endometrial pattern

Besides, the association between endometrial pattern and endometrial receptivity has also has been discussed for many years with conflicting results. Some studies revealed an association between endometrial pattern and IVF outcome,[8,45,46] while other studies showed no significant correlation.[47,48] Our data showed no significant association between endometrial pattern and IVF outcome.

4.5. Strength and limitations

We acknowledge there are also some limitations in the present investigation. An important limitation of our study was that although morphological good-quality blastocysts were used in our study, we were still limited by the unknown genetic composition of embryos. Embryo morphology might not really represent the real quality of embryos. Another limitation is the retrospective design of this study. However, we believe that these findings were of interest as previous similar studies revealed conflicting conclusions. A well-designed and powered randomized clinical trial will be needed for further study.

5. Conclusion

In conclusion, our study suggested that endometrial thickness during fresh IVF cycles was a better predictor of endometrial receptivity in subsequent FET cycles than FET cycle endometrial thickness. For those females with thin endometrium in fresh cycles, additional estradiol stimulation might be helpful for adequate endometrial development.

6. Authors' contributions

Carried out the analysis and interpretation of data, and writing of the manuscript: Tao Zhang.

Involved in the ultrasound examination and critical manuscript revisions: Zhou Li, Bo Huang, and Xinling Ren.

Participated in the conception and design of the study: Wei Yang.

Participated in its design and coordination, and helped to draft the manuscript: Lei Jin and Guijin Zhu.

Read and approved the final version of the manuscript: All authors.

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