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Original Article

Hormonal replacement treatment improves clinical pregnancy in frozen-thawed embryos transfer cycles: a retrospective cohort study

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Abstract: This study aimed to assess frozen-thawed embryo transfer (FET) outcomes in natural, hormone replacement treatment (HRT) and semi-HRT cycles. This was a retrospective cohort study of 5414 cycles of patients in an academic hospital. Patients were grouped as 2216 natural cycles, 1180 semi-HRT cycles, and 2018 HRT cycles. Primary outcome measures were implantation rate, clinical pregnancy rate and live birth rate. Other parameters, such as peak endometria-thickness, were also analyzed. Patients undergoing FET with HRT obtained higher implantation rate and clinical pregnancy rate than patients with natural or semi-HR cycles (29.3% vs. 21.5% vs. 25.6%, *P*=0.01, and 48.7% vs. 42.7% vs. 36.1%, *P*=0.01, respectively). This finding was not changed in patients with thin endometrium (≤8 mm). A Subanalysis in patients with HRT showed that the implantation and clinical pregnancy rate was higher in patients without ovulation than ovulatory patients (29.8% vs. 16.9%, *P*<0.01, and 49.5% vs. 26.3%, *P*<0.01, respectively). This study suggests that HRT increases the possibility of pregnancy. Further, our data showed that ovulation in HRT cycle has a detrimental effect on pregnancy. Therefore, we recommend that HRT should be used in FET cycles, and ovulation of patients should be evaluated during the treatment.

Keywords: Frozen-thawed embryo transfer, natural cycle, hormone replacement treatment, implantation rate, clinical pregnancy rate

Introduction

The first successful pregnancy after use of frozen-thawed embryo transfer (FET) was reported in 1983 [1]. Thereafter, FET treatment was rapidly expanded. FET can effectively prevent IVF associated complications, such as ovarian hyperstimulation syndrome and multiple births. In addition, FET serves as an safe and cost-effective way to increase cumulative pregnancy rate [2]. Further, FET enables a strategy of supporting an elective single embryo transfer policy, which has been adapted in many countries. In 2006, FET has accounted for around 20% of all assisted reproductive technology cycles in Europe [3].

Embryo implantation is an important step in human reproduction. A successful implantation depends on the reciprocal interaction between the embryo and receptive uterus [4, 5]. Some parameters have been reported as prognostics in evaluating endometrial receptivity, such as endometrial volume, endometrial thickness, and artery blood flow [6]. Numerous biomarkers were also found to be associated with endometrial receptivity, including LIF, Integrin, and HOXA10 [7-9]. Due to the important role of receptive endometrium, various endometrial preparation protocols are applied, aiming to improve FET success. FET has been successfully performed in natural cycles after spontaneous ovulation and in hormone replacement treatment (HRT) cycles [10-12]. Previous studies were performed to compare FET outcomes in different endometrial preparation protocols. However, the results still remain conflicting. Therefore, the present study was conducted to explore the optimal endometrial preparation protocol in FET treatment.

Materials and methods

Patients

This was a retrospective, non-interventional, single-center cohort study of patients undergoing FET treatment at reproductive medicine center in Tongji hospital, Wuhan, China, between January 2008 and December 2011. A total of 5414 FET cycles were enrolled. In order to avoid potential bias, only the first two FET cycles of each patient was included. Non-autologous and cancelled cycles were excluded. Institutional Review Board (IRB) approval was not necessary, because all women in the present study underwent the routine FET treatment in our center and no additional intervention or sampling was performed.

Natural cycles

Transvaginal ultrasound scan and measurement of serum progesterone level were initiated from cycle day 10-12 to assess endometrial thickness, follicle growth, and ovulation. FET was planned 3 days after ovulation, indicated by a serum progesterone >5 ng/mL. Embryo transfer was performed 1 day after collapse, and progesterone administration was commenced as luteal support.

Semi-HRT cycles

Oral estradiol Valerate (Progynova, Bayer, Germany) were administrated from the cycle day 10-12, dosage of estradiol was adjusted according to the endometrial-thickness assessed by transvaginal ultrasound. Administration of 40 mg progesterone (progesterone injection, Sansheng, China) intramuscularly was given when the endometrium reached a thickness of 8 mm or maximum. Administration with 60 mg, 80 mg and 80 mg progesterone were used respectively in the following 3 days. Embryos were collapsed and transferred after 4 days of progesterone administration.

HRT cycles

Oral estradiol was taken at 2 mg/d from cycle day 1-4, 4 mg/d from day 5-8, 6 mg/d from day 9-12. Transvaginal ultrasound scan was performed to assess endometrial-thickness and ovulation from day 13 and estradiol dosage was adjusted based on the endometrial-thickness. Administration with 40 mg progesterone intramuscularly was given when the endometrium reached a thickness of 8 mm or maximum. Administration with 60 mg, 80 mg, and 80 mg progesterone were used respectively in the following 3 days. Embryo transfer was performed after 4 days of progesterone administration.

FET protocols

Day 3 embryos were classified as Grade 1 to Grade 4, according to cleavage stage, blastomere size and shape, and fragmentation [13]. The assessment criteria of blastocyst quality were based on blastocoele cavity, inner cell mass, trophectodermal cells, and volume of zona pellucida [14]. Surplus embryos in fresh cycles were cryopreserved for subsequent FET cycles. Cryopreserve and thawing of the embryos were implemented as previously published [15].

Pregnancy outcomes

In the present study, biochemical pregnancy was defined as a serum hCG >20 IU/L 2 weeks after transfer but then declined to negative. Pregnancy was defined as a serum hCG level >20 IU/L and confirmed by observation of gestational sac on transvaginal ultrasound scan 5-7 weeks after transfer. Implantation rate was defined as the number of gestational sacs present on ultrasound divided by the number of embryos transferred. All pregnant women were followed up to obtain the delivery data.

Statistical analysis

The continuous data were given as mean and SD. Groups were compared with one-way analysis of variance (ANOVA) with Bonferroni adjustment or student's *t*-test as appropriate. Categorical variables were presented as number and percentage. Differences were evaluated with chi-square test and the Fisher exact test. A P value <.05 was considered statistical significant. SPSS version 13.0 (SPSS Inc.) was used for statistical analysis.

Results

Demographic data were presented in **Table 1**. The age and BMI were higher in natural cycle group than those in semi-HRT and HRT Group,

Table 1. Demographics

	Natural cycle	Semi-HRT cycle	HRT cycle
No. of cycles	2216	1180	2018
Mean age (year)	31.3±3.8[a]	30.8±3.8	30.8±4.0
BMI (kg/m^2)	22.6±3.3[b]	22.3±3.7	22.4±3.5
Duration of infertility (year)	4.9±3.3	4.8±2.8	4.8±3.1
Primary infertility (%)	49.2 (1090/2216)	48.9 (577/1180)	50.1 (1011/2018)
Basal FSH (mIU/mL)	5.7±2.2	5.6±2.2	5.8±2.0

Note: [a]$P<0.01$; [b]$P=0.04$.

whereas these differences were small. No differences were found in duration of infertility, infertility type, and basal FSH level.

FET parameters and outcomes were shown in **Table 2**. There were no differences in delay between cycles and the number of embryo transferred. The peak endometrial-thickness was higher in patients with natural cycles (10.4±4.2, $P<0.01$), as compared to patients with semi-HRT cycles (9.0±2.5) and HRT cycles (9.0±2.1). As for FET clinical outcomes, patients in the three group obtained comparable biochemical pregnancy rate. Patients undergoing HRT obtained higher implantation rate (29.3%, $P=0.01$) and clinical pregnancy rate (48.7%, $P=0.01$) than patients in natural cycle (21.5% and 36.1%, respectively) and semi-HRT (25.6% and 42.7%, respectively) group. No difference was found regarding live birth rate among the three groups. Similar results were found in patients with thin endometrial thickness ≤8 mm on the day of transfer (**Table 3**). Patients undergoing HRT obtained elevated implantation rate and clinical pregnancy rate than patients with natural cycles and semi-HRT cycles (27.8% vs. 22.1% vs. 19.9%, $P<0.01$, and 47.6% vs. 33.7% vs. 34.4%, $P<0.01$, respectively).

A subanalysis was performed on patients undergoing HRT. Dividing patients into patients with and without ovulation, our data showed that implantation rate and clinical pregnancy rate in anovulatory patients were higher than patients with ovulation (29.8% vs. 16.9%, $P<0.01$ and 49.5% vs. 26.3%, $P<0.01$, respectively). Patients in the two groups obtained similar live birth rate (**Table 4**).

Discussion

Our study found that HRT improved FET outcomes, in terms of implantation rate and clinical pregnancy rate. Furthermore, in HRT cycles, ovulation negatively affected the pregnancy results.

In the present study, HRT was applied without prior GnRHa down-regulation but still yielded favorable pregnancy results, which was consistent with the study by Queenan et al. [16]. They reported that prior suppression with a GnRH-a is not necessary for endometrial preparation. Multiple studies were performed to assess the efficacy of various endometrium preparation protocols. However, controversies still remain on the issue of optimal protocol in FET treatment. The study by Morozov et al. [17] showed that HRT was associated with decreased pregnancy rate in comparison to natural cycle. The alteration of implantation window span in FET cycles may contribute to the reduced pregnancy rate at supraphysiologic estrogen level [18]. In contrast, some studies reported superior pregnancy outcomes in HRT cycles [11, 19]. Additionally, several studies suggested that patients in natural cycles and HRT cycles obtained comparable FET outcomes [10, 20, 21]. Our study found better FET outcomes in patients who underwent HRT, which may attribute to the following reasons. Firstly, scheduling the day of embryo thawing and transfer was difficult in natural cycles, because the length of follicular phases varies among patients. In contrast, in HRT cycles, the day of embryo transfer can be programmed in advance, which favors embryo implantation. Secondly, it was difficult to identify a positive serum LH surge as the ascending or the descending limb of the LH surge, and thus the planned day of embryo transfer may not match the implantation window in natural cycles. Lastly, it was a dilemma in some cases with natural cycles that the ovulation was completed, whereas the endometrium failed to reach suitable thickness.

Endometrial-thickness provides an effective approach to assess endometrial development and predict endometrial receptivity [6]. The study by Gonen et al. [22] suggested pregnancy was unlikely to occur when the endometrial-

Table 2. FET characteristics and outcomes

	Natural cycle	Semi-HRT cycle	HRT cycle
No. of cycles	2216	1180	2018
Delay between cycles (months)	5.6±2.7	5.2±2.0	5.3±2.5
Peak endometria thickness (mm)	10.4±4.2[a]	9.0±2.5	9.0±2.1
No. of embryo transferred	2.1±0.5	2.0±0.5	2.0±0.5
Biochemical pregnancy rate (%)	3.3 (74/2216)	3.6 (43/1180)	3.9 (79/2018)
Implantation rate (%)	21.5 (977/4552)	25.6 (616/2408)	29.3 (1197/4080)[b]
Clinical Pregnancy rate (%)	36.1 (801/2216)	42.7 (504/1180)	48.7 (982/2018)[b]
Live birth rate (%)	85.7 (837/977)	84.3 (519/616)	87.0 (1041/1197)

Note: [a]$P<0.01$; [b]$P=0.01$.

Table 3. FET results in patients with endometrial-thickness ≤8 mm

	Natural cycle	Semi-HRT cycle	HRT cycle
No. of cycles	199	302	502
Delay between treatments (months)	5.5±5.2	5.7±4.5	5.5±5.3
Endometrial thickness (mm)	7.4±0.7	7.4±0.6	7.4±0.7
No. of embryo transferred	2.0±0.5	2.0±0.5	2.0±0.5
Implantation rate (%)	22.1 (86/389)	19.9 (123/617)	27.8 (281/1012)[a]
Clinical pregnancy rate (%)	33.7 (67/199)	34.4 (104/302)	47.6 (239/502)[a]
Live birth rate (%)	77.9 (67/86)	79.7 (98/123)	86.5 (243/281)

Note: [a]$P<0.01$.

Table 4. FET parameters and outcomes in patients undergoing HRT with or without ovulation

	With ovulation	Without ovulation	*P* value
No. of cycles	76	1942	
Delay between treatments (months)	5.8±3.4	5.2±4.5	NS
Endometrial thickness (mm)	9.1±1.8	8.9±2.1	NS
No. of embryo transferred	2.0±0.4	2.0±0.5	NS
Implantation rate (%)	16.9 (25/148)	29.8 (1172/3932)	<0.01
Clinical pregnancy rate (%)	26.3 (20/76)	49.5 (962/1942)	<0.01
Live birth rate (%)	84.0 (21/25)	87.0 (1020/1172)	NS

Note: NS=not significant.

thickness was <6 mm. Other studies reported FET success rates were higher when endometrial-thickness was greater than 8 mm on the day of transfer [10, 17]. In patients with poor endometrial response, exogenous estradiol treatment constitutes as a common practice to improve endometrial thickness. Our data showed that patients with thin endometrium undergoing different protocols had similar endometrial thickness, indicating that administration with estradiol is insufficient to increase endometrial thickness in patients with poor endometrial response. Indeed, it has been reported that aberrant expression pattern of estrogen receptors was associated with thin endometrium and infertility [23]. Interestingly, despite the lack of ability to increase endometrial thickness, HRT yielded better pregnancy results than other protocols. Therefore, it should be recommended that patients with thin endometrium take HRT.

HRT carries numerous advantages over natural cycles. Based on the results of our study, HRT increases the possibility of pregnancy. By using HRT, fewer times of visits were needed during the cycles, which considerably benefits the patients. The days of embryos thawing and transfer can be scheduled in advance. Treatment cancellation rate can be limited low in HRT, due to the programmed exogenous hormone replacement and suppression of ovulation. HRT constitutes a more suitable option for some specific patients, such as women with >40 age old, premature ovarian failure, and oligomenorrhea [12, 24].

Whether an ovulatory HRT cycle should be cancelled constitutes a dilemma in practice of FET treatment. The unsuppressed follicle development and ovulation may alter the hormone profile, and thus impair the endometrial receptivity and pregnancy. In our study, we found decreased implantation rate and pregnancy rate in ovulatory patients. This finding has clinical implication that the HRT cycle with unsuppressed ovulation may be considered to be cancelled. However, it should be noted that the arm of ovulatory patients included only 76 cycles. A prospective observational study with large sample size is needed to confirm this finding.

The strength of the present study is the large size of the cohort: a total of 5414 FET cycles were analyzed. In addition, the patients data was collected in a relative short period of time (Jan. 2008 to Dec. 2011), during this period of time, the protocols of endometrium preparation, as well as FET, were relative consolidate, which favors intra-group homogeneity in our study. To discriminate the effect of embryo-quality on pregnancy, only the FET cycles with good quality embryo transferred were enrolled. To alleviate the confounding influence of the factor that the same patient with excessive embryos may underwent multiple FET cycles, only the first 2 FET cycles were analyzed. Limitation of the present study is its respective nature, and confounding factors may interfere with the results.

In conclusion, we found that HRT increases the possibility of pregnancy. This result was not changed in patients with thin endometrium. In addition, ovulation in HRT cycle may defer the FET success. Therefore, we recommended that HRT should be applied in patients who plan to take FET treatment, and ovulation in HRT cycle should be evaluated during the treatment.

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