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Effect of different luteal support schemes on clinical outcome in frozen-thawed embryos transfer cycles

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Summary

Objective: To evaluate the clinical outcome of frozen-thawed embryo transfer (FET) when using different luteal support schemes. *Study Design:* Retrospective analysis of FET cycles was performed from June 2013 and December 2013. Infertile women, who underwent FET cycles utilizing embryos cryopreserved on day 3 post-insemination following an initial fresh IVF cycle. Patients were divided into three groups according to the luteal support scheme. Grade A (oral administration of progesterone, n=156), Group B (vaginal administration of progesterone, n=345), Group C (dissolved progesterone in oil with intramuscular infection, n=885), and group C was divided into two subgroups according to with (subgroup C1, n=521) or without (subgroup C2 ,n=364) human chorionic gonadotrophin (hCG) injected intramuscularly. The authors compared patients' characteristics and the pregnancy outcomes of each group. *Results:* There was no difference in the patient characteristics of each group. There was no difference in the implantation rate or clinical and ongoing pregnancy rate among oral, vaginal, and intramuscular progesterone groups. The abortion and ectopic pregnancy rates were not significantly different among the three groups. *Conclusion:* Oral progesterone in the FET cycles is convenient and has similar pregnancy outcomes compared with intramuscular or vaginal administration.

Key words: Frozen-thawed embryo transfer; Luteal support scheme; Pregnancy outcome.

Introduction

The first successful pregnancy undergoing frozen-thawed embryo transfer (FET) was reported in 1983 and this strategy has been progressively used in assisted reproductive technology (ART) [1]. It is well known that FET has become a routine treatment of infertility. Surplus embryos in fresh cycles were cryopreserved for subsequent FET cycles. FET was often a better choice when mild stimulation or luteal phase ovarian stimulation was used in fresh cycles [2].

FET can provide several benefits in ART such as decreasing risk of multiple pregnancy and ovarian hyperstimulation syndrome, increasing cumulative pregnancy rate [3, 4]. FET perhaps avoids embryo-endometrium asynchrony which is a major cause of impaired endometrial receptivity after ovarian stimulation [5]. FET is safe and has similar neonatal outcome in terms of prematurity, low birth weight, stillbirth, neonatal death, and major malformation compared with fresh ET [6].

Due to the important role of corpus luteum, various luteal phase support schemes are applied, aiming to improve the FET success rate. Several medicinal products containing progesterone are in widespread use orally, intramuscular injection or vaginally for support of luteal function during assisted reproduction. In fresh IVF cycles, both intramuscular and vaginal progesterone have become the standard of care for luteal phase support. However, the dosage, type, route of administration, and the length of treatment for luteal phase support in FET cycles still remains conflicting. Therefore, the present study was conducted to evaluate clinical outcomes of FET with different luteal support schemes.

Materials and Methods

Retrospective analysis was carried out with FET cycles between June 2013 and December 2013 in Children's Hospital of Shanxi & Women Health Center of Shanxi. A total of 1,386 cases with FET cycles were enrolled. In order to avoid potential bias, only the first two FET cycles of each patient was included. The cycles with preimplantation genetic diagnosis and cancelled cycles were excluded.

All FET cycles were classified as Grade A to Grade C, according to luteal support scheme. These main outcomes were analyzed including the implantation rate, clinical pregnancy rate, ongoing pregnancy rate, abortion rate and ectopic pregnancy rate. Other parameters, such as age, duration of infertility, and endometrium thickness on the day of ovulation or day 3 before FET in HRT cycles were also analyzed.

FET protocol

A transvaginal sonography was performed in all patients on day 2 or 3 of the cycle for exclusion of ovarian cysts. Women with a spontaneous cycle did not have any medication during their follicular phase, and second transvaginal sonography was performed

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Table 1. — *Patient characteristics of FETs ($\bar{x} \pm sd$).*

Characteristics	Group A	Group B	Group C		p-value
			C1	C2	
Age (years)	31.19 ± 4.59	30.58 ± 4.31	30.42 ± 4.54	30.20 ± 4.49	NS
Duration of infertility (years)	4.11 ± 0.42	4.03 ± 0.36	3.91 ± 0.38	4.04 ± 0.40	NS
*EM thickness (mm)	10.45 ± 1.75	10.22 ± 1.88	10.08 ± 1.63	10.23 ± 1.78	NS

Values are presented as mean ± standard deviation. FET: frozen-thawed embryo transfer; NS: not significant; EM: endometrium.
* presents the value of the day of ovulation or day 3 before FET in HRT cycles.

on day 10. Women undergoing an ovarian stimulation cycles started on the day 3 and received hMG (75I U/d i.m.).Then ultrasounds were performed until the endometrium thickness was at least eight mm and triple line in ultrasonography and the main follicle reached 18–22 mm. At this point the ovulation was induced with 10,000 IU hCG or triptorelin (triptorelin acetate injection) 0.2mg s.c. Women undergoing an artificial cycle started on day 3 of their natural menstrual cycle and received oral estradiol valerate, with an analogous protocol: two pill on days 3, 4, 5, 6, and 7; four pills on days 8, 9, and 10; six pills on days 11, 12, and 13; then the endometrium was transformed; and six pills on day 14 to 30. If the endometrial thickness was less than eight mm the transfer was cancelled and shifted to the next cycle.

Evaluation of thawed embryos

Using vitrification freezing and rapid thawing protocol, all the frozen-thawed embryos underwent three days culture in vitro. Embryos were assessed for quality from the following aspects: morphology of cells, and the number of cell and cellular debris. All transferred embryos are no less than seven blastomeres and cellular debris <10%.

Luteal support

Progesterone administration was commenced to transform endometrium on the day of ovulation or day 3 before FET in HRT cycles. Administration of 40 mg progesterone (progesterone injection) intramuscularly was given when the endometrium reached a thickness of eight mm or maximum. Administration with 40 mg, and 80 mg or 40 mg progesterone were used, respectively, in the following two days. Embryos were thawed and transferred after three days of progesterone administration. Then the different progesterone administration scheme was used as luteal phase support.

Group A: Oral administration of progesterone. The luteal phase was supported with oral micronized progesterone tablets (200mg, three times daily) or duphaston (20 mg, three times daily), n= 156.

Group B: Vaginal administration of progesterone. The luteal phase was supported with vaginal micronized progesterone tablets (200 mg, three times daily) or vaginal progesterone gel (90 mg/d) n= 345.

Group C: Progesterone in oil injected intramuscularly (IMP). Progesterone injection (80 mg, im, qd), n = 885.Subgroup C1: IMP with human chorionic gonadotrophin (hCG). Beside IMP, the luteal phase was supported with hCG for Injection (2,000 I.U., im.QoD×3). n=521.Subgroup C2: IMP only, n=364. The luteal phase support continued until the 10th gestational week.

Diagnosis of pregnancy and follow-up

In the present study, biochemical pregnancy was defined as a serum hCG >35 IU/L two weeks after transfer but then declined to negative. If fetus with fetal heart activity in the seventh gestational week was visualized by ultrasonography, it was considered clinical pregnancy. Ongoing pregnancy was defined as the presence of a gestational sac on transvaginal ultrasound at the fifth to seventh weeks of gestation and the existence of a fetal heartbeat at approximately 12 weeks gestation. Spontaneous abortion: loss of fetus with gestational age before 20 weeks. Ectopic pregnancy: the diagnosis of extra uterine pregnancy confirmed by laparoscopy or ultrasound. The authors compared patients' characteristics and the pregnancy outcomes of each group.

Table 2. — *Pregnancy outcomes of FETs.*

	Group A	Group B	Group C	*p*-value
Implantation rate (%)	18.47	18.50	18.32	0.992
Biochemical pregnancy rate (%)	39.10	40.00	36.61	0.508
Clinical pregnancy rate (%)	36.54	37.10	32.99	0.332
Ongoing pregnancy rate (%)	30.13	29.86	23.95	0.051
Abortion rate (%)	17.54	16.41	22.95	0.261
Ectopic pregnancy rate (%)	0.00	3.13	2.05	0.420

Table 3. — *Pregnancy outcomes of subgroups C1 and C2.*

	Subgroup C1	Subgroup C2	*p*-value
Implantation rate (%)	17.54	18.86	0.415
Biochemical pregnancy rate (%)	35.99	37.04	0.748
Clinical pregnancy rate (%)	32.42	33.40	0.760
Ongoing pregnancy rate (%)	25.27	23.03	0.442
Abortion rate (%)	19.49	25.29	0.248
Ectopic pregnancy rate (%)	2.54	1.72	0.689

Statistical analysis

Statistical analysis was performed using SPSS 13.0. Continuous characteristic values were compared using t-test. Ordinary were analyzed by the χ^2-test. A p-value < 0.05 was reported as statistically significant.

Results

The results showed there was no significant differences in the patient characteristics such as age, duration of infertility, and endometrium thickness on day 3 before the transfer was noted among groups (Table 1).

Data on early pregnancy outcome in terms of implantation rate, biochemical pregnancy, ongoing pregnancy rate, spontaneous abortion, and ectopic pregnancy are detailed in Tables 2 and 3. There were no losses to follow-up pregnancies in this study.

Discussion

It is well known that luteal support with progesterone is necessary for successful implantation of the embryo following egg collection and embryo transfer in IVF cycle. Early studies showed the effect of different luteal support scheme on clinical outcome in fresh embryos transfer cycles.

Progesterone can be administered by several routes. The oral, intramuscular (IM), and vaginal routes have been chosen frequently in the past. There are disadvantages to each method. The oral route is ineffective, since progesterone has a liver first-pass effect, and is associated with a high rate of metabolites which may result in side effects such as a somnolent effect [7]. Friedler *et al.* and Licciardi *et al.* showed oral progesterone to be associated with significantly lower implantation and pregnancy rates, and higher miscarriage rates, or both, compared with IM or vaginal administration [8,9].

Progesterone in oil injections serve as an effective route for without first passing through the liver. It results in a depot effect and continuous release of progesterone over time, with a long elimination half-life, allowing once-daily injections, but in some cases intramuscular progesterone injections may be painful, especially if prolonged for up to ten weeks.

Vaginal progesterone may be inconvenient, though progesterone reaches the uterus directly and endometrial progesterone concentration reaches a steady state within five hours, the short half-life of natural progesterone and intermittent peaks of absorption with vaginal administration, it is generally required to be inserted multiple times daily and may cause vaginal irritation in some women. Uterine contractility and endometrial wave activity are higher after vaginal administration compared with intramuscular administration, which might adversely affect the implantation of the embryo [10]. However, the effect of different luteal support scheme for FET cycles still remains conflicting. Smitz *et al.* showed that vaginal administration had shown to be at least as effective as IM administration, and significantly more effective then oral treatment [11]. Shapiro *et al.* retrospective analyzed 682 FET cycles in which IMP was used for luteal support and 238 FET cycles in which vaginal progesterone gel was used. They found that the implantation, clinical pregnancy, and live birth rates were not significantly different between IMP and vaginal progesterone gel group [12]. Guo *et al.* reported that there were no significant differences in the clinical outcomes between the patients receiving dydrogesterone and intramuscular progesterone as luteal phase support in either natural cycle FET or HRT FET [13]. Lee *et al.* found that the pregnancy outcomes of natural cycle FET were similar with or without luteal phase support [14].

In the present study, the authors found that there was no difference in implantation rate, clinical and ongoing pregnancy rates, and abortion and ectopic pregnancy rates among oral, vaginal and intramuscular progesterone groups. Consequently oral progesterone in FET cycles is convenient and has similar pregnancy outcomes compared with IM or vaginal administration.

Both hCG, which stimulates steroid production in the corpus luteum, and progesterone administration in the luteal phase are effective and significantly improve the clinical pregnancy rate [15]. However, in this study, the authors found that there was no difference in the clinical outcomes between groups C1 and C2.

In this study, the authors did not compare neonatal outcome among the groups and different clinical outcomes among different types of cycles. Aflatoonian *et al.* showed that if the pregnancy reached 20 weeks of gestation, FET did not adversely affect neonatal outcome in terms of birth weight prematurity, LBW, stillbirth, neonatal death, and major malformation compared with fresh ET [6]. Early studies showed a trend towards similar pregnancy rates and live birth rates with the administration of FET during a spontaneous cycle compared to FET during an artificial cycle or using GnRH in the artificial cycle [16, 17].

Conclusion

The present results showed that oral progesterone in the FET cycles as luteal support had similar clinical outcomes in terms of the implantation, clinical and ongoing pregnancy rates, and abortion and ectopic pregnancy rates compared with intramuscular and vaginal routes. An oral route has the advantage of convenient manipulation and better compliance, so it probably has a good prospect for clinical application.

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