Original Article
The effect of accompanying dominant follicle development/ovulation on the outcomes of frozen-thawed blastocyst transfer in HRT cycle

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Abstract: The artificial regime was widespread used in frozen-thawed embryo transfer (FET). Some researchers asserted that if dominant follicles developed or ovulation occurred in hormone replacement FET cycles, this cycle should be cancelled because the fitting timing of transfer was hard to determine. In this study, we compared the difference between the outcome of frozen-thawed blastula transfer in hormone replacement treatment cycle (HRT) with or without dominant follicle development/ovulation. A total of 171 cases of frozen-thawed blastula transferred successfully in HRT cycle were retrospectively analyzed. Patients were divided into three groups according to dominant follicle development, ovulation or not: Group A, cycles without dominant follicle developing. Group B, cycles with dominant follicle developing but without ovulation. Group C, ovulated cycles. The results showed that there was no significant difference in the pregnancy rates or other parameters among the three groups, but the abortion rate was higher in group C than those of other two groups. To conclude, dominant follicle development/ovulation was not the necessary indication to cancel transfer cycles in HRT cycles, and our cautious decision would save many valuable cycles.

Introduction

Recently great advances have been made in cryopreservation culture technique for embryo since the first pregnancy of frozen-thawed embryo transfer (FET) was successful in 1983 [1]. This technique was acted as a supplement to in vitro fertilization and embryo transfer (IVF-ET) and accepted by every center. The methods of endometrial preparation for frozen embryo transfer in women with functioning ovaries were various, mainly including monitoring ovulation in natural cycle or hormone replacement therapy (HRT) imitating the natural endocrine curves with exogenous hormones. The fit time of embryo implantation is limited and short, which lasts for only a few days. This period of time is called as "implantation window", and if an embryo is transferred within this window, the chance of conceiving are greater, so the selection of the time of the transfer day was essential to achieving pregnancy. The exact timing of ovulation is often difficult to determine, particularly in women with irregular cycle or poor follicle development, therefore the risk of cycle cancellation is high. So the artificial step-up regime with exogenous steroids was more advantageous [2].

In early stage, HRT was performed following to the pituitary suppressionby gonadotropin-releasing hormone agonist (GnRHa), which would suppress follicular recruitment and endogenous secretion of luteinizing hormone (LH). LH might lead to a rise in progestogen level ordirectly affect endometrial receptivity and implantation [3, 4]. Subsequent reports declared that although the HRT without pituitary suppression may lead to a rise in serum LH level, which is similar to the phenomenon pre-ovulation in natural cycles, estrogen administration may suppress most follicular recruitment as well, except for few cycles about 4~10% [5, 6], and this LH rise is usually not accompa-

nied by a rise in progesterone [7]. Indeed, the artificial endometrium preparation in the absence of pituitary suppression would achieve the similar pregnancy rates and live birth rates [8]. Some researchers asserted that if dominant follicles developed or ovulation occurred in hormone replacement FET cycles without pituitary suppression, endogenous serum progesterone might raise , this cycle had to be cancelled because the fitting timing of transfer was hard to determine [9]. However, it means that numerous cycles might be missed based on this viewpiont. Does the development of dominant follicles or ovulation have any significant effect in artificial endometrial preparation? Currently there is still no consensus concerning this subject and few articles supplied insufficient evidence. The present study aimed to explore an appropriate criteria of cancelling cycles when dominant follicles grew or ovulated in frozen blastocysts transfer cycle (B-FET) with hormone replacement.

Materials and methods

Patients

This is a retrospective cohort study of patients who underwent B-FET treatment in reproductive medicine center of Tongji Hospital, in the period from January 2012 to September 2012. A total of 171 women who had normal ovulatory cycles but also histories of lack of significant pre-ovulatory serum LH peak or Luteinized Unruptured Follicle Syndrome (LUFS) were preferred step-up hormone replacement protocol. Besides, the patients whose endometrial thickness did not reach sufficient level to a successful transfer in previous nature cycles were also enrolled. The cycles with donated oocytes and preimplantation genetic diagnosis were excluded.

Endometrial preparation and ET

Briefly, all patients started estrogen treatment on the first day of their menstrual period without previous down regulation of GnRH-a. The standard oral estrogen replacement was 2 mg per day, 4 days; 4 mg per day, 4 days; and 6 mg per day, 4 days. 12 days later, patients' endometrial thickness was evaluated through vaginal ultrasound. The maximal distance between two outer strong echo lines were defined as the standard measure of endometrial thickness. If

the thickness was <8 mm, the same dose of estrogen was continued or increased to 8 mg/d for another 3 days. When the endometrium reached a thickness of 8mm or approached to the thickness in the follicular aspiration cycles, progesterone was injected IM 20 mg, and then progesterone was administration in the dose of 40 mg/d, 60 mg/d, 80 mg/d, 80 mg/d, respectively from the next day to the day of ET. The endometrial thickness on the day of initiation of progestone administration was recorded and analyzed.

If the endometrium didn't reach the thickness of 8 mm after 20 days of continuous estrogen administration, or there was a premature rise of serum progesterone (>2.25ng/ml) [10], the cycle was canceled. The day 5 blastocysts were thawed and transferred on day 5 of progesterone treatment. The same doses of estrogen and progesterone were continued until obtaining a serum β -hCG assay 11 days after ET. If the pregnancy test was positive, the hormone replacement were given for another 2 weeks and patients were followed with serial ultrasonography to determine fetal viability. Clinical pregnancy was defined as the presence of a gestational sac on vaginal ultrasound. The dose of progesterone was reduced by 20mg every week, and that of estrogen by one third of the current dose every week till drug withdrawal, if there was no sign of threatened abortion.

Participants group

The patients were divided into three groups according to the development of follicles through vaginal ultrasound till the initiation of the first progesterone administration. Group A: cycles without growing follicle (the maximum diameter of follicles in both ovaries <10 mm); Group B: cycles with dominant follicles (the diameter of any follicle in alternative ovary >10 mm) which had not ovulated; Group C: cycles with advantageous follicular ovulation (the evidence of ovulation by consecutive ultrasound examination and/or the LH surge by the use of serum or urine LH monitoring).

Blastocyst freezing-thawing and grade

According to the quality of the sperm, insemination (conventional IVF) or microinjection (ICSI) was performed after ovum pick-up (OPU) in fresh cycles. Then, fresh ET was performed on

Table 1. The characteristic of patient in each group

	Group A	Group B	Group C	P-value
Age at transfer (years)	30.26±3.57	30.21±4.01	31.01±10.61	0.53
Duration of infertility (yeras)	5.34±2.89	5.28±3.13	5.54±4.19	0.81
Basal FSH[*] (IU/L)	5.53±1.96	5.61±1.25	5.35±1.97	0.92
No. of blastocyst transferred	1.61±0.43	1.47±0.49	1.79±0.52	0.87
The higher scores of two blastocyst in each single patient	7.93±1.87	7.89±1.56	8.08±1.29	0.32
The mean scores of the blastocyst	7.26±1.89	7.21±1.93	7.31±1.48	0.72

[*]basal serum Follicle Stimulating Hormone.

Table 2. Dose of estrogen and clinical result

	Group A	Group B	Group C	P-value
Dose of estrogen (mg)	78.56±6.05	76.98±8.47	79.97±5.37	0.34
Thickness of endometrium (mm)[a]	9.01±1.30	9.08±1.65	9.06±1.75	1.98
Serum P (ng/ml)[b]	1.52±0.62	1.51±0.82	1.59±1.09	0.81
No. of blastocyst implantion	0.96±0.80	0.97±0.79	0.98±0.82	0.75
Cycle cancelled rate (%)	3.13 (4/128)	3.45 (1/29)	7.14(1/14)	0.79
Clinical pregnancy rate (%)	66.13 (82/124)	71.43 (20/28)	61.54(8/13)	0.80
Early abortion rate (%)	8.54 (7/82)	5.00 (1/20)	37.5(3/8)	0.00[*]

[a]The thickness of endometrium on the day of the first progestone injection. [b]The serum progestoneon the day of the first progestone injection. [*]P-value presents the significance of the difference between the groups C and other two groups ($p < 0.05$).

day 3 (cleavage stage ET) and excess day 3 high-quality embryos were cultured further to day 5-6, then high-quality blastocysts were frozen. Fewer than two blastocysts were transferred in subsequent FET cycles. The characteristic of viable blastocyst was the dilatation of cavity. The grading was performed according to Gardner [11]: blastocyst stage I-VI corresponding to 1-6, grade A, B, C of inner cell mass (ICM) were scored to 3, 2, 1 respectively, and grade A, B, C of the trophectoderm to 3, 2, 1. The accumulation of the 3 sub scores was the total score, which was carried on the comparison.

Statistical analysis

Statistical Package for Social Sciences (SPSS v 13.0 for windows) software was used for data analysis. Continuous characteristic values of each group, such as age were presented as the mean (± SD). Means were compared by one way ANOVA. Ordinary values such as the pregnancy rate were analyzed by the X^2 test and the Fisher exact test. A *P*-value <0.05 was considered statistically significant.

Result

Table 1 details the patients' demographic of each group. There were no differences in age,

duration of infertility, basal FSH, the number of blastocyst transferred, the higher score of the blastocyst in each single patient and the mean blastocyst score between groups.

Table 2 listed the comparisons of the outcomes of groups and clinical parameters between every groups. The indicators including the total dose of estrogen administration, the thickness of endometrium and the serum progestone on the day of the first progestone injection, the number of blastocyst implantion were similar between groups. The number of cancelled cycles due to too thin endometrium or the elevated serum progestone in advance was 4, 3, 1 in group A, B, C, respectively, which has no significant difference. Of all the cancelled cycles, except for 2 cycles separately from group A and group C for elevated serum progestone in advance, other cycles were called off because of the thin endometrium. There was no notable difference of clinical pregnancy rates, but the rate of abortion of group C was markedly higher than other two groups.

Discussion

The success of a frozen-thawed embryo transfer program is closely linked to exacted synchronization between endometrial maturation

and embryo development [12]. Such synchronization may be achieved in a natural cycle after spontaneous ovulation [12-14] or after artificial preparation of the endometrium with exogenous steroids [13]. Endometrial preparation with HRT in FET cycles was most suitable for women with ovarian dysfunction, such as ovulation failure, irregular menstruation, and decreased ovarian reserve [14]. Sometimes it was also used in patients with normal menstruation. Supplementation with estrogen administration simulating physiological status would suppress follicular recruitment, inhibit the rise of endogenous LH, and accelerate the growth of endometrium to synchronize with embryo to achieve higher implantation rate and pregnancy rate [15].

According to some investigators, supplementation with estrogen from the early follicular phase without pituitary suppression in an artificial endometrial preparation cycles for frozen-thawed embryo transfer would lead to a rise in LH level, which may lead to the rise of progestone in turn, affect the endometrial receptivity, and reduce the pregnancy rate in result [16]. In our study, the growing follicles and ovulation during the course of artificial endometrial preparation without pituitary suppression were not accompanied by a significant rise in serum progestone. The prevalence of the cycle with a premature rise of serum progestone without growing follicle were 1 in 128 cycles, 0 in 29 cycles with dominant follicles but without ovulation and 1 in 14 with advantageous follicular ovulation, which has no significant difference between groups.

Other researchers considered that although HRT using estrogen only is lack of sufficient suppression effect, it would not reduce the pregnancy rate and live birth rate [13, 17]. Nowadays, HRT without pituitary suppression was widely used, whereas cycles were cancelled when spontaneous ovulation occurred, and the therapy had to be conducted afresh, which would increase the return visit rate [9].

The present study addressed the question whether growing follicles and ovulation are associated with the likelihood of clinical pregnancy or the rate of cycle cancelling, in patients undergoing a B-FET cycle in which endometrium is prepared with oral estrogen step-up artificial endometrial preparation protocol in the

absence of pituitary down-regulation. The data presented herein showed that clinical pregnancy likelihood is similar in B-FET cycles in the presence of dominant follicles, ovulation to those in the absence of growing follicles.

It was interesting that the group with the highest pregnancy rate among the groups was not that without growing follicle as we had anticipated. The order from highest to lowest pregnancy rate was the group with dominant follicles but without ovulation, the group without growing follicle and that with advantage follicular ovulation. The order from the lowest to the highest abortion rate was the comparable. To explain the reasons, we proposed that although the follicles recruitment and dominance could not be suppressed by exogenous hormone in the patient with dominant follicles but without ovulation, the endogenous estrogen rose in the course of the follicle development, which accelerated the growth of the endometrium accompanying with exogenous estrogen. What's more, the endogenous progestone did not rise because the granule in the follicles had not developed enough to ovulate or to luteinize, which made it possible that the timing of endometrial decidualization could be controlled by exogenous progestone completely to select the most suitable time to transfer. That could be explained by our previous result which the serum progestone level would not affect the result of ET if it was <2.25ng/ml [10]. In this way, it was maybe achieve better results than which without growing follicle. Nevertheless, in the cycles with advantageous follicular ovulation, the endogenous estrogen rose and advanced the growth of the endometrium as mentioned before, but the rise of endogenous progestone following the ovulation would disturb the doctors' decision of administration of progestone, which made it hesitated to determine the exact beginning and ending of "the window of implantation", and influenced the implantation and growth of the blastocyst. Moreover, it would let the doctors ignore the occurrence of luteal phase defect and performed the shortage administration of luteal support, leading to the rise of abortion rate. In accord with our study, some published studies declared that the appearance of LUFS had no influence on the result of FET cycles [18].

In conclusion, our study suggest that in estrogen step-up artificial endometrial preparation

protocol without pituitary down-regulation, the appearance of growing follicle of ovulation could not predict the FET outcome. When deciding whether a FET cycle should be cancelled, cautious assessment of serum estrogen and progestone should be taken into account, instead of the appearance of growing follicle or ovulation. It seems more possible to get higher pregnancy rate and live birth rate in cycles with dominant follicles but no ovulation. More strengthen luteal support should be performed in cycles with advantageous follicular ovulation to avoid the occurrence of early abortion. The strength of our study: we proposed that the actual effect of follicle growing and ovulation on the outcomes of B-FET in HRT cycles without pituitary down-regulation. To our knowledge, this is the first evidence on the subject. The limitation of the study is the relatively small size of cohort and its retrospective nature, and randomized controlled trials are needed to address the impact of accompanying dominant follicle development/ovulation on the outcomes of frozen-thawed blastocyst transfer in HRT cycle.

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