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Transfer of Cryopreserved-Thawed Embryos in a Cycle Using Exogenous Steroids with or Without Prior Gonadotropihin-Releasing Hormone Agonist

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The aim of this study was to investigate the outcome of frozen-thawed embryo transfer in exogenous estrogen plus progesterone without GnRH agonist and with GnRH agonist cycles for endometrial preparation in women with regular menstrual cycles. This study was designed as a prospective randomized clinical trial. In total, 60 patients were randomly divided into two treatment groups. In both groups, Estradiol Valerate was taken orally at 2 mg day^{-1} from day 1 to day 4, at 4 mg day^{-1} from day 5 to day 9 and at 6 mg day^{-1} from day 10 onwards up to the day of the pregnancy test. In day 13 of cycle, an ultrasound examination was performed. If the endometrial thickness was more than 8 mm, progesterone would be administered, i.m, at a dose of 100 mg day^{-1} and the dose of estradiol would be increased to 8 mg day^{-1} in all women if the endometrial thickness was less than 8mm. Group A (n = 30) commenced steroid supplementation without prior pituitary desensitization; whereas group B (n = 30) had pituitary suppression with using Busereline acetate (0.5 mg SC), a GnRH agonist, prior to steroid hormone administration that it was started in the mid luteal phase (day 21) of the menstrual cycle and was continued until day 11 of cycle. Present results showed that there was no significant difference in the woman's age, duration and etiology of infertility, number of embryos transferred and the score of transferred embryos between the groups. The implantation rate, chemical and clinical pregnancy rates were 1.6, 10 and 6.6% in the group A and 3, 13.3 and 10% in the group B. There was no significant difference in implantation and pregnancy rates between both groups. In conclusion, endometrial preparation for FET based exclusively on steroid administration appears to be as effective as the protocol involving preliminary desensitization with a GnRH agonist.

Key words: Embryo cryopreservation, endometrial preparation, frozen-thawed embryo transfer, GnRH agonist, artificial cycle, pregnancy outcome

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INTRODUCTION

An important Factor for Implantation in frozen-thawed embryo transfer (FET) is exact synchronization between endometrial maturation and embryo development (Nardo *et al*., 2003). In fact, cryopreservation allows the transfer of embryos at a point in time remote from ovarian stimulation and offers a variety of options for the timing of embryo transfer and the method of endometrial preparation (Cohen *et al*., 1988).

FET has been successfully performed after artificial preparation of endometrium with exogenous steroids. The advantages of the programmed embryo transfer cycles with using estrogen and progesterone alone are simplicity and decreased expenses (Muasher *et al*., 1991; Schmidt *et al*., 1989).

Several studies have reported the successful use of exogenous estrogen and progesterone without previous ovarian suppression by Gonadotropin Releasing Hormone (GnRH) agonist for artificial preparation of endometrium in women with functioning ovaries who were undergoing FET (Lelaidier *et al*., 1992; Queenan *et al*., 1997).

Currently, the most popular protocol involves pituitary down-regulation with a GnRH agonist to avoid spontaneous ovulation before sequential administration of 17-β estradiol and progesterone (Younis *et al*., 1996). El-Toukhy *et al*. (2004) have showed that artificial preparation of the endometrium with estrogen and progesterone following pituitary desensitization with a GnRH agonist may play a crucial role. The main advantage of pretreatment with GnRH agonist is that the women are able to select the date of embryo thawing and transfer, thus it minimizes the fear and anxiety of cycle cancellation. In addition; the risk of cycle cancellation is avoided by this treatment. However, treatment with a GnRH agonist is costly; there is a risk of hypoestrogenic side effects before hormonal replacement and the preparation is lengthy (Schmidt *et al*., 1989).

The present study investigated the outcome (such as implantation, chemical and clinical pregnancy rates) of frozen-thawed embryo transfer in exogenous estrogen plus progesterone without GnRH agonist and with GnRH agonist cycles for endometrial preparation in women with regular menstrual cycles.

MATERIALS AND METHODS

The study protocol was approved by committee of Yazd Research and Clinical Center for Infertility. All patients (<30 years) had previously undergone IVF or ICSI with embryo cryopreservation, had regular menstrual cycles and gave written informed consent prior to inclusion in the study.

Cryopreservation was performed at the pronuclear stage with employing 1, 2-propanediol and sucrose as cryoprotectants (Edgar *et al*., 2000). Freezing and thawing solutions consisted of the cryoprotectants in a PBS plus HEPES medium with 20% W/V human serum albumin.

Patients who volunteered to participate were randomly divided into two treatment groups between January 2005 and October 2006 using a computer generated randomization list.

Group A (n = 30) commenced steroid supplementation without prior pituitary desensitization; whereas group B (n = 30) had pituitary suppression prior to steroid hormone administration with using Busereline acetate, a GnRH agonist (suprefact; Hoechst AG, Germany) (0.5 mg SC) starting in the mid luteal phase (day 21) of the menstrual cycle and was continued until day 11 of cycle.

In both groups, Estradiol Valerate (Elleste Solo; Shire pharmaceuticals, Hants, UK) was taken orally at the dose of 2 mg day^{-1} from day 1 to day 4, at the dose of 4 mg day^{-1} from day 5 to day 9 and at the dose of 6 mg day^{-1} from day 10 onwards up to the day of pregnancy test.

In day 13 of cycle, an ultrasound examination was performed. It was used to assess endometrial thickness. The endometrial thickness (mm) was measured at the greatest diameter perpendicular to the midsagittal plane in the fundal region, including both layers of the endometrial cavity. If the endometrial thickness was more than 8 mm, progesterone in oil (prontogest; Amsa, Rome, Italy) would be administered i.m, at a dose of 100 mg day^{-1} and transfer of two frozen-thawed Embryos to the uterus was performed on day 2 after Progesterone administration by using Labotect catheters (Labor-technik, Germany).

The dose of estradiol would be increased to 8 mg day^{-1} in all women if the endometrial thickness was less than 8 mm. Then the women followed up with ultrasonography and embryo transfer would be performed if the endometrial thickness was greater than 8 mm.

In this study, only frozen-thawed embryos with best quality (score = 18) were transferred.

Hormonal treatment was continued for up to 14 days. If the pregnancy was confirmed by serum BHCG>50 m IU mL^{-1}, 2 weeks after thawed embryo transfer, the estradiol valerate and progesterone would be continued (the progesterone was increased to 100 mg three times a day) until approximately 12 weeks gestation. If this test was negative, the medications would be discontinued.

Implantation rate was defined as the Number of sacs with fetal heart beat per number of embryos transferred.

Biochemical pregnancy was determined by a serum β-HCG greater than 50 m IU mL^{-1} and clinical pregnancy was defined as the observation on ultrasound scanning of a gestational sac with fetal heart beat between 4 and 5 weeks after the positive pregnancy test.

Table 1: Comparison of baseline characteristics in the group A and group B

Variables	Group A	Group B	p-value*
No. of subjects	30	30	
Mean (±SD) age of women, years	28.10±6.3	27.80±4.4	NS*
Mean (±SD) duration of infertility, years	8.70±5.4	8.8±3.8	NS
Endometrial thickness (mm)	9.06±1.7	9.47±1.2	NS
No. of embryos transferred	2.17±0.7	2.13±0.7	NS
Score of embryos transferred	18.10±1.5	19.14±1.7	NS
Causes of infertility			
Male factor (%)	16(53.3)	20(66.7)	
Tubal factor (%)	4(13.3)	3(10)	
Ovary factor (%)	9(30)	2(6.7)	
Unexplained (%)	1(3.3)	4(13.3)	
Mixed (%)		-1(3.3)	NS

Note: Data are expressed as mean±standard deviation, * T-test and chi-square test. *NS = Not Statistically significant

Table 2: Pregnancy outcome of frozen-thawed embryo transfer in both groups

Variables	Group A n = 30	Group B n = 30	p-value*
Implantation rate	1.6	3	0.07
Chemical pregnancy rate	10%(3/30)	13.3%(4/30)	0.50
Clinical pregnancy rate	6.6%(2/30)	10%(3/30)	0.37

*Exact test

Miscarriage rate was defined as the Termination of pregnancy before 20 weeks based upon the date of the first day of the last normal menses.

The data were analyzed with the student's Statistical Package for Social Sciences (SPSS) version 13. Rates were compared using the Chi-square test or the exact test when it was necessary. Groups of values were compared using the t-test. A p-value of <0.05 was considered statistically significant.

RESULTS

In this study, the woman's age, duration and etiology of infertility, number of embryos transferred and the score of transferred embryos had no significant differences between the groups (Table 1).

The implantation rate, chemical and clinical pregnancy rates were 1.6, 10 and 6.6% in the group A and 3, 13.3 and 10% in the group B. There was no significant difference in implantation and pregnancy rates between both groups (Table 2). In addition; the number of miscarriage was one in both groups. Twin pregnancies were not seen in any groups.

DISCUSSION

The success of a frozen-thawed embryo transfer program is closely linked to exact synchronization between endometrial maturation and embryo development (Cohen *et al*., 1988; Nardo *et al*., 2003).

This is a prospective randomized study that compared the results of frozen-thawed embryo transfer cycles in an artificially prepared endometrium with and without prior use of GnRH agonist for pituitary suppression in women with functioning ovaries.

Several studies have reported a successful use of exogenous estrogen and progesterone with no previous ovarian suppression by GnRH agonist for artificial preparation of endometrium in women with functioning ovaries who were undergoing frozen-thawed embryo transfer (Lelaidier *et al*., 1992; Queenan *et al*., 1997). However, Simon *et al*. (1998) compared endometrial preparation with and without previous GnRH agonist suppression. In that trial micronized estradiol was given orally at a fixed daily dosage of 6 mg in women not treated with GnRH analogue and 4 mg in women with previous GnRH analogue treatment. In addition; they used micronized progesterone tablet as vaginally in a dose of 300 mg three times a day. Dal Prato *et al*. (2002) compared endometrial preparation with transdermal estradiol in a step-up protocol, with and without GnRH agonist pretreatment. In present study, estradiol valerate was given in both groups orally at the dose of 2 mg day^{-1} for 4 days, at the dose of 4 mg day^{-1} for 5 days and at the dose of 6 mg day^{-1} until a pregnancy test was performed. In addition to, progesterone was administered with dose of 100 mg day^{-1} as i.m.

The pregnancy rate was assessed after the transfer of FET in a natural or down regulated hormonally cycle in some studies. Their findings suggested that both EFT protocols were equally effective in terms of implantation rate and pregnancy outcome in women with regular menstrual cycle (Gelbaya *et al*., 2006; Sathanandan *et al*., 1991; Queenan *et al*., 1994, Al-Shawaf *et al*., 1993). Present results indicated that endometrial preparation with GnRH agonist pretreatment did not increase the success rate of frozen-thawed embryo transfer. In fact, the success rate was similar to that achieved exogenous steroid alone. Present results were similar to that reported by Dal Prato *et al*. (2002) and Simon *et al*. (1998).

Some studies suggested that suppression with a GnRH agonist was not necessary for endometrial preparation for frozen-thawed embryo transfer (Simon *et al*., 1998; Queenan *et al*., 1997; Simon *et al*., 1999).

In this study, at first the low dose of estradiol valerate was used for endometrial preparation. Simon *et al.* (1999) chose a high fixed dose of micronized estradiol (6 mg day^{-1}); whereas Pattinson *et al.* (1992) who used a lower fixed dose of micronized estradiol (2 mg day^{-1}) starting from the second to fifth day of the cycle. The results of above studies about pregnancy outcome were similar. It indicated that the starting dose of estradiol did not seem to be as important.

In conclusion, endometrial preparation for frozen-thawed embryo transfer based exclusively on steroid administration appeared to be as effective as the protocol involving preliminary desensitization with a GnRH agonist and had a similar success rate. The treatment with exogenous steroid with no previous GnRH agonist was convenient and reduced the cost of the procedure and minimized pharmacological treatment.

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