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ARTICLE

Spontaneous ovulation versus HCG triggering for timing natural-cycle frozen–thawed embryo transfer: a randomized study

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Dr Ariel Weissman graduated from the Hadassah-Hebrew University Medical School in Jerusalem in 1988. In 1994 he completed his residency in obstetrics and gynaecology at the Kaplan Medical Centre, Rehovot, where he spent another 2 years working as a senior physician in the IVF unit. He then pursued a 2-year research and clinical fellowship with Bob Casper at the Division of Reproductive Sciences, University of Toronto, Canada. His main focus of research was transplantation of human ovarian tissue in immunodeficient mice. In 1998, he joined the IVF unit at the Wolfson Medical Centre, where he currently holds a position of senior lecturer.

Abstract In ovulatory patients, frozen–thawed embryo transfer (FET) is commonly performed during a natural cycle (NC). The objective was to compare serial monitoring until documentation of ovulation with human chorionic gonadotrophin (HCG) triggering, for timing NC-FET. Sixty women with regular menstrual cycles undergoing NC-FET were randomized into two groups: group A ($n = 30$) had FET in a natural cycle after ovulation triggering with HCG; group B ($n = 30$) had FET in a natural cycle after detection of spontaneous ovulation. The main outcome measure was the number of monitoring visits at the clinic per cycle. Secondary outcome measures included implantation rate, clinical pregnancy and live-birth rates. Both groups were similar in terms of demographic characteristics and reproductive history. Clinical and laboratory characteristics of fresh and frozen cycles and pregnancy and delivery rates were comparable for both groups. The number of monitoring visits in group A (3.2 ± 1.4) was significantly lower than in group B (4.7 ± 1.6) ($P = 0.002$). In patients undergoing NC-FET, triggering ovulation by HCG can significantly reduce the number of visits necessary for cycle monitoring without an adverse effect on cycle outcome. Ovulation triggering can increase both patient convenience and cycle cost effectiveness.

KEYWORDS: frozen–thawed embryo transfer, IVF, monitoring, natural cycle, ovulation

Introduction

Cryopreservation of supernumerary embryos following IVF has become an important feature in assisted reproduction technology and is widely practiced as a safe and cost-effective method to increase cumulative pregnancy rates per oocyte retrieval. The current trend towards decreasing the number of embryos being transferred and the increased

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implementation of single-embryo transfer (SET) policy in many IVF programmes emphasize the relevance and importance of frozen–thawed embryo transfer (FET).

Synchronization between embryonic and endometrial development is a major consideration in preparation for FET. Care needs to be taken so that the age of the embryos after thawing corresponds to the age of the endometrium on the day of embryo transfer. FET has been carried out through different cycle regimens including: spontaneous ovulatory cycles (natural cycle); cycles in which ovulation is induced by drugs (ovulation induction cycle); and cycles in which the endometrium is artificially prepared by exogenous oestrogen and progesterone (artificial cycle). All regimens seem to be similar in their efficacy. A recent Cochrane review comparing cycle regimens for FET has concluded that at the present time there is insufficient evidence to support the use of one intervention in preference to another (Ghobara and Vandekerckhove, 2008).

In ovulatory patients, FET is commonly performed during a natural cycle (NC) (Byrd, 2002). The NC offers the advantages of utilizing the natural physiological process of endometrial preparation for implantation and decreases medical intervention as compared with hormone replacement or stimulated cycles. These advantages make NC-FET preferable to many women. For the purpose of synchronization between the endometrium and the frozen embryos, the day of spontaneous ovulation in the NC corresponds to the day of egg retrieval in the 'fresh' IVF cycle. Thawing and transferring of embryos is scheduled according to the stage at which embryos were frozen. Patient monitoring prior to FET in a NC consists of serial blood and ultrasound testing until the detection of ovulation (al-Shawaf et al., 1993). Alternatively, human chorionic gonadotrophin (HCG) may be utilized to trigger ovulation in the presence of a mature follicle and satisfactory endometrial development. This latter approach may simplify the monitoring process by saving the patients and the clinic the time and expense involved in extra visits necessary for documentation of ovulation.

A recent retrospective study compared serial monitoring until documentation of ovulation, with HCG triggering, for timing FET in natural cycles (Weissman et al., 2009). It was demonstrated that triggering ovulation by HCG can significantly reduce the number of visits necessary for cycle monitoring without an adverse effect on cycle outcome.

In order to validate these findings, this study conducted a randomized trial focusing on the same outcome measures. The aim was to compare the number of clinic visits and cycle outcome between ovulation triggering by HCG and serial monitoring until documentation of ovulation in patient preparation for NC-FET.

Materials and methods

The study design was a prospective, randomized trial conducted at a tertiary referral university hospital.

Patients

Sixty patients that underwent NC-FET in the study centre's IVF unit between April 2006 and December 2008 were included in the study. All women had regular ovulatory cycles and had previously undergone either conventional IVF or intracytoplasmic sperm injection (ICSI) as indicated, with cryopreservation of supernumerary embryos. Patients had to be $\leq$38 years old at the time of embryo freezing. Exclusion criteria were: (i) the use of testicular spermatozoa for ICSI; and (ii) basal FSH concentrations $\geq$12 IU/l. Patients could participate in the study only once. With a sample size of 28 subjects in each group, the present study was designed to have 80% power to detect a true between-group difference of 1 ± 1.3 monitoring visits by intervention group, using the t-test for independent samples and assuming a two-sided alpha of 0.05. The actual sample size of 30 reflects 'insurance' against missing data.

The study was approved by the Institutional Review Board and each patient gave her written informed consent. The study was a non-blinded open trial. Patients were randomized into two groups before entering the treatment cycle according to a computer-generated list by using opaque sealed envelopes: Group A (HCG group) included 30 cycles, in which women were administered HCG to trigger ovulation upon visualization of a dominant follicle on transvaginal sonography (TVS); and Group B (expectant management) included 30 cycles, in which HCG was not administered and monitoring was continued until ovulation was detected and documented.

Fresh cycle characteristics

The stimulation protocols of 'fresh' cycles leading to egg retrieval and embryo cryopreservation were standard protocols utilized in the study unit as described previously (Levran et al., 2002; Weissman et al., 2003; Weissman et al., 2009). Patients' and fresh cycle characteristics are summarized in **Table 1**. A slow freezing protocol was used. Only high-quality surplus embryos were cryopreserved. The technique for freezing and thawing of embryos has also been recently described in detail (Weissman et al., 2009).

NC-FET protocols

In group A, 30 cycles were monitored until the criteria for ovulation triggering by HCG were met. Criteria for HCG administration included: (i) visualization of a leading follicle $>$17 mm in diameter by TVS; (ii) serum oestradiol concentration $>$150 pg/ml; and (iii) serum progesterone concentration $<$1 ng/ml. In group B, 30 cycles were monitored until documentation of ovulation. Criteria for ovulation detection included: (i) drop of serum oestradiol concentration compared with the previous test; (ii) rise of serum progesterone concentration $>$1.5 ng/ml; and (iii) disappearance or typical change in the shape of the leading follicle. In both groups, endometrial thickness $\geq$7 mm was considered mandatory for proceeding with embryo thawing.

Embryos were transferred in relation to day of development when cryopreserved. Embryo transfer was performed using Cook catheter (Soft Pass, J-SPPE; Cook Ob/Gyn, Spencer, IN, USA) by a standard technique under ultrasound guidance. The number of embryos transferred was individualized based on patients' age, number of previous IVF cycles and embryo quality. The luteal phase was supported with

Table 1 Demographic characteristics and fresh cycle outcomes.

Variable	*Group A (HCG)*	*Group B (no HCG)*
Cycles (*n*)	25	30
Age (years)[a]	29.5 ± 4.5	33.0 ± 3.3
Gravidity	1.8 ± 1.8	1.5 ± 1.0
Parity	0.4 ± 0.5	0.8 ± 0.8
Day-3 FSH (IU/l)	6.7 ± 2.0	6.5 ± 2.1
Infertility duration (years)	2.7 ± 1.2	3.3 ± 2.0
No. of previous failed IVF cycles	1.7 ± 1.0	1.6 ± 0.9
No. of oocytes retrieved	11.4 ± 3.9	12.6 ± 5.0
Cycles with ICSI	26/30 (86.7)	17/25 (68.0)
No. of oocytes fertilized	8.3 ± 2.7	8.9 ± 3.5
Fertilization rate (fresh cycles)	0.77 ± 0.18	0.72 ± 0.13
Clinical pregnancy (fresh cycles)	11/25 (44.0)	17/30 (56.7)
No. of embryos frozen	5.2 ± 2.5	5.1 ± 2.4

Values are mean ± standard deviation or *n* (%) unless otherwise stated.
HCG = human chorionic gonadotrophin; ICSI = intracytoplasmic sperm injection.
[a]$P = 0.002$.

vaginal progesterone tablets (Endometrin, 100 mg b.i.d.; Ferring, Cesarea, Israel) or single daily application of vaginal progesterone gel (Crinone; Merck Serono, Herzlya, Israel) from the presumed day of ovulation until pregnancy testing, 12 days after FET. In cases of pregnancy, luteal support was continued until 8 weeks of gestation.

Data analysis

Data recorded for analysis included age, gravidity and parity, infertility duration, day-3 FSH concentrations and the number of previous failed IVF attempts (Table 1). Fresh cycle characteristics included the number of oocytes retrieved and fertilized, the use of ICSI, fertilization rate, cycle outcome and the number of embryos cryopreserved (Table 1). For the frozen–thawed embryo transfer cycles, comparisons were made for the number of monitoring visits at the clinic (primary outcome measure), the number of thawed embryos, the number of cycles with embryo transfer, the number of embryos transferred, endometrial thickness, pregnancy and live-birth rate per started cycle and per embryo transfer and implantation rate (secondary outcome measure). A clinical pregnancy was defined as ultrasonographic visualization of an intrauterine gestational sac with fetal heartbeat. A spontaneous abortion was defined as a clinical pregnancy lost before 20 weeks of gestation. Implantation rate was defined as the number of observed intrauterine gestational sacs divided by the number of embryos transferred.

Serum oestradiol and progesterone were measured by means of the automated Elecsys Immunoanalyser (Roche Diagnostics, Mannheim, Germany). Intra- and interassay coefficients of variation were $<5\%$ and $<10\%$ for oestradiol and $<3\%$ and $<5\%$ for progesterone, respectively.

The statistical package Sigmastat (Jandel Corporation, San Raphael, CA) was used for data analysis. Comparisons were made using the unpaired Student's t-test, the Mann–Whitney rank sum test, chi-squared analysis and Fisher's Exact test, where appropriate. $P < 0.05$ was considered statistically significant. Results are expressed as mean ± standard deviation.

Results

In group A, five patients did not meet the criteria for HCG administration and were withdrawn from the study. Of the 25 left, only one had no embryos available for transfer after thawing. In group B, all patients completed the study and 27 had FET. In three patients there were no embryos available for transfer after thawing.

Demographic characteristics and reproductive history of patients in groups A and B were similar (Table 1). The fresh cycles leading to embryo freezing were found comparable with regard to clinical and embryology data such as duration of infertility, basal FSH concentrations, number of previous IVF cycles, number of oocytes retrieved, fertilization rate, clinical pregnancy rate and number of embryos frozen (Table 1). Patients in group A were significantly younger (29.5 ± 4.5 versus 33.0 ± 3.3 years; $P = 0.002$).

Characteristics of the cycles in which frozen–thawed embryos were transferred are summarized in Table 2. Clinical and embryology characteristics are similar in both groups with no statistically significant difference in terms of number of thawed embryos, number of cycles without embryo transfer, number of embryos transferred, clinical pregnancy rate per cycle and per transfer and live-birth rates. Endometrial thickness was significantly lower in group

Table 2 Characteristics and outcome of frozen–thawed embryo transfer cycles.

Variable	*Group A (HCG)*	*Group B (no HCG)*	*P-value*
Cycles (*n*)	25	30	
No. of monitoring visits	3.2 ± 1.4	4.7 ± 1.6	0.002
Follicle size maximum (mm)	19.2 ± 1.3	20.5 ± 3.3	NS
Oestradiol maximum (pg/ml)	258.7 ± 81.8	285.2 ± 123.3	NS
Patients with transfer	24/25 (96.0%)	27/30 (90.0%)	NS
Endometrial thickness (mm)	9.2 ± 1.6	10.2 ± 1.6	0.016
No. of embryos thawed	3.9 ± 1.9	3.5 ± 1.17	NS
No. of embryos transferred	2.24 ± 0.9	2.0 ± 1.05	NS
Clinical pregnancy rate per cycle	8/25 (32.0)	8/30 (26.7)	NS
Clinical pregnancy rate per transfer	8/24 (33.3)	8/27 (29.6)	NS
Live-birth rate per cycle	8/25 (32.0)	5/30 (16.7)	NS
Live-birth rate per transfer	8/24 (33.3)	5/27 (18.5)	NS
Implantation rate	9/55 (16.4)	8/60 (13.3)	NS

Values are mean ± standard deviation or *n* (%) unless otherwise stated.
HCG = human chorionic gonadotrophin; NS = not statistically significant.

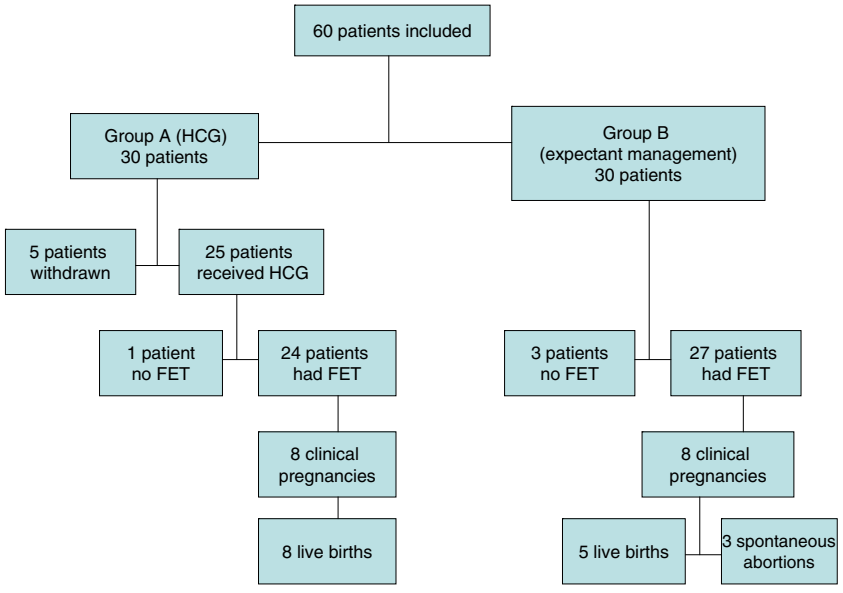

Figure 1 Number of women included in and excluded from the study and clinical pregnancy data and outcomes. FET = frozen–thawed embryo transfer; HCG = human chorionic gonadotrophin.

A patients as compared with group B patients (9.2 ± 1.6 versus 10.2 ± 1.6 mm; $P = 0.016$) The mean number of monitoring visits was significantly reduced in group A as compared with group B (3.2 ± .4 versus 4.7 ± 1.6; $P = 0.002$). **Figure 1** summarizes the outcome for all patients who entered the study.

Discussion

This study suggests that triggering of ovulation with HCG is as efficient as serial monitoring until ovulation detection in patient preparation for NC-FET in terms of implantation, pregnancy and live-birth rates. Since ovulation triggering by HCG significantly reduces the number of monitoring visits that are necessary to schedule the day of FET, this approach may be superior in terms of patient convenience and cost-effectiveness of the cycle.

For many years, FET has been successfully performed in natural cycles following spontaneous ovulation (al-Shawaf et al., 1993; Sathanandan et al., 1991). Since the NC protocol is relatively simple and obviates the need for prolonged hormonal supplementation in early pregnancy, it is preferred by many patients and clinics (Kolibianakis et al., 2003). However, problems often occur when this protocol is used. The timing of transfer requires accurate determination of ovulation. Daily hormone determinations and ultrasonographic monitoring are required, which can become inconvenient for patients. Natural cycle transfers have a 6% cancellation rate (Sathanandan et al., 1991) because of the inability to determine the exact time of ovulation. Finally, unlike in artificial cycles, the date of embryo thaw and transfer cannot be chosen, a particularly vexing problem in centres that do not operate 7 days a week.

Because of the above limitations, only patients with regular ovulatory cycles can be included in NC-FET programmes. Among the ovulatory patients that were included in the study, the number of monitoring visits before FET was found to be significantly lower in the induced ovulation group compared with the expectant monitoring group (3.2 ± 1.4 versus 4.7 ± 1.6; $P = 0.002$). Each patient that received HCG had saved approximately 1.5 visits to the clinic. Indeed, all studies published so far that have compared the number of monitoring visits after spontaneous ovulation versus HCG triggering in patient preparation for NC-FET have concluded that HCG triggering significantly reduces the number of monitoring visits (**Table 3**).

Two differences were found in the demographic and clinical characteristics of the study groups. First, patients in group A (HCG group) were significantly younger as compared with group B (expectant management), and second, endometrial thickness was significantly lower in group A as compared with group B. Although these differences reached statistical significance, they are probably of no clinical significance, because for both groups, patient age and endometrial thickness are in the range considered favourable for the achievement of implantation and pregnancy. Indeed, pregnancy and implantation were found to be comparable for both groups, and therefore, these differences most probably arise from the relatively small size of this study.

Table 3 Number of monitoring visits in trials comparing spontaneous ovulation with human chorionic gonadotrophin (HCG) triggering.

Study	*Spontaneous ovulation*	*HCG triggering*	*P-value*
Weissman et al. (2009)	4.4 ± 1.4	3.5 ± 1.8	<0.0001
Fatemi et al. (2010)	4.1 ± 1.4	2.6 ± 1.1	0.001
Current study	4.7 ± 1.6	3.2 ± 1.4	0.002

Values are mean ± standard deviation.
HCG = human chorionic gonadotrophin.

Very few studies in the literature have looked closely at the use of HCG for ovulation triggering in timing NC-FET. Kyrou et al. have recently reported a retrospective analysis comparing NC with clomiphene citrate in patient preparation for FET (Kyrou et al., 2010a) and a retrospective study evaluating the role of luteal support in NC-FET (Kyrou et al., 2010b). In both studies HCG was used for ovulation triggering and a favourable ongoing pregnancy rate (23% and 21.5%, respectively) was achieved. Recently, the same group published the results of a randomized, controlled trial comparing timing of NC-FET by detection of a spontaneous LH/progesterone rise with HCG for triggering ovulation (Fatemi et al., 2010). The study was terminated early since an interim analysis found a significantly higher ongoing pregnancy rate in the spontaneous LH surge group (31.1% versus 14.3%; $P = 0.025$). The low ongoing pregnancy rates in the HCG arm (14.3%) are also considerably lower than those reported by the same group in other trials where the ongoing pregnancy rate following HCG administration was 22–23% (Kyrou et al., 2010a,b).

This study was compared with that of Fatemi et al. (2010) for differences in methodology. The current study's criteria for HCG administration were very strict. Patients who demonstrated findings suggestive of impending ovulation ($n = 5$) were not eligible for HCG administration and were withdrawn from the study, since ill-timed administration of HCG can cause less than optimal synchronization between endometrium and embryo development. In the study by Fatemi *et al.*, patients in the HCG group had LH concentrations that were 3.7 times higher on the day of HCG administration (17.5 ± 16.7 IU/l) compared with baseline concentrations (4.7 ± 1.3 IU/l) and with a very wide standard deviation. Without doubt, in many of these patients the ovulation process had already started when HCG was administered, a fact that could seriously compromise the accuracy of timing FET. Such patients should have been withdrawn from the study rather than receiving HCG. The overall ongoing pregnancy rate in the HCG group in the paper of Fatemi et al. was 14.3%. If patients that received HCG despite a documented LH surge are withdrawn from the study ($n = 23$), then ongoing pregnancy rates in the remaining (40) patients become 20% (8/40) and the difference in pregnancy rates between the two study arms is non-significant.

Fatemi et al. (2010) postulate that a detrimental effect of exogenous HCG on implantation may exist. This is surprising as HCG has been widely used with great success for many years for triggering ovulation and for luteal support in both spontaneous and induced cycles. For a detailed review of the actions and interactions of HCG in the management of infertility, the reader is referred elsewhere (Filicori et al., 2005). In the current study, HCG for ovulation triggering simplifies the monitoring process without compromising

live-birth rates. A live-birth rate of 33.3% in the HCG study arm is proof of the concept. It is hoped that the negative findings on HCG administration by Fatemi et al. (2010) will stimulate further studies on this subject rather than the abandonment of this treatment modality.

Many practices in the field of assisted reproduction are not scientifically proven or evidence based. Among these are the protocols for endometrial preparation for FET. In a recent large-scale web-based survey (http://www.ivf-worldwide.com/survey/survey-fet-results.html), representing nearly 40,000 FET cycles worldwide, it was revealed that NC-FET is the preferred method for endometrial preparation in 45% of FET cycles. HCG is used for triggering ovulation in 24% of cycles and is added after ovulation has occurred in another 37% of cycles. In 54% of cycles luteal support is used. While it is difficult to draw solid scientific conclusions from a survey, these results suggest that the wide use of HCG for triggering ovulation and luteal support in NC-FET simply represents the satisfaction and success of the many centres and clinics where it is being used.

The cost–benefit evaluation for the use of HCG for ovulation triggering differs by country, and even by clinic, and thus should be individually assessed in every clinical set-up. In the study centre's experience, patients were happy to trade extra monitoring visits with a single self-administered subcutaneous injection of HCG, although patient preference was not evaluated in the current study. Further studies are required to draw firm conclusions about patient preferences.

In summary, in patients undergoing NC-FET, triggering ovulation by HCG can significantly reduce the number of visits necessary for scheduling embryo transfer without an adverse effect on cycle outcome. Ovulation triggering can increase both patient convenience and cycle cost effectiveness. Utilizing this new treatment approach can therefore be attractive to both patients and clinics.

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