

Effect of luteinized unruptured follicle cycles on clinical outcomes of frozen thawed embryo transfer in Chinese women

Lina Wang · Jie Qiao · Ping Liu · Ying Lian

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Abstract

Objective To determine the effect of luteinized unruptured follicle (LUF) cycles on frozen thawed embryo transfer (FET). **Design** A retrospective analysis comparing the clinical outcomes after FET among 144 cases of luteinized unruptured follicle (LUF) cycles and 866 cases of ovulation cycles. **Setting** Reproductive medical center, Beijing China. **Patient(s)** Chinese infertile women who underwent FET. **Intervention(s)** None. **Main outcome measure(s)** Clinical pregnancy rate (PR), implantation rate. **Result(s)** The implantation rate, clinical pregnancy rate, ongoing pregnancy rate and live birth rate in LUF group were 12.76% (49/384), 27.78% (40/144), 24.31% (35/144) and 19.44% (28/144), respectively, and in ovulation group, 14.74% (332/2251), 31.29% (271/866), 28.29% (245/866) and 22.23% (193/866), respectively ($p > 0.05$). **Conclusion(s)** LUF does not affect the clinical outcomes of FET. Patients of LUF should be included in FET treatment.

Keywords Luteinized unruptured follicle · Ovulation · Frozen thawed embryo transfer · Clinical outcomes

Introduction

Luteinized unruptured follicle (LUF), a failure of ovulation due to luteinization of follicle under the action of luteinizing hormone (LH), is one of the major causes of infertility for women [1]. Like the women with ovulation cycles,

women with LUF have normal menstrual period and biphasic of basal body temperature (BBT): they exhibit similar cervical mucus and secretory changes to endometrium, and have almost the same ultrasonographic findings on endometrium as well as. Hence monitoring of follicle development and assessment of blood hormone were rarely conducted in clinical settings except for women with specific clinical indications. However, with the development of new diagnostic tools such as B type ultrasound, the diagnostic rate of LUF has been on the rise. In addition, recent data have shown that the endometrium proliferates more slowly in LUF cycles than in ovulation cycles, and women with LUF cycles have a shortened luteal phase, reduced P level and inadequate luteal function [2]. In spite of these new developments, to our knowledge, to date, there have been no reports in the world about the feasibility of FET in women having LUF cycle and many reproductive medical centers do not perform FET for women in LUF cycles.

It has been reported that the incidence of LUF is 11.2% in women with regular menstrual cycle [3], and 25% to 43% in women of infertility. The recurrent rate of LUF is much higher, ranges from 78.6% to 90% [4]: patients with recurrent LUF have to undergo repeated cancellation of FET leading to increased anxieties. It is therefore important to evaluate the effect of LUF cycles on FET. This study compares the clinical outcomes after FET between Chinese women in LUF cycles and women in ovulation cycles.

Materials and methods

Selection criteria

Included in the study were 1,016 infertile women with natural cycle (none of them was from induction of

L. Wang · J. Qiao (✉) · P. Liu · Y. Lian
Reproductive Medicine Center, Peking University Third Hospital,
Beijing 100083, China
e-mail: jie.qiao@263.net

ovulation cycles) who had undergone FET treatment between January 2004 and December 2004 at Reproductive Medicine Center, Peking University Third Hospital. The subjects were categorized into either LUF group or ovulation group (144 and 866 cases, respectively) based on the criteria described below. On the day of FET and day 3 after FET, serum E₂ and P level were measured and compared for patients performed FET between November 2004 and December 2004.

Diagnostic standards of ovulation

B type ultrasonography was used to monitor the process of ovulation starting at the time of the enlargement of follicles. If the midluteal P level was greater than 10 ng/ml (31.8 nmol/l) and a sudden disappearance of the preovulatory follicle or the follicle shrinking by 5 mm in diameter was observed, and/or the corpus luteum with or without the presence of fluid in the pelvic cavity was seen on ultrasound follow-up, the cycle was considered to be ovulatory.

Diagnostic standards of LUF

The following findings were diagnostic criteria of LUF [5, 6]: On ultrasonographic screening which a normal follicular development and a observation of a preovulatory follicle failed to rupture, instead: (1) continued to grow in size with an thickened follicular wall and increased internal resonance; meanwhile, extensive intrafollicular bright spots would be detected after 2 to 4 days; (2) rapidly enlargement to the size of 30 to 35 mm in diameter with strong internal resonance, persistent till the next cycle or even longer intrafollicular echoes might be (1) low, medium or high; (2) echoless; (3) diffuse and linear; (4) reticular or band-like. The presence of lowered cervical score, biphasic of BBT, elevated serum LH, E₂ and P level at the time together with the above findings established the diagnosis of LUF. The serum P level were >3 ng/ml (9.5 nmol/l) in LUF cycles [7].

Monitoring of ovulation

In the current study the patients were asked to monitor their follicles and sometimes cervical mucous score and serum E₂ and P assessment to determine the day of ovulation and LUF. The size of follicles was assessed with transvaginal ultrasonography using 5.0 MHz section type testing probe. From the day 8 or day 10 of menstrual cycle, the size of follicles was measured continuously. Ovulation and LUF were established by ultrasonographic findings, the cervical mucous score and serum LH, E₂, P level on the day of ovulation.

Luteal support

All the patients received the same luteal support by progesterone since the day of embryos transfer in which progesterone 20 mg were given daily from the day of ET.

FET therapy and evaluation of thawed embryos

Using slow freezing and rapid thawing protocol [8], all the frozen thawed embryos underwent 3 days culture in vitro. Embryos were assessed for quality from the following aspects [9]: morphology of cells, the number of cell and cellular debris. The embryos in G1 and G2 grade with no less than six blastomeres are defined as good quality embryos. Cumulative embryo score (CES) score was done in all patients according to embryonic morphology [10].

FET outcomes

The outcomes of FET were divided into two statuses: no pregnancy and clinical pregnancy. No pregnancy mean women whose blood HCG level was less than 35 IU/l. Clinical pregnancy mean women who had positive blood HCG, visible gestation cyst on ultrasound 30 days after FET or visible abortus. Clinical pregnancy was redefined into ongoing pregnancy (pregnancies continued over 20 gestation weeks) and embryonic diapause (women with empty gestational cysts by ultrasound on more than two consecutive observation, or presence of gestational sac without primary fetal heartbeat).

Statistical analysis

Statistical analysis was performed by using SPSS software, version 10.0 (SPSS, Inc., Chicago, IL, USA). χ^2 tests or Fisher exact tests were used for qualitative variables and nonparametric analysis of variance was used for the quantitative variables. The probability value used to identify significance was given a test of $p < 0.05$.

Results

Parameters of LUF and ovulation cycles

There were no significant differences between LUF and ovulation group in basic characteristics including age, course of infertility, infertility types, frozen time of embryos, the number of embryos transferred, the number of embryonic cells, mean number of cleavage cells, the number of top quality embryos between LUF and ovulation group (Table 1).

Table 1 Parameters related to both groups

Group	LUF (n=144)	Ovulation (n=866)	p value
Age (years)	31.97±3.79	32.37±3.89	0.242
Course of infertility (years)	5.96±3.16	5.98±3.40	0.958
Period of embryos thawed (years)	4.85±4.35	4.95±3.30	0.802
Type of infertility			0.202
Primary	76 (52.78)	508 (58.67)	
Secondary (%)	68 (47.22)	358 (41.34)	
Protocol of fertilization			0.058
Routine	105 (72.92)	561 (64.78)	
ICSI (%)	39 (27.08)	305 (35.22)	
Numbers of embryos transfer	2.48±0.56	2.36±0.58	0.149
Average cleavage cells	6.12±1.27	6.15±1.49	0.811
CES score	62.73±22.73	62.80±22.19	0.638
Embryos injury			0.689
Complete survival (%)	8 (5.56)	40 (4.62)	
Partial damaged (%)	89 (61.81)	566 (65.77)	
Mixed (%)	47 (32.64)	260 (29.63)	
Embryos quality			0.987
All good quality embryos (%)	45 (31.25)	276 (31.87)	
Only one good quality embryo (%)	32 (22.22)	189 (21.82)	
No good quality embryo (%)	67 (46.53)	401 (46.30)	

Relationship between outcomes of FET and pregnancy in LUF cycle

Out of the 1,016 Chinese infertile women included in the study 1,010 were followed up during their FET protocol, including 144 LUF patients and 866 ovulation patients (99.40% of the enrolled patients were followed up). A total of 2,635 embryos were transferred (384 in LUF group, and 2,251 in ovulation group), of which 369 (14.00%) embryos were implanted. As a result, 311 (30.79%) patients had clinical pregnancy. The pregnancy rate was 27.78% in LUF group (40/144 cases) and 31.29% in ovulation group (271/866 cases) and the implantation rate was 12.76% (49/384 cases) and 14.74% (332/2,251 cases) in LUF and ovulation group, respectively. Neither difference between the two groups was significant (for clinical pregnancy rate, $p=0.436$; implantation rate, $p=0.346$). There were no significant differences in ongoing pregnancy rate, term birth rate or premature delivery rate and late abortion rate between LUF and ovulation group. Also shown in Table 2 there was

no significant difference in live birth rate from ongoing pregnancy between the two groups (19.44% vs. 22.23%, 28/144 cases vs. 193/866 cases, $p=0.514$) noted in Table 2.

Endometrial ultrasonographic findings and serum hormone levels in LUF cycle

The ultrasonographic examination showed that there was no significant difference in the thickness of the endometrium between LUF group and ovulation group ($1.01±0.14$ vs. $1.02±0.14$ cm, $p=0.534$); There was no significant difference in the distribution of A type endometrial lining in LUF and ovulation group (46 vs. 625, 31.94% vs. 72.17%) but there were significantly more cases with B endometrial lining in LUF group than in ovulation group (98 vs. 241, 68.06% vs. 27.83%, $p=0.000$).

The serum E₂ and P level were measured for LUF patients undergoing FET between November 2004 and December 2004 on the day of FET and day 3 after FET, and the findings were compared with those in ovulation cycles.

Table 2 The outcomes of FET between the two groups

Group	Outcome of FET			Outcome of ongoing pregnancy				
	n	Embryo diapause/abortion (%)	Ongoing pregnancy (%)	n	Live birth	Term birth (%)	Premature delivery (%)	Late abortion (%)
LUF	144	5 (3.47)	35 (24.31)	33	1.21±0.54	27 (81.82)	2 (6.06)	4 (12.12)
Ovulation	866	26 (3.00)	245 (28.29)	230	1.13±0.33	185 (80.43)	9 (3.91)	36 (15.65)
P value		0.572			0.572		0.755	

The comparison showed no significant differences in E_2 , P level and P/E_2 ratio on the day of FET and day 3 after FET between LUF and ovulation group (Table 3).

Discussion

In 1975 Jewelwicz [11] first reported that well developed follicles could persisted unruptured and have the typically luteinization as observed in normal menstrual cycle. Marik and Hulka [12] demonstrated by laparoscope that in some individuals there failed to show cleavage for ovulation on the surface of the ovaries in the early luteal phase, indicating the process of luteinization didn't necessarily result from ovulation. The mechanism of LUF was attributed to endocrinal and mechanical causes. The former referred to internal environmental factors leading to ovulation disorders, such as elevated level of cyclic adenosine monophosphate due to insufficient secretion of LH, reduced secretion of P, lowered activity of profibrinolysin activators, decreased fibrinolysis and autolysis of the wall of the ovaries leading to disorders in the maturation of follicles, cleavage and discharge oocytes [13]. LUF cycles might also result from such defects as collagens and vascularized graft, which usually lead to lowered P level [14, 15]. The later condition was also known as "false LUF". It was usually caused by adhesion, thickening of pelvic soft tissues resulting from infarction of fallopian tube, endometriosis, pelvic surgeries or inflammation [16]. Other implicated causes of LUF included the use of certain non-steroid anti-inflammatory drugs, such as mezinol, meloxicam etc [17, 18]. The endocrinal mechanism of LUF has been gradually elucidated after numerous studies of the relationship between LUF and infertility. Westfahl [19] observed the absence of P surge and shortened P duration in animal experiments in LUF cycles, the level of androstenedione and testosterone rose and backed to basal level 48 h after HCG administration. A study on ovarian blood flow by ultrasonography, it demonstrated that intraovarian resistance indexes (RI) of LUF cycles was similar to those of anovulatory cycles but was much higher as compared with the ovulatory cycles [3, 20]. The high impedance of flow was partially caused by the defective corpus luteum formation and angiogenesis [21]. Yun et al. [22] reported that more than half of LUF cycles

had luteal phase shorter than 11 days, suggesting inadequate luteal functions presenting in LUF cycle. There was evidence that the incidence of LUF in ovulation cycles were higher than that in natural cycle [23]. In a study, the incidence of LUF was reported 58.9% in ovulation induction cycle (clomiphene citrate cycle) [4]. But another study that monitored ultrasonographically 45 cycles of 40 infertile patients treated with clomiphene showed that no LUF occurrence [24]. Later study showed that after ovulation induction with CC/HMG/HCG, patients with LUF had similar ovulation development, the estrogen, LH and FSH levels before HCG administration were similar with those in ovulation cycles. But in LUF cycles the P level in mid-luteal phase was significantly lowered [25].

Inadequate luteal functions and retarded development of endometrium might influence the embryos implantation and their survival rate. Given the possible controversy effect on the application of FET in LUF cycles, some reproductive medical centers gave up performing FET in LUF cycles. But the recurrence of LUF was said to be as high as 90% [4], infeasibility of FET due to the recurrent LUF will lead to consumption of time, increasing mental and economical burdens. Assuming that by adopting luteal support protocols, we could increase the pregnancy and implantation rate of FET in LUF cycles. To reach this goal, luteal support protocols were given in LUF cycles to correct the possible inadequate luteal functions and facilitate the implantation and development of embryos; also the same luteal supports were given in ovulation cycles as control. The results of this study showed that the levels of blood hormones in LUF cycles were closed to that in ovulation cycles. Yimin and Hefeng [26] reported a case of FET in a LUF patient, that achieved a successful pregnancy and delivery and concluded that FET would be applicable to patients with intact endometrium and appropriate luteal support. But this assumption had not yet supported by experiments of larger sampling size. The present study reviewed the outcomes of FET in 144 Chinese LUF women. The results showed that no significant differences in serum E_2 , P and P/E_2 measured on the day of transfer and day 3 after the transfer were noted between LUF group and ovulation group. Besides, no significant differences in implantation rate, clinical pregnancy rate, ongoing pregnancy rate and live birth rate between LUF and ovulation group were noted. In conclu-

Table 3 The serum E_2 and P level on the day of FET and day 3 after FET between the two groups

Group	On the day of FET				Day 3 after FET			
	<i>n</i>	E_2 (pmol/l)	P (nmol/l)	P/E_2 (median)	<i>n</i>	E_2 (pmol/l)	P (nmol/l)	P/E_2 (median)
LUF	35	507.0±371.9	62.1±58.2	112.9	23	686.9±557.2	114.1±94.9	187.9
Ovulation	155	436.2±298.5	46.7±39.6	112.5	110	586.6±363.8	116.1±75.2	183.3
<i>p</i> value		0.067	0.066	0.477		0.436	0.915	0.568

sion, our data suggest that FET might be a safe and workable procedure in LUF cycle if luteal phase support is given. Further study on FET for LUF patient with relapsing risks was warranted.

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