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Administration of estrogen by different delivery routes in frozen-thawed embryo transfer

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【Abstract】

Objective: To compare the influence of different delivery routes of estrogen therapy in the frozen-thawed embryo transfer(FET).

Methods: A total of 244 patients underwent hormone replacement treatment for FET were divided to two groups:120 patients used 17beta-estrogen(Estradiol gel)by transdermal route in the transdermal group and 124 patients used oral route of estrogen(Estradiol valerate tablet, Progynova)in the oral group.

Results: There was no statistical difference in the mean endometrial thickness($P=0.63$)between the two groups. Comparing with oral group, the estradiol levels were significantly higher($P=0.04$)and drug consumption was significantly lower($P=0.03$)in transdermal group. The period cancellation rate of the transdermal group was significantly lower ($P=0.02$). The biochemical pregnancy rate and clinical pregnancy rate were higher, abortion rate was lower in transdermal group, but there were no statistical difference($P=0.90, 0.54, 0.16$ respectively).

Conclusions: The transdermal route is an effective method for the patients using hormone replace treatment in FET.

Key words: FET; Hormone replace treatment; Endometrial preparation

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Froze-thawed embryo transfer (FET) is an adjunctive method of assisted reproductive technology and plays an important role in periodical FET and the protection of limited fertility. Cycle regimes frequently used for FET are natural cycle, hormone replacement cycle (also called artificial cycle) and ovulation induction cycle [1-2]. For patients with irregular menstrual cycle, hormone replacement cycle is often used to control the synchronization of endometrium and embryo more easily, and to increase implantation rate of frozen-thawed embryos [3, 4]. The most frequently used estrogen for hormone replacement cycle is oral estradiol valerate, a micronized and esterified estradiol that can be resolved quickly and degraded *in vivo* into valeric acid and estradiol. It was observed in clinical practice that when oral dose was up to 15 mg/d, the incidence of common side effects of this drug, such as nausea, dizziness and vomiting etc., increased, or the patients had to discontinue the use of this drug due to irregular vaginal bleeding caused by drug malabsorption. Also, studies have shown that oral estradiol valerate was metabolized into folliculin liver and small intestine [5, 6]. It is known that folliculin can activate oncogenes in endometrium and breast tissue and increase the risk of venous thrombosis [7-11]. Estradiol gel is a topical formulation of 17β-estradiol that is bioactive

without further degradation or metabolism. Transdermal route is an ideal way to deliver the drug as it can avoid the side effects arising from the enterohepatic circulation of drug [12]. This route of administration is at present mainly used in the hormone replacement therapy for postmenopausal and perimenopausal patients. Specifically, the drug is applied over the skin to make it form an subcutaneous estradiol depot that can release the drug slowly into the body and thus maintain a stable serum estradiol level [13] and reduce the risk of venous thromboembolism (VTE). This article was to compare the clinical application of oral estradiol valerate and transdermal 17β-estradiol in FET and to preliminarily discuss the safety and suitability of these two different routes of administration in hormone replacement cycle in FET.

Materials and methods

I. Data source

Three-hundred-and-twenty-one patients who received FET in our center from January 2013 to June 2013 were screened first. Then, based on the exclusion criteria of excluding patients with endometrium diseases such as endometrial polyps and endometriosis, and inclusion criteria of age 23 ~40 (33.25 ± 5.12), duration of infertility of 1~11 years (5.12 ± 2.43), 244 patients with irregular menstrual cycle and who received hormone replacement therapy to prepare the endometrium for FET were selected finally. The patients were randomized into study group i.e., 17β-estradiol (Estradiol gel) transdermal group (120 patients) and control group i.e., oral estradiol valerate (Progynova) group (124 patients). This study was approved by the Medical Ethics Committee of our hospital, and all patients included in the study signed the informed consent form. The general information of the patients in these two groups showed no statistical difference (Table 1).

Table 1 General information of patients in two groups ($\bar{x} \pm s$)

Group	*n*	Age (year)	Duration of infertility (year)	Component ratio of primary and secondary infertility	Number of implanted embryos	Number of implanted good quality embryos
Study group	120	33.52 ± 4.46	5.12 ± 2.57	69/51	1.92 ± 0.68	1.29 ± 0.56
Control group	124	32.70 ± 3.92	5.18 ± 2.63	72/52	2.11 ± 0.48	1.42 ± 0.72

Note: P > 0.05 for all parameters compared in two groups.

II. Methods

1. Drug administration regime: Patients in two groups received B ultrasound examination

on the second day of menstruation to observe if there were dominant follicles in both ovaries and the drugs were given. Patients in control group were given oral estradiol valerate (Progynova, 1 mg/tablet, Bayer, Germany), starting dose 3 mg, once daily. Then the dose was increased to 5 mg t.i.d. based on the serum estradiol level and endometrium thickness. Patients in study group received transdermal 17β-estradiol (Estradiol gel, estradiol 0.06%, Laboratoires Besins International, France), with starting dose of 1.5 mg (2.5 g gel) given on the second day of menstruation, once daily. Then, the dose was gradually increased to 3 mg, b.i.d. Application method of this gel: wash cubital fossa and its surrounding skin using lukewarm water, measure using the ruler in the package, apply the drug evenly over an area of 8 cm in length and 4 cm in width around the center of cubital fossa, massage gently till the gel was adequately absorbed. When blood estradiol concentration was up to 1830 pmol/L [14], the drug was used over 12 days [15-17] and endometrium thickness was ≥ 8 mm [18, 19], progesterone soft capsules (Laboratoires Besins International, France) to further enhance the proliferation of endometrial glands and blood vessels, making endometrium change into secretory phase and thus preparing for embryo implantation. See table 2 for detailed drug administration regime and table 3 for comparison of various parameters in two groups on progesterone day.

Table 2 Different drug administration regime

Drug administration regime	1d	4d	6d	8d	10d	12d	Progesterone day	ET day
Estradiol gel (mg)	1.5	3	3	4.5	4.5	6	6	6
Progynova (mg)	3	6	8	10	12	15	15	15
Progesterone (mg)	-	-	-	-	-	-	400	500

Table 3 Comparison of various parameters in two groups on progesterone day

Group	*n*	Endometrium thickness (mm)	Serum estradiol (pmol/L)	Serum folliculin concentration (pmol/L)	Total amount of drug used (mg)
Study group	120	10.52 ± 1.86*	2362.2 ± 118.7*	556.8 ± 44.6*	42.51 ± 5.33*
Control group	124	10.76 ± 1.99	1920.1 ± 78.5	1850.9 ± 59.4	108.64 ± 8.46

Note: *P < 0.05, compared with control group.

2. Selection of frozen-thawed embryos: Vitrification and rapid thawing method was used to select embryos. The third day embryo that contained 6~8 evenly sized cells, with fragmentation of <15% and scored as A or B, or the fifth day embryo that contained grade 3-4 blastocyst was chosen. The third day embryo was cultured till it changed into

blastocyst and then it was implanted. The third day blastocyst was thawed for 2 hours before implantation.

3. Cycle cancellation: For patients who had endometrium < 8 mm or >14 cm, and serum estradiol level < 1098 pmol/L after the use of the drug more than 30 days, this cycle of implantation was cancelled.

4. Observation parameters: Total amount of estradiol used on progesterone day in two groups was calculated, and endometrium thickness and shape on progesterone day in two groups was measured. In addition, serum estradiol and folliculin concentration during the use of drugs, FET outcomes in both groups, cycle cancelation rate, drug side effects were evaluated.

III. Statistic methods

SPSS 16.0 software was used for data analysis. *t* test was used for measurement data and χ^2 test for enumeration data. P<0.05 signified statistical difference.

Results

On progesterone day, endometrium thickness in two groups showed no significant difference. Serum estradiol level in study group was higher than that in control group with statistical significance (P = 0.04), and serum folliulin concentration decreased markedly (P = 0.03) (Table 3). Cycle cancellation rate in study group decreased markedly than that in control group (P = 0.02); biochemical pregnancy rate and clinical pregnancy rate increased, and abortion rate decreased, but showed no statistical difference (Table 4). Twelve patients in control group had swelling pain in the breasts, and 5 patients experienced nausea and vomiting.

Table 4 Clinical outcome in two groups [*n* (%)]

Group	*n*	Cycle cancellation rate	Biochemistry pregnancy rate	Clinical pregnancy rate	Abortion rate
Study group	120	1 (0.83)*	59 (49.17)	54 (45.00)	2 (1.67)
Control group	124	8 (6.45)	60 (48.39)	51 (41.13)	6 (4.84)

Note: *P < 0.05, compared with control group.

Discussion

For patients with regular menstruation cycles, natural cycle can avoid side effects of exogenous hormones, reduce the patients' economic burden, and make synchronization of endometrium and embryo more similar to natural pregnancy. For patients with irregular menstruation cycles, ovulation disorders and low ovarian reserve, hormone replacement

cycle is frequently used clinically to simulate artificial endometrium, or ovulation cycle in order to perform FET [1, 2]. Of these, the former method can avoid a series of side effects, such as waste of follicles and ovarian hyperstimulation caused by ovulation, and can also control the synchronization of endometrium and embryo well and thus increase clinical pregnancy rate. Furthermore, recent studies have shown that the use of hormone replacement cycle produces similar implantation rate and pregnancy rate to natural cycle [20, 21].

I. Bioavailability of different routes of administration and effects on endometrium

At present, oral route is the most frequently used administration route for FET hormone replacement cycle. The bioavailability of oral estradiol is 3% ~ 5%. As different people have various absorption and metabolism rates, serum estradiol level fluctuates in a big range. Patients with ovulation disorders may have unsatisfactory reaction to exogenous estrogen due to their own characteristics in endocrine system, and thus endometrium thickness, shape and serum estradiol level are not satisfactory despite the use of large quantity of estrogen. In addition, some patients have drug malabsorption during the use of the drug, the decrease in the blood estrogen concentration leads to endometrial breakthrough bleeding and subsequent cancellation of implantation cycle. Duponta et al [22] reported that when 17β-estradiol was given transdermally, estradiol was absorbed through the skin and stored in the cuticle, then slowly permeated into dermis and blood vessels. The bioavailability was up to 10%. As transdermal estradiol has higher bioavailability than oral estradiol, transdermal route generates ideal increase in serum estradiol level, reduce the amount of drug used and the patient's economic burden, and increase the patient's compliance. When estradiol reached a certain level, further increase might not increase pregnancy rate, and endometrium thickness and shape in two groups showed no significant difference. However, the special sustained release system of transdermal route can better simulate human estradiol level in natural cycle, and increase endometrial receptivity. The specific mechanism needs further studies. This study showed that the patients in study group had higher clinical pregnancy rate and lower abortion rate than control group, but the difference was not significant. This conclusion needs further studies of large samples for confirmation.

II. Side effects of different routes of administration

Studies on the use of the two drugs as hormone replacement therapy in perimenopausal women showed that hormone replacement therapy could increase the risk of venous thrombosis in perimenopausal women 2-4 folds [8, 9]. The main reason is that oral route accelerated the thrombin production. Large dose of oral estradiol valerate are transformed

into a large amount of folliculin in liver and intestines, inducing the production of coagulation proteins. Bagot et al [8] also reported their study that folliculin concentration was significantly associated with the rate of thrombin production. In the meantime, a large amount of folliculin resulted in breast hyperplasia and proto-oncogene expression. Our study showed that the serum folliculin concentration in the control group was 3 fold that of study group and the difference was significant. Thus it was concluded that transdermal route might produce lower risk of venous thrombosis than oral route. However, during the performance of FET, whether the large amount of folliculin produced by oral Progynova increases the risk of cardiovascular diseases and venous thrombosis needs further large-sampled studies, in-depth follow-up and risk evaluation. In our study, during the use of the drugs, 12 patients in control group had breast swelling and pain, and 5 patients had nausea and vomiting, while no patients in study group experienced any side effects. This result indicated that transdermal route could be used in FET hormone replacement cycle.

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