Running title

Frozen embryo replacement: Natural v HRT

Title

A randomized controlled pilot trial of natural versus hormone replacement therapy cycles in frozen embryo replacement IVF

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Capsule

This randomized controlled trial suggests that, for women with regular ovulatory cycles undergoing Frozen Embryo Replacement (FER) IVF, the live birth rate is similar whether the endometrium is prepared using a natural menstrual cycle or GnRH-agonist pituitary suppression and HRT.

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Abstract

Objective: To determine whether there is any difference between the outcomes of two standard treatment protocols for frozen embryo replacement (FER): natural and down-regulated hormone replacement treatment (HRT).

Design: Prospective, open, single-center randomized controlled pilot trial.

Setting: Private Fertility Clinic

Patient(s): Women (n=159) planning a frozen embryo replacement cycle at the Oxford Fertility Unit, less than 40 years old at the time their embryos were frozen; had at least 1 blastocyst or 2 cleavage stage embryos in storage; regular ovulatory cycles; and at most 2 previous FER cycles.

Intervention(s): Eligible participants were recruited and randomized between March 2010 and July 2012 into one of 2 standard FER treatment groups, natural (n=80) menstrual or Gonadotropin-Releasing Hormone (GnRH) agonist/HRT (n=79) cycles.

Main outcome measures: Live birth rate following replacement of frozen-thawed embryos, clinical pregnancy rate, implantation rate and cycle cancellations.

Result (s): 159 women were randomized (80 Natural; 79 HRT) and 145 had embryo transfer (ET) and completed the study (72 Natural; 73 HRT). Pregnancy outcomes were not significantly different between the two groups. The live birth rates were 26.3% (Natural) and 31.7% (HRT) per randomized patient ~~(P=NS)~~. Per ET/protocol the live birth rates were 29.2% and 34.2% ~~(P=NS)~~. The implantation rates were 24.3% and 26.0% ~~(P=NS)~~ and there were 3 twin births in the Natural and 5 in the HRT arms.

Conclusion(s): The findings of this pilot study support the suggestion that for women with ovulatory cycles undergoing FER, the outcomes are similar between natural and HRT protocols.

Clinical Trial Registration Number: NCT00843570. Registered at www.clinicaltrials.gov

Key Words: In Vitro Fertilization; IVF; frozen embryo replacement

Introduction

Frozen embryo replacement (FER) *in vitro* fertilization (IVF) cycles are increasingly carried out in fertility practice worldwide, now accounting for 26% of all IVF cycles (1).

This increase is explained by the concurrent trend for fewer fresh embryos being transferred and improved laboratory technologies, including embryo vitrification (2, 3). Indeed a small number of reports claim superior pregnancy (4) and perinatal outcomes (5) for frozen cycles.

FER treatment is concerned with preparing the endometrium for receiving the thawed embryos and with ensuring that the timing of endometrial and embryo development coincide. There are broadly two methods to achieve this; in natural cycles following spontaneous ovulation (6) or in cycles in which the endometrium is artificially prepared with exogenous steroids (7). In practice, natural cycles may be supplemented with hormones and medicated cycles may be with or without first down-regulation with a GnRH agonist.

Comparisons of the outcomes of the two treatment approaches have so far been mainly retrospective, with no published prospective randomized controlled trials. The need for a direct comparison of the two treatments by a prospective RCT has been indicated by a Cochrane review (8), the British Fertility Society (9) and, subsequent to the initiation of this study, a recent systematic review (10).

The aim of the study was to compare two standard treatment protocols for frozen embryo replacement using live birth rate as the main outcome measure. The secondary objectives

were to investigate if there are differences between other outcome measures (clinical pregnancy rate, implantation rate and cycle cancellations) between the two protocols.

Materials and Methods

Patient population

Women planning a frozen embryo replacement cycle at the Oxford Fertility Unit were invited to take part in the trial. They were eligible to participate if they were < 40 years old at the time their embryos were frozen; had at least 1 blastocyst or 2 cleavage stage embryos in storage; regular ovulatory cycles; and at most 2 previous FER cycles. Women were excluded from participating in the trial more than once.

Study Design

The study protocol was approved by a NHS Ethics Committee (09/H0604/89), Medicines and Healthcare Regulatory Authority (EudraCT 2009-009323-11) and registered with Clinicaltrials.gov before patient recruitment started. All procedures took place at the Oxford Fertility Unit and patients gave consent to participate in the study between March 2010 and July 2012.

The study design was a single centre open randomized controlled trial comparing the efficacy of natural and HRT cycles in frozen embryo replacement treatment. Both treatment protocols were standard protocols in use at the Oxford Fertility Unit at the time. Due to the size of the trial, this was a pilot study, with the data from the study being used to decide whether a larger study should be conducted. No other randomized controlled trials comparing these two types of cycle have been published to date.

Following initial screening and fulfilment of inclusion criteria, research participants were randomized using a minimization algorithm (to balance for age, BMI, number of previous cycles) to one of 2 groups: Group 1: Natural FER and Group 2: Down-regulated HRT FER.

Study Procedures

All patients intending to commence treatment and meeting the study inclusion criteria were invited to the unit between Day 1-5 of their monthly cycle for a baseline scan and study enrollment.

At this visit those who wished to take part completed a study consent form and were randomized into either Group 1 (Natural FER) or Group 2 (HRT-FER). The study procedures then followed the standard Oxford Fertility Unit FER treatment protocols according to Group. Group 1 participants had an ultrasound assessment between day 10-13 of their cycle to confirm follicular growth and endometrial thickness, followed by additional ultrasound monitoring in subsequent days if necessary. Participants then commenced self-monitoring of impending ovulation by using urinary dipsticks (supplied by the Oxford Fertility Unit) for the endogenous surge of Luteinising Hormone (LH). On detection of the LH surge, the unit was informed and embryo transfer scheduled for up to one week later depending on the stage of embryo development at freezing (i.e. day 2 cleavage embryos, day 3 cleavage embryos or day 5 blastocysts). The transfer day was 4 days after LH surge for day 2 embryos, 5 days later for day 3 embryos and 7 days later for blastocysts. In the event of the endogenous LH surge not being detected, further visits were arranged. No luteal support was given to Group 1 participants.

One or two thawed frozen embryos were transferred to the uterus by abdominal ultrasound guided transfer, dependent on patient choice. A urinary pregnancy test was performed at home 11 – 14 days later, dependent on embryo stage, and Oxford Fertility Unit informed of outcome. The results of the pregnancy test were recorded. If the pregnancy test was positive then further visits to the unit were arranged to confirm clinical pregnancy (by presence of fetal heart activity). Pregnancy outcomes (live births) were collected after patients had notified the unit.

Group 2 (HRT FER) participants commenced daily nasal administration of GnRH agonist nafarelin (Synarel; Pharmacia Ltd, Milton Keynes, UK) 400mcg b.d. on day 21 of their menstrual cycle until advised to stop, dependent on stage of embryo, before the embryo transfer procedure. In total this was 5-6 weeks. GnRH agonist down-regulation was used to prevent premature endometrial luteinisation. A blood test 2-3 weeks later confirmed down regulation (oestradiol levels <150 pmol/L). Once confirmed, patients started oral administration of 2mg/day oestradiol valerate (Progynova, Schering Health Care, Burgess Hill, UK) for endometrial preparation which was increased by a step-up protocol to 6mg/day. An ultrasound assessment 12-13 days later assessed the uterus ready for the embryo transfer procedure when the endometrial thickness > 8mm. If not adequate, endometrial priming continued and monitoring scans undertaken to confirm further endometrial thickening. Participants commenced luteal support via vaginal administration of progesterone pessaries (Cyclogest, Actavis UK, Barnstaple, UK) 400mg, b.d according to the proposed day of embryo thawing and transfer; patients with embryos cryopreserved at the cleavage day 2 stage started pessaries 2 days before the transfer day, patients with cryopreserved day 3 embryos started pessaries 3 days before and patients with cryopreserved blastocysts started their pessaries 5 days before. The embryo transfer was correspondingly scheduled for up to a week after the scan dependent

on embryo stage. Embryo transfer and pregnancy testing was as for Group 1 participants. If positive, HRT (oestradiol valerate and progesterone) administration continued for a further 6 weeks.

Randomization

Subject numbers were assigned sequentially as each woman entered the study. Study participants were allocated to one of two treatment groups using a minimisation algorithm (to balance for age, BMI and number of previous cycles). This is a form of randomization which allocates subjects to the group that 'minimises' the current imbalance in terms of their specific characteristics. Therefore, similar numbers of patients were allocated to each treatment group. Minimisation is an effective method of allocating participants to treatment groups within a randomized controlled trial (11), an example being Tave's minimization (12). The study was not blinded as study participants and clinic staff were aware of which study protocol they were following.

Sample size

Ideally, sufficient numbers of patients would have been recruited to this study to determine equivalence between the two types of cycles. The number of subjects needed in each arm with alpha = 0.05 and beta = 0.20 (power = 80%) is 973 (i.e., 1,946 overall). This emphasizes the size of a formal RCT required to demonstrate equivalence and why this study can thus only be viewed as a pilot study.

Sample size was determined by pragmatic considerations alone: we initially aimed to recruit 150 patients. Although this number is underpowered for an equivalence trial, its results should still enable us to determine if a larger scale, long-term trial will be of value.

Statistical analysis

The trial data was analysed using both an intention to treat analysis (where patients are analysed according to their randomized group, irrespective of whether they actually received the allocated treatment) and a per protocol analysis (where patients are only analysed if they fully comply with the study protocol, including embryo transfer). The primary outcome measure was live birth rate per cycle and per embryo transfer procedure.

Clinical pregnancy rate was defined as a pregnancy with fetal heart visible on ultrasound. Implantation rate was defined as the number of fetal hearts visible on ultrasound divided by the number of embryos replaced and expressed as a percentage.

Data was analysed using the chi-squared test for categorical outcome data Statistical significance was taken at the 5% level throughout, with 95% confidence intervals used to express the uncertainty around the estimates.

Since this was a pilot study with well characterised interventions we did not anticipate early termination of the project to be likely. However, an interim analysis of clinical pregnancy rates was carried out after 50 participants had had pregnancy test to ensure that it had not fallen significantly in either group.

Expenses

Study participants had their study drugs supplied by the Oxford Fertility Unit so that the treatment costs for both groups of study participants remained the same for both natural-FER and HRT FER cycles.

Results

159 women were randomized (80 Natural; 79 HRT) and 145 had ET and completed the study (72 Natural; 73 HRT) (Figure 1). Of the 14 women who did not have an ET, 4 withdrew preferring the alternative arm (2 Natural; 2 HRT), 3 had a failed embryo thaw (HRT group) and the rest were cancelled cycles. The baseline characteristics of the two groups including age when embryos were frozen, age at inclusion in study, body mass index and number of previous FER cycles are described in Table 1. No significant differences were detected between the two groups. Characteristics of the original stimulated cycle in which the embryos were frozen are shown in Supplemental Table 1 and, as no significant differences were detected between the two groups, this suggests both were similar in their pregnancy potential.

Treatment outcomes for the randomized groups are shown in Table 2. In the Natural arm there were 24 clinical pregnancies and 21 live births, while in the HRT arm this was 26 clinical pregnancies and 25 live births. The live birth rates were 26.3% (Natural) and 31.6% (HRT) per randomized patient (P=NS). Per ET/protocol the live birth rates were 29.2% and 34.2% (P=NS). The clinical pregnancy rates were 30.0% (Natural) and 32.9% (HRT) per randomized patient (P=NS) and 33.3% and 35.6% (P=NS) per ET/protocol. There were no differences between the two groups observed in the interim analysis following the pregnancy test of the first 50 participants. 12 positive tests were recorded in each group.

Other cycle characteristics including implantation rate, numbers of embryos transferred and

day of transfer are represented in Table 3. There were no significant differences in implantation rates between the two groups at 24.3% (Natural) and 26.0% (HRT) respectively. In total 234 embryos were transferred, with 157 blastocyst and 77 cleavage stage embryos replaced in the 145 cycles. Similar numbers of blastocyst transfers took place across both groups; with 50 in the Natural group and 51 in the HRT group. Overall there were 37 live births (36.6%) following blastocyst transfer and 9 (20.4%) after cleavage stage embryo transfers, but no significant differences between live birth rate and stage of embryo transfer and randomized group. There appeared to be a greater proportion of single embryo transfers in the Natural group, as in this arm there were 33 single and 39 two-embryo transfers compared to 23 single and 50 two-embryo transfers in the HRT group. However this was not significantly different. Overall there were 8 live twin births, giving a multiple birth rate of 17.4%; 3 in the Natural and 5 in the HRT arms.

Discussion

Clinical pregnancy and live birth rates were remarkably similar between the two groups of randomized patients. This is in keeping with previous non-randomized studies. For example in a retrospective comparison of 417 women with regular menstrual cycles undergoing natural versus HRT agonist FER cycles, there was no difference in implantation rate, clinical pregnancy rate or live birth rate per cycle or per embryo transfer (13). Similarly in a partly randomized trial comparing these groups but allocating women with irregular cycles, oligomenorrhoea or amenorrhoea to the HRT group, pregnancy rates were similar between HRT and natural cycle groups (14). This was seen in other studies where patients had been allocated according to their cycle characteristics i.e. ovulatory and anovulatory (15, 16) or preference (17). A further retrospective study showed higher live birth rates in 1151 GnRH

agonist HRT cycles as compared with 240 natural cycles, supplemented with progesterone, but cycle type was determined by preference and cycle characteristics (18). In our study the overall the live birth rates in both groups compares favourably to that for FER cycles across all age groups in both the UK (18.1%) (19) and Europe (13.3%) (1).

During the study the trend for blastocyst cryopreservation became apparent as increasing numbers of blastocyst transfers were undertaken. However, as this was a RCT these were distributed randomly and a chi-squared test showed no difference in numbers of blastocyst transfers between groups (50 natural, 51 HRT). Similarly we were unable to control for numbers of embryos transferred and although more single embryo transfers were carried out for the natural group (33 vs 23), this was not significantly different. Indeed the implantation rates were very similar for both groups.

Very few participants withdrew from the study or had their cycle cancelled. Only 3 cycles were cancelled due to failed embryo thaw (2%), and 4 participants withdrew due to a desire for the alternative treatment arm. Both approaches to FER are used routinely in practice and both have advantages. Cycles using HRT may confer the advantage of enabling cycle control and this may help with workforce planning in timing of embryo transfer. It is primarily for this reason that the vast majority of FER treatment (1580 cycles compared to 60 for the period 2003-2008 at the Oxford Fertility Unit) used this method. On the other hand natural cycles avoid the use of exogenous steroids, the side effects of down-regulation and are of shorter duration and therefore they may be more ‘patient friendly’. Additionally, and in particular for self-funded cycles, the absence of medication reduces the financial cost of each natural cycle by upwards of $300 compared to HRT cycles. However, although there is evidence that progesterone

supplementation is not necessary for optimum luteal support following spontaneous ovulation (20), protocols for natural cycle FER in other centers may include the administration of exogenous progesterones (10) and thus these potential advantages are less apparent.

As indicated earlier, the small sample size is a limitation of this study and it is difficult to draw firm conclusions from specific secondary outcomes. However, cycle cancellations were greatest in the natural group (6 compared to 1), due to failure in ovulation or its detection, an inability to conveniently timetable transfers or inadequate endometrial thickness. This finding merits further evaluation in future studies as it is an important finding for clinicians and patients. There is wide variation in the timing of ovulation in individual patients and some reports of difficulties in interpretation of the self-administered urine-dipstick tests used for this purpose (21, 22). This underscores the importance of selection of appropriate candidates for natural cycle FER treatment and patient counselling.

Our participants had self-reported ‘regular’ cycles and may not necessarily have been ideal candidates for natural FER protocol. Furthermore, as the difficulties of ovulation detection and the wide variation between cycle and ovulation length between women is known this must be communicated to patients offered this protocol. Despite this, the cancellation rate, including withdrawn participants, was just under 9%, which is similar to overall cancellation rates at the Oxford Fertility Unit at the time.

In conclusion, this is, to our knowledge, the first published RCT comparing the live birth rates of true Natural and GnRH agonist HRT frozen embryo replacement cycles. The main limitation of this study is its small size and since it is underpowered to demonstrate equivalence it can only be viewed as a pilot study. However, our results suggest that, for women with regular

ovulatory cycles wishing to undergo FER, the live birth rate is similar whether the endometrium is prepared using a natural menstrual cycle or GnRH-agonist pituitary suppression and HRT.

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Author's contribution

T.C. conceived the idea, initiated the project, designed the study, analysed and interpreted the data, and gave final approval of the manuscript. G.M. was involved in study execution and analysis, protocol development, randomization, and manuscript drafting. E.M. was involved in initiation of the project and revision of the manuscript. K.T. provided clinical expertise/data and critically revised the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Figure legend

Figure 1

Flowchart showing of enrollment and randomization of study patients

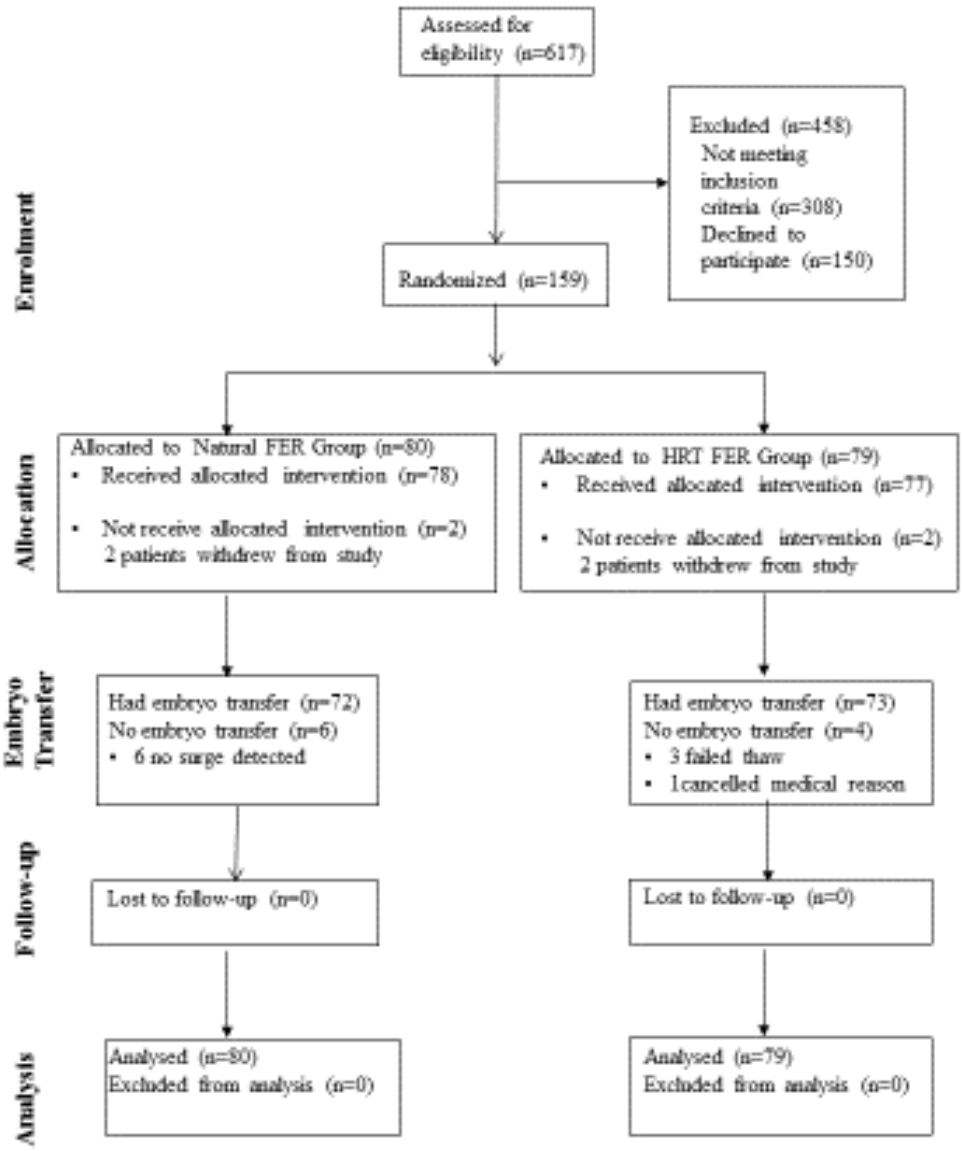

Table I. Baseline characteristics of the Natural and HRT groups

	Natural FER (n=72)	HRT FER (n=73)	P-value
Mean age at freeze Y (SD)	33.7 (3.1)	33.5 (3.6)	0.68
Mean age in study Y (SD)	35.3 (3.9)	34.7 (3.7)	0.37
Mean BMI kg/m2 (SD)			
< 26	22.2 (2.1)	22.1 (1.8)	0.70
≥ 26	28.7 (2.2)	29.0 (2.5)	0.72
Number of previous FER cycles			0.93
0	64 (40.3)	63 (39.6)	
1	11 (6.9)	10 (6.3)	
2	5 (3.1)	6 (3.8)	

Note: Data expressed as number (percentage) unless stated otherwise

Y,years; SD, standard deviation; BMI, body mass index

Table 2. Treatment outcomes of the Natural and HRT groups

	Natural FER	HRT FER	P-value
	(n=72)	(n=73)	
Live Birth			
Per randomised patient	21/80 (26.3)	25/79 (31.6)	0.45
Per embryo transfer	21/72 (29.2)	25/73 (34.2)	0.51
Clinical pregnancy			
Per randomised patient	24/80 (30.0)	26/79 (32.9)	0.69
Per embryo transfer	27/72 (33.3)	26/73 (35.6)	0.77

Note: Data expressed as number (percentage) unless stated otherwise

Table 3. Cycle characteristics of the Natural and HRT groups

	Natural FER	HRT FER		P-value
	(n=72)	(n=73)		
Implantation rate (IR)	27/111(24.3)	32/123 (26.0)		0.77
Embryo Transfers				
Embryo stage				0.96
Blastocyst	50	51		
Cleavage	22	22		
Number replaced				0.08
Single embryo	33	23		
Two embryos	39	50		
	Natural FER	**HRT FER**	**Overall**	**P-value**
Live Birth				
Embryo stage				0.74
Blastocyst	15/50 (30.0)	22/51(43.1)	37/101 (36.6)	
Cleavage	6/22 (27.2)	3/22 (13.6)	9/44 (20.4)	
Number replaced				0.17
Single embryo	10/33 (30.0)	7/23 (30.4)	17/56 (30.4)	
Two embryos	11/39 (28.2)	18/50 (36.0)	29/89 (32.6)	
Multiple (Twins)	3 (4.2)	5 (6.8)		

Note: Data expressed as number (percentage) unless stated otherwise

IR = Number of fetal heart/number embryos replaced (%)

Supplemental Table 1. Cycle characteristics of the original stimulated cycles of the Natural and HRT groups

	Natural FER	HRT FER	P-value
	(n=80)	(n=79)	
Mean number oocytes collected (SD)	13.4 (5.7)	14.0 (5.9)	0.50
Mean number mature Oocytes (SD)	8.8 (3.5)	9.6 (4.5)	0.21
Mean number embryos frozen (SD)	3.3 (2.1)	3.8 (3.6)	0.27
Live Births (at original cycle)	28	21	0.25

Note: Data expressed as number unless stated otherwise

SD, standard deviation