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ASSISTED REPRODUCTION TECHNOLOGIES

# Natural cycle frozen-thawed embryo transfer—can we improve cycle outcome?

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**Abstract**
*Purpose* Several replacement protocols for frozen-thawed ET (FET) exist, with no advantage of one protocol over the others. In the present study, we aim to evaluate the outcome of natural cycle FET with modified luteal support.
*Methods* All consecutive patients undergoing natural or artificial hormone replacement (AHR) day-2/3 FET cycles between May 2012 and June 2015 in our IVF unit were evaluated. While AHR FET cycles were consistent, those undergoing natural cycle FET received progesterone luteal support, and from June 2014, patients received two additional injections, one of recombinant hCG and the other of GnRH-agonist, on day of transfer and 4 days later, respectively (modified luteal support).
*Results* Patients' clinical characteristics and laboratory/embryological variables were comparable between those undergoing natural vs. AHR cycles, during the earlier as compared to the later period. Moreover, while implantation, clinical, and ongoing pregnancy rates were significantly higher during the later period in patients undergoing the natural cycle FET with the modified luteal support (31, 51, and 46 %, respectively), as compared to natural (17, 26, and 20 %, respectively), or AHR FET in the late study period (15, 22, and 17 %, respectively), the natural cycle FET without the additional two injections yielded the same results, as the AHR cycles.
*Conclusions* We therefore suggest that in ovulatory patients undergoing FET, natural cycle FET with the modified luteal support should be the preparation protocol of choice. Further large prospective studies are needed to elucidate the aforementioned recommendation prior to its routine implementation.

**Keywords** Cryopreservation · IVF · Luteal support · hCG · GnRH-agonist · Pregnancy rate

*Capsule* Two additional injections, one of rhCG and the other of GnRH-agonist, on day of transfer and 4 days later, respectively, may improve FET outcome.

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## Introduction

With the recent trend toward single embryo transfer (ET) adopted in an attempt to reduce the risk of multiple pregnancy [1, 2], the remaining extra embryos are cryopreserved, allowing further possibilities for conception following the subsequent frozen-thawed embryo transfer (FET) cycles. While there are several currently employed replacement protocols for FET [3], no compelling advantage for one protocol over another has been hitherto established [4]. The choice of protocol depends on the individual woman's ovarian function and convenience of the method, as well as on the experience gained with the method by the physicians.

Recently, we have described a "new" preparation protocol, the natural FET with modified luteal support [5]. The protocol has been offered to our ovulatory patients and consists of daily vaginal progesterone started on the day of ovulation (determined by the spontaneous LH peak), with two additional injections, one of recombinant hCG (Ovitrelle, Merck Serono, Herzliya, Israel; s.c. 250 mcg) and the other of GnRH-agonist (Triptorelin, Ferring Lapidot, Netanya, Israel; s.c. 0.1 mg), on day of transfer and 4 days later, respectively [5, 6]. We could

demonstrate significantly higher implantation, clinical, and ongoing pregnancy rates while comparing this protocol to a previously used natural FET protocol without the additional two injections.

The criticisms and main concerns regarding our aforementioned observation were whether these significant differences are biologically true or result from the potential differences between the two study periods, specifically, better laboratory methodology and techniques and improved embryologists' skills.

In an attempt to overcome and control for the aforementioned concerns, we sought to extend our previous study period and to investigate two additional control groups, i.e., patients undergoing FET following artificial hormone replacement (AHR) cycle using only estrogen and progesterone, in the comparable study periods.

## Patients and methods

All consecutive patients undergoing FET cycles, following either natural cycle (NC) or AHR preparation protocols, between May 2012 and June 2015 in our IVF unit, were evaluated. The selection of type of endometrial preparation used was the decision of the treating physician and largely dependent on the fashion at the time. Moreover, the elimination of bias in this selection, for the purposes of this study, was achieved by including only patients undergoing a day 2 or 3 frozen-thawed embryo transfer. Moreover, only embryos cryopreseved by vitrification, using a vitrification kit (SAGE Vitrification Kit, SAGE Media, USA), were included. The study was approved by the Institutional Research Ethics Board of our Medical Center.

From May 2012 to May 2014, 74 patients underwent natural cycle FET in our IVF unit (NC-early group). Following spontaneous menstruation, patients were monitored by serial ultrasound for endometrial thickness, follicular development, and LH and progesterone levels, until a rise in LH level was observed (LH level exceeds 180 % of the baseline value [7]), corresponding to a day prior to OPU/ovulation. On the following day, progesterone luteal support was started with either daily 600 mg micronized progesterone soft gel vaginal capsules (Utrogestan, Besins, Iscovesco, C.T.S., Petach Tikva, Israel) in three divided doses or vaginal progesterone 90 mg (Crinone; Merck Serono, Hellerup, Denmark) once a day.

From June 2014, 59 patients underwent the same aforementioned natural cycle FET cycles, with two additional injections, one of recombinant hCG (250 mcg) and the other of GnRH-agonist (triptorelin 0.1 mg), on day of transfer and 4 days later, respectively (NC-late group).

During the same aforementioned study periods (May 2012 to May 2014 and June 2014 to June 2015), 113 and 54 patients underwent artificial hormone replacement FET cycles (AHR-early and AHR-late groups, respectively). Patients received daily oral β-estradiol or estradiol valerate (6 mg) in three divided doses, starting on days 2–3 of the menstrual cycle. After 10 days of estrogenic exposure, the patients were asked to attend the clinic, and from this point, they were monitored by serial ultrasound scanning for endometrial thickness and serum estradiol and progesterone levels. Progesterone supplementation was added whenever a triple-line pattern endometrium reaches 8 mm thickness concomitant with follicular level of plasma progesterone. The dose of progesterone supplementations were either daily 900 mg micronized progesterone soft gel vaginal capsules (Utrogestan) in three divided doses or vaginal progesterone 90 mg (Crinone) twice a day.

The clinical outcomes of the natural and AHR FET cycles were compared between the same and the different periods. While a top quality embryo (TQE) was defined as 3/4, or 7/8 blastomeres on day 2 or 3, respectively, equally sized blastomeres and <20 % fragmentation, poor quality embryos consist of all the rest. Clinical pregnancy was defined as visualization of a gestational sac, while ongoing pregnancy necessitated the visualization of fetal cardiac activity on transvaginal ultrasound.

Statistical analysis was performed with chi-square and independent *t* test to compare categorical and continuous patient or clinical variables, respectively. All analyses have been performed using SAS software (version 9.3 of the SAS System for Windows; SAS Institute Inc., Cary, NC, USA). Results are presented as means ± standard deviations; $p < 0.05$ was considered significant. Based on our previous experience and assuming a $p < 0.05$ and 80 % power, it was calculated that 54 FET cycles were required to demonstrate a difference of 25 % in ongoing pregnancy rate between NC FET with the modified luteal support and the NC FET and AHR controls.

## Results

Tables 1 and 2 detail the clinical outcome of the four different FET groups in the two study periods. Mean patients' age during the study groups were comparable, except for statistically, but not clinically significant, older age of patients undergoing the artificial FET in the later (AHR-late), as compared to early (AHR-early), or to those who underwent natural cycle FET at the same period (NC-late) (35.1 ± 4.7 vs 32.4 ± 5.3 and 32.3 ± 5.5 years, respectively) (Table 1).

### Natural cycles FET

While the patients' clinical characteristics, the etiologies of infertility (Table 1), the prevalence of embryos that survived the thawing process, and the number of embryos and TQE transferred were comparable between the two study periods, implantation rate (31 vs 17 %; $p < 0.02$), positive β-hCG (52 vs 30 %; $p < 0.01$, respectively), and clinical (51 vs 26 %; $p < 0.01$, respectively) and ongoing (46 vs 20 %; $p < 0.01$,

**Table 1** Patients' characteristics of the four different FET groups in the two study periods

| Study group | 1 NC-late | 2 NC-early | *p* value between groups 1 and 2 | 3 AHR-late | 4 AHR-early | *p* value between groups 3 and 4 | *p* value between groups 2 and 4 | *p* value between groups 1 and 3 |
|---|---|---|---|---|---|---|---|---|
| Study period | June 2014–June 2015 natural cycle+ | May 2012–May 2014 natural cycle | | June 2014–June 2015 E2+ | May 2012–May 2014 E2+ | | | |
| No. of cycles | 59 | 74 | – | 54 | 113 | – | – | – |
| Mean patient age at OPU (years) | 32.3 ± 5.5 | 33.4 ± 5.0 | NS | 35.1 ± 4.7 | 32.4 + 5.3 | <0.01 | NS | <0.01 |
| Etiology of infertility: | | | | | | | | |
| Male factor | 39 | 46 | NS | 20 | 45 | NS | | |
| Unexplained | 14 | 18 | NS | 8 | 25 | NS | | |
| Endometriosis | 2 | 2 | NS | 4 | 3 | NS | | |
| Non-ovulatory | 0 | 0 | NS | 23 | 31 | NS | | |
| Others | 4 | 8 | NS | 2 | 11 | NS | | |

*NS* not significant

**Table 2** Clinical outcomes of the frozen-thawed ET cycles during the two periods

| Study group | 1 NC-late | 2 NC-early | *p* value between groups 1 and 2 | 3 AHR-late | 4 AHR-early | *p* value between groups 3 and 4 | *p* value between groups 2 and 4 | *p* value between groups 1 and 3 |
|---|---|---|---|---|---|---|---|---|
| Study period | June 2014–June 2015 natural cycle+ | May 2012–May 2014 natural cycle | | June 2014–June 2015 E2+ | May 2012-May 2014 E2+ | | | |
| No. of FET cycles | 59 | 74 | – | 54 | 113 | – | – | – |
| Total no. of surviving embryos/no. of embryos thawed (%) | 106/116 (91 %) | 141/155 (91 %) | NS | 98/117 (84 %) | 211/244 (86 %) | NS | NS | NS |
| No. of embryos thawed per cycle | 2 ± 0.7 | 2.1 ± 0.7 | NS | 2.2 ± 1.2 | 2.2 ± 1 | NS | NS | NS |
| No. of embryos transferred per cycle | 1.8 ± 0.5 | 1.8 ± 0.5 | NS | 1.8 ± 0.8 | 1.9 ± 0.6 | NS | NS | NS |
| No. of TQE transferred per cycle | 1.3 ± 0.7 | 1.1 ± 0.7 | NS | 1.4 ± 0.8 | 1.3 ± 0.9 | NS | NS | NS |
| Implantation rate (%) | 33/106 (31 %) | 24/139 (17 %) | <0.012 | 15/98 (15 %) | 29/213 (14 %) | NS | NS | <0.006 |
| No. of positive β-hCG per no. of FET cycle | 31/59 (52 %) | 22/74 (30 %) | <0.006 | 18/54 (33 %) | 42/113 (37 %) | NS | NS | <0.035 |
| No. of clinical pregnancy per no. of FET cycles | 30/59 (51 %) | 19/74 (26 %) | <0.002 | 12/54 (22 %) | 26/113 (23 %) | NS | NS | <0.000 |
| No. of ongoing pregnancy per no. of FET cycles | 27/59 (46 %) | 15/74 (20 %) | <0.001 | 9/54 (17 %) | 18/113 (16 %) | NS | NS | <0.000 |

*NS* not significant

respectively) pregnancy rates were significantly higher during the later period, characterized by the administration of additional two injections of recombinant hCG and GnRH-agonist, on day of transfer and 4 days later, respectively (Table 2).

### Artificial hormone replacement cycles FET

There were no in-between group (AHR-early vs AHR-late) differences in patients' clinical characteristics, the etiologies of infertility, the prevalence of embryos that survived the thawing process, and the number of embryos and TQE transferred, implantation, clinical, or ongoing pregnancy rates.

### Natural vs artificial hormone replacement cycles in the late period

While the patients' clinical characteristics, the prevalence of embryos that survived the thawing process, and the number of embryos and TQE transferred were comparable between the two study groups (NC-late vs AHR-late), implantation rate (31 vs 15 %; $p<0.01$), positive β-hCG (52 vs 33 %; $p<0.04$, respectively), and clinical (51 vs 22 %; $p<0.01$, respectively) and ongoing (46 vs 17 %; $p<0.01$, respectively) pregnancy rates were significantly higher in the natural cycle who received two additional injections of recombinant hCG and GnRH-agonist, on day of transfer and 4 days later, respectively (natural-late), as compared to patient undergoing the AHR group (AHR-late).

## Discussion

In the present study, we clearly observed significantly higher implantation, clinical, and ongoing pregnancy rates in natural cycle FET, with two additional injections, one of recombinant hCG (250 mcg) and the other of GnRH-agonist (triptorelin 0.1 mg), on day of transfer and 4 days later, respectively (NC-late). This novel protocol yields a significantly better outcome when compared to natural cycle FET without the additional two injections (NC-early) or the artificial hormone replacement FET cycles (AHR-early and AHR-late). Moreover, in order to control for laboratory and embryologists contributions in the different two study periods, we compared the outcome of artificial hormone replacement FET in the two study periods (AHR-early vs AHR-late), same intervention in different periods, which were found to be comparable.

In the present study, we could also compare the two natural cycle FET, with and without the additional two injection, to the artificial hormone replacement FET. In agreement with Mounce et al. [8], we could not find any significant differences in FET cycle outcomes between patients undergoing the natural cycle FET without the additional injections (Table 2) as compared to artificial hormone replacement FET. On the contrary, Levron et al. [9] demonstrated a better outcome using the natural cycle FET without the additional injections compared to artificial hormone replacement FET, difference that might be explained by the improvement in laboratory methodology in the later years and the use of vitrified embryos only (in the present study) compared to slow freezing [9].

A recent meta-analysis evaluating the different methods of endometrial preparation prior to FET could not identify one method of endometrial preparation in FET, as being more effective than another [4]. However, in accordance with our observation, a recent Cochrane review [10] demonstrated that while progesterone for luteal phase support was shown to improve live birth rate, co-treatments with GnRH agonists yield an additional benefit.

Perinatal morbidity following IVF cycles is significantly influenced by the high incidence of multiple births, which relates to the number of embryos transferred. Therefore, strategies of single fresh embryo transfer, or freeze-all, followed by a single FET cycle can dramatically reduce the rate of multiple births, without compromising the cumulative live birth rates [1]. This trend toward single embryo transfer results in the cryopreservation of more extra embryos for future replacement. Moreover, while presenting the cons and pros of FET, compared with fresh transfer, Shapiro et al. [11] highlighted the advantages of FET vs fresh cycles, which were related to the detrimental effect of controlled ovarian hyperstimulation on endometrial receptivity, leading to advancement of the receptive phase, which resulted in embryo–endometrium asynchrony.

The rationale behind choosing the aforementioned approach is based on the following observations:

*A natural cycle* was chosen based on the previous study demonstrating higher ongoing pregnancy rate following the transfer of frozen-thawed embryos in natural cycles with spontaneous LH rise compared with natural cycles controlled by hCG for final oocyte maturation [12]; *the administration of hCG injection* on day of transfer was chosen based on the ability of hCG to further improve the function of the corpus luteum [13]; and *the administration of GnRH-agonist* relied on the previous observed higher pregnancy rate in patients who received a mid-luteal injection of a GnRH-agonist [14, 15]. These latter effects were explained by a putative direct or indirect effect of the GnRH direct effect on the endometrium and/or corpus luteum. Moreover, the increase in LH levels following GnRH administration precedes several pathways, which result in the secretion of growth factors, cytokines, angiogenic, and adhesion molecules, all involved in the implantation process [14].

## Conclusions

The choice of endometrial preparation protocol for frozen-thawed ET cycle depends on the individual woman's ovarian

function and convenience of the method, as well as on the experience gained with the method by the team. When natural cycle FET is offered, our data suggest that the addition of two injections of recombinant hCG and GnRH-agonist, on day of transfer and 4 days later, respectively, might increase clinical pregnancy rates as compared to natural cycle FET without the additional injections or artificial hormone replacement FET. Further large prospective studies are needed to elucidate the aforementioned recommendation prior to its routine implementation.

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**Compliance with ethical standards** All authors read and approved the final manuscript.

**Conflict of interest** The authors declare that they have no competing interests.

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