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Original Article

Comparison between oral and vaginal estrogen usage in inadequate endometrial patients for frozen-thawed blastocysts transfer

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Abstract: Endometrial preparation with exogenous estrogen is a common practice in frozen-thawed embryo transfer (FET) cycles. The objective of this study was to compare the clinical outcomes of two endometrial preparation groups, oral estradiol valerate tablets (OEV) group versus vaginal estradiol (VE) tablets group, in inadequate endometrium patients. This retrospective, single-center, cohort study of patients undergoing FET treatment between Jan. 2012 and Jun. 2013, at an academic IVF center, included 247 patients (cycles) with endometrial thickness < 8 mm on day 13 of the hormone replacement cycle: OEV group included 69 patients (cycles) who received continuous OEV from day 1 onwards up to the day of progesterone supplement, while VE group included 178 patients (cycles) who taken OEV from day 1 to day 12, and used VE tablets from day 13 till the day of progesterone supplement. Patients in VE group required more days and higher dosage of estradiol, but had thinner endometrium on the day of transfer. However, the increase of endometrial thickness was more, when compared to OEV-treated patients. The implantation rate and pregnancy rate were, though not significantly, higher in VE group. Conclusions: Longer time of administration and higher dosage of estradiol usage did not have adverse effects on the clinical pregnancy rate. VE tablets may promote endometrial development and pregnancy success in FET cycles could not verify. Further study is needed to confirm the vaginal estradiol action on frozen-thawed embryo transfer cycles.

Keywords: Frozen-thawed embryo transfer (FET), endometrial preparation, estradiol supplement duration, clinical pregnancy rate

Introduction

Cryopreservation of spare embryos after controlled ovarian stimulation has been increasingly used during the last decade, coinciding with an increase in the elective single embryo transfer policy and the improvement of freezing technologies [1]. As a result, an increase in the cumulative pregnancy rate per oocyte retrieval has been obtained [1-3]. Cryopreservation of embryos crested during fresh IVF cycles provides a less expensive and time-intensive opportunity for pregnancy. If a stimulated fresh cycle is unsuccessful and there are frozen embryos available, a frozen-thawed embryo transfer is performed.

Frozen embryo transfer (FET) was successfully performed in natural cycles with spontaneous ovulation, ovulation induction cycles, and hormonal replacement cycles with different agents, such as estrogen and/or progesterone, with and without GnRH-a down-regulation [4, 5]. Adequate hormonal preparation of the endometrium is of outmost importance in frozen embryo replacement cycles to provide the optimal chances of pregnancy. The endometrium is frequently artificially prepared with estrogen and progesterone supplementation in order to match the endometrial stage during the critical implantation window [6]. Many drugs and various modes of administration have been tried by several investigators in order to optimize implantation rates and consequently improve the success rates of the embryo transfer procedures [5]. While the currently available data show no significant difference in pregnancy rates among these methods [7, 8]. The optimal

Table 1. Demographic data of patients with inadequate endometrium

	OE group	VE group	*P* value
No. of cycles	69	178	
Maternal age (years)	28.9±3.2	29.7±3.0	NS
BMI (kg/m^2)	22.5±3.1	21.7±3.0	NS
Duration of infertility (years)	5.3±1.9	5.5±2.1	NS

Note: NS = not significant; OE: oral estradiol; VE: vaginal estradiol.

endometrial thickness is unclear. Several studies suggested a thickness < 8 mm may be associated with implantation failure in both fresh and frozen embryo transfer cycles [9-12]. Women preparing for FET will often require additional estrogen supplementation, or other intervention, if their endometrium is inadequate (< 8 mm). The objective of this study is to compare the clinical outcomes of two artificial cycles for FET treatment in patients undergoing endometrial preparation with oral estrogen and with vaginal estrogen tablet, who were inadequate of endometrial thickness after 12 days of estrogen supplementation.

Materials and methods

Study population

This was a retrospective, single-center, cohort study at the reproductive medicine center in an academic hospital. A total of 247 patients (cycles) who underwent FET treatment were enrolled. Inclusion criteria: (1) patients who underwent frozen-thawed blastocyst transfer treatment between Jan. 2012 and Jun. 2013; (2) The endometrial preparation was initiated with oral estradiol valerate from cycle day 1 to day 12; (3) The endometrial thickness was less than 8 mm on cycle day 13 measured by ultrasonography. Exclusion criteria: (1) donor oocyte recipients; (2) gestational carriers; (3) Day 3 embryo transfer cycles; (4) >35 years. Patients were divided into two groups: OEV group included 69 patients (cycles) who were administrated with oral estradiol valerate continuously. VE group included 178 patients (cycles) who used oral estradiol valerate from cycle day 1 to 13 but adding vaginal estradiol after day 13. Institutional Review Board approval for the study was not necessary because subjects underwent routine FET treatments in our center, and no additional intervention was applied. Written informed consent was provided by each subject prior to their participation in the study. Patient information and data were anonymized and de-identified prior to analysis. This study was approved by the Institute Review Board (IRB) of Tongji Hospital.

IVF/ICSI treatment protocol

Ovarian stimulation was performed by using follicle-stimulating hormone (Gonal-F, Serono, Sweden), human menopausal gonadotropin (HMG). Women were administed human chorionic gonadotropin (HCG, Profasi, Serono, Sweden) when dominant follicles were 18 mm or three or more follicles reached a diameter of ≥17 mm. Oocyte retrieval was performed within 34 to 36 hours after HCG administration using a vaginal ultrasound-guided procedure. IVF or ICSI was carried out 4-6 hours after oocyte retrieval. Embryos were cultured by conventional method in our center. Embryos had been cryopreserved by a vitrification method following IVF fresh cycle.

Endometrium preparation and FET protocols

The protocols of blastocyst culture, embryo vitrification and warming were implemented as previously published [13]. Endometrial preparation protocols were as follow: oral estradiol valerate (Progynova, Bayer) was taken 2 mg/d from on cycle day 1-4, 4 mg/d on day 5-8, 6 mg/d on day 9-12. After 12 days of administration with estradiol valerate, patients continued the administration with estradiol valerate 6-8 mg/d, or used vaginal estradiol (Femoston, Solvay pharmaceuticals B.V.) 1-2 mg/d, the dosage was adjusted according to the response of endometrium evaluated by ultrasonography. Adding estradiol valerate or vaginal estradiol depended on the clinical doctors. Progesterone I.M. was given to transform the endometrium, provided the endometrial thickness reached 8 mm or maximum. Embryo transfer was performed after five days of progesterone administration. The luteal phase was supported with 60 mg progesterone injections I.M. from the day of transfer.

Cycle outcome

Serum β-hCG levels were measured 14 days after embryos transfer. Subsequent ultrasound examinations were performed 4 weeks after embryo transfer. Clinical pregnancy was defined as pregnancy diagnosed by ultrasonography visualization of gestational sac (s) or definitive clinical signs of pregnancy. Early miscarriage was

Table 2. Endometrial preparation parameters

	OE group	VE group	*P* value
No. of cycles	69	178	
Duration of estradiol supplementation (days)	20.4±2.0	23.9±3.4	<0.01
Total estrogen dose (mg)	119.0±23.2	175.2±51.4	<0.01
Serum progesterone level (ng/mL)	0.6±0.7	0.4±0.6	NS
No. of transferred embryos	1.9±0.4	1.9±0.4	NS
ET on day 13 (mm)	7.3±0.5	6.3 ±0.9	<0.01
ET on the day of transfer (mm)	8.7±0.9	8.2±1.0	<0.01
ET increase after day 13	1.5±0.9	1.9±1.1	<0.01
ET increase rate	0.2±0.1	0.3±0.2	<0.01

Note: NS = not significant; OE: oral estradiol; VE: vaginal estradiol; ET: endometrial thickness; ET increase after day 13 = ET on the day of transfer (mm) - ET on day 13 (mm); ET increase rate = ET increase after day 13/ET on day 13.

defined as pregnancy ending before 12 weeks of gestation. Ectopic pregnancy was defined as a pregnancy in which implantation takes place outside the uterine cavity [14].

Statistical analysis

Continuous data are expressed as mean ± standard deviation. Categorical data are presented as Count and percentage (%). Results were analyzed using χ^2 analysis, two tailed Student's t-test, or Fisher's exact test where appropriate. Unless stated otherwise, alpha was set at 0.05. All analyses were performed in the Statistical Package for the Social Science (SPSS) version 16.0.

Results

Demographic data was illustrated in **Table 1**. No differences were found in age, BMI, duration of infertility between these two groups.

Endometrial preparation parameters were shown in **Table 2**. Patients in VE group required longer duration and higher dosage of estradiol (23.9 ± 3.4 vs. 20.4 ± 2.0, $P < 0.01$ and 175.2 ± 51.4 vs. 119.0 ± 23.2, $P < 0.01$, respectively). Endometrial thickness on day 13 and endometrial thickness on the day of transfer in the endometrial thickness group were lower than those in OE group. However, the endometrial thickness increase after day 13 and the endometrial thickness increase rate (the endometrial thickness increase after day 13/the endometrial thickness on day 13) were higher in VE group (1.9 ± 1.1 vs. 1.5 ± 0.9, $P < 0.01$ and 0.3 ± 0.2 vs. 0.2 ± 0.1, $P < 0.01$). Serum progesterone level on the day of progesterone injection and the number of transferred embryos were similar between the two groups.

The FET outcomes were illustrated in **Table 3**. Patients in the two groups obtained comparable implantation rate (46.1% vs. 50.8%). Patients in VE group and OE groups obtained comparable clinical pregnancy rate, live birth/ongoing pregnancy rate. No difference was found in miscarriage rate between the two groups.

Discussion

Previous studies were undertaken to investigate the optimal endometrial preparation methods for FET. However, there is still insufficient evidence to recommend any one particular protocol over another for endometrial preparation with regard to pregnancy rates after embryo transfer [15-18]. The results of our study showed that OEV and VE yielded similar pregnancy results in FET cycles, as indicated by comparable implantation rate and clinical pregnancy rate per transfer cycle. Nevertheless, the data suggested a tendency that in lower endometrial thickness group adding vaginal estradiol can get similar pregnancy rate and implantation rate in inadequate endometrium patients. And we also got another result, which was different from other study [19], the estradiol supplement duration did not influence the implantation rate and clinical pregnancy rate in inadequate endometrium patients.

Estradiol valerate, contained in OEV tablets, is structural similar to 17 beta-estradiol. The bioavailability of estradiol valerate is low, only 3% of ingested estradiol valerate can be metabolized to be available as 17 beta-estradiol. After absorption in the small intestine and first pass through the liver, estradiol valerate is metabolized to 17 beta-estradiol and valeric acid, 17 beta-estradiol is then metabolized to mostly inactive metabolites by cytochrome P450 3A enzymes in the liver and intestinal mucosa,

Table 3. Implantation rate and clinical pregnancy rate per cycle

	OE group	VE group	*P* value
No. of cycles	69	178	
Implantation rate (%)	59/128 (46.1%)	168/331 (50.8%)	NS
Clinical pregnancy rate (%)	40/69 (58.0%)	119/178 (66.9%)	NS
Early miscarriage rate (%)	4/59 (6.8%)	22/168 (11.5%)	NS
Ongoing pregnancy rate (%)	36/69 (52.2%)	97/178 (54.5%)	NS

Note: NS= not significant; OE: oral estradiol; VE: vaginal estradiol; ET: endometrial thickness.

only around 5% of the ingested dose reaches the circulation intact. In contrast, the component in VE tablets is 17 beta-estradiol, which can directly exert its local effect on endometrium after absorption by vaginal epithelium without undergoing liver metabolism, resulting in relative higher bioavailability [20, 21]. Therefore, VE tablets were used in hormonal preparation for patients of with inadequate endometrial thickness.

The success of a frozen-thawed embryo transfer program is closely linked to exact synchronization between endometrial maturation and embryo development [22]. Endometrial preparation can be achieved in a natural cycle after spontaneous ovulation [22, 23] or after artificial preparation of the endometrium with exogenous steroids [23, 24]. Endometrial thickness on the day of transfer is commonly used to predict pregnancy success in FET cycles. Previous studies reported that higher pregnancy rates were achieved when peak endometrial thickness of more than 8 mm [25-30]. A possible explanation for this phenomenon may be that optimal endometrial thickness represents appropriate development in proliferation phase and suitable receptivity after transformation, which is crucial to successful implantation, and therefore to pregnancy outcomes. Indeed, it has been reported that an optimal endometrial proliferation is necessary to enable transformation into receptive endometrium [31-33]. In the present study, the endometrial thickness on cycle day 13 and embryo transfer day were lower in VE group. It may result from the clinicians' decision. However, the endometrium developed better in VE group after cycle day 13, as indicated by higher absolute increase and increasing ratio of endometrial thickness. The clinical and ongoing pregnancy rate did not differ between the VE group and OE group. Therefore, we inferred that vaginal estradiol may improve the endometrial development not only via influencing the endometrial thickness but also the endometrial microenvironment. However, we should also note that endometrial development observed in VE group may also be related to longer duration of administration with estradiol. The higher dosage estradiol in VE group is mainly due to the longer duration of estradiol administration.

FET is an effective, efficient and affordable means of attaining pregnancy for the patient undergoing IVF. It is concluded that adding vaginal 17-β estradiol pills cycles produce acceptable pregnancy rates, ongoing pregnancy rate, implantation rates and early miscarriage rate. Vaginal estradiol supplementation seems to be convenient. For further conclusion, better quality and large randomized controlled trials are needed.

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Disclosure of conflict of interest

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