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Frozen embryo transfer: the present practice and beyond

Abstract

Background: With the success of oocyte donation in vitro fertilization (IVF) in postmenopausal women, the importance of endometrial preparation became obvious. This appears also very crucial for achieving pregnancy after frozen embryo transfer (FET) after failed fresh IVF cycles and FET in subsequent natural cycles. Although there are different methods of preparing endometrium, in this study, another method of preparing the implantation bed using letrozole was scrutinized for its usefulness.

Methods: Two hundred and seventy patients between 28 and 40 years of age undergoing IVF due to bilateral tubal blocks were chosen for FET. They had a previous unsuccessful single IVF attempt or had postponed embryo transfer due to the threat of ovarian hyperstimulation syndrome or poor endometrial development. Informed consents were obtained from the participants. One hundred patients had endometrial preparation by gonadotrophin-releasing hormone agonist down-regulation and with hormone replacement therapy, 55 had natural cycle FET, and the remaining 115 patients had letrozole-induced ovulation induction (OI) for endometrial preparation.

Results: The clinical and biochemical pregnancy rate or live birth rate was higher in the letrozole OI group than in the other two groups.

Conclusions: OI by letrozole and subsequent endometrial preparation as a result of it may be utilized for FET, and this will be cheap and easy to administer, giving better success rates in FET cycles.

Keywords: down-regulated; endometrial preparation; frozen embryo transfer; letrozole; natural cycle.

Introduction

Immense effort is ongoing to increase the success rate in assisted reproductive technology (ART) procedures. A large number of variable factors that interact in in vitro fertilization (IVF) programs need to be in equilibrium, to achieve success in these procedures. Controlled ovarian hyperstimulation (COH) is no doubt of prime importance to obtain multiple eggs, but this also affects fertilization as well as the endometrial environment, which might affect the success rate. Associated factors for infertility, like endometriosis or tubercular infestation of the endometrium [1] or hydrosalpinx, may affect the endometrial receptivity along with embryo quality. It has been proven by many investigators that the success rate of ART increases with endometrial measurement (EM) in optimal endometrial/endocrinological condition, which may be affected in the stimulated cycle. This requires the transfer of a frozen embryo in a natural/down-regulated or minimally stimulated cycle, where ideally endometrial preparation can be performed.

The primary goal in establishing an appropriate freezing protocol is to do as little damage as possible while exposing specimens to non-physiologic, ultra-low temperatures. The popular protocols essentially freeze-dry or dehydrate blastocysts, to prevent intracellular ice from forming.

The transfer of cryopreserved embryos in women with functioning ovaries can be timed with ovulation in natural cycle or after artificially preparing the endometrium with exogenous hormones [2, 3]. Although ET in a natural cycle is often simple and obviates the need for prolonged hormonal supplementation in early pregnancy, the monitoring of ovulation can sometimes be pragmatically difficult, particularly in women with irregular menstrual cycles.
endometrial and embryo development have been developed based on the experience gained from oocyte donation programs [3–7]. This strategy has allowed the achievement of pregnancy rates comparable with those obtained from fresh transfer [8] as well as after cryo-thawed embryo replacement in a natural cycle [9, 10]. Moreover, attempts to simplify cycle programming have suggested that omitting the down-regulation phase does not adversely affect cycle outcome, provided that ultrasound (US) and/or endocrine monitoring of ovarian activity is performed [11–13].

Frozen embryo transfer (FET) is performed in the following conditions:
1. Failed fresh ET when excess embryos are kept frozen. In a subsequent cycle, FET can be performed.
2. In a stimulation cycle, when endometrial development is suboptimal or poor, fresh ET is postponed and FET is performed later on.
3. In cases of oocyte donation or surrogacy, if the recipient is not prepared for ET in corresponding cycles.

The most important procedure for FET is endometrial preparation. Frozen/thawed embryos may be transferred to the uterus either in a natural monitored cycle or in a hormone controlled cycle. The chance of achieving a pregnancy has been reported to be the same as for fresh ET [10, 14].

Replacement in a natural cycle

Natural cycle replacement is generally recommended when the woman is young and has normal, regular menstrual cycles, with previously confirmed ovulation. The women in this group attended the clinic from day 10 in the menstrual cycle, to monitor follicular growth and ovulation by serial US scanning, with measurement of the serial levels of serum estradiol, luteinizing hormone (LH), and progesterone. The US scan should also confirm appropriate endometrial development; the cycle should be cancelled if the EM is <8 mm at the time of LH surge. When the spontaneous LH surge has been detected, the day of embryo replacement is timed for 3 days later. No luteal phase support is given in these cycles.

Protocol for transfer in a natural menstrual cycle

An ovulation “kit” such as Clearpan can also be used in a previous cycle to confirm that the patient has regular ovulatory cycles.

Timing of the ET is as follows:
- Pro-nucleate embryos: thaw on day 1 after ovulation (3 days after the LH surge: LH+3), culture overnight
- Cleavage stage embryos: thaw and transfer on day 2 or 3 after ovulation (LH+4/5)
- Blastocysts: thaw and transfer on day 4 or 5 after ovulation (LH+6/7).

Replacement in a hormone controlled cycle

Older women and those with irregular cycles will usually be treated in cycles brought under control by the use of GnRH agonists such as buserelin, nafarelin, or leuprolide depot. Daily doses of the agonist are usually started on or about the preceding cycle day 21. Pituitary down-regulation is confirmed on the second day of menstruation. If baseline levels have been reached and the ovaries are quiescent on US scanning, the dose of agonist is reduced and hormone replacement therapy (HRT) with estradiol valerate tablets are given in doses of 2 mg orally daily, increasing to 4 mg on day 6 and 6 mg on day 10, reducing again to 4 mg from day 14 onward. Progesterone in the form of cyclogest or, more recently, crinone 8% is given from cycle day 15 onward [14].

Natural micronized progesterone 100 mg injection daily/200 mg vaginal capsule thrice daily/8% gel vaginally twice daily.

Embryo transfer

- Pro-nucleate: thaw on day 16 of the artificial cycle, culture overnight before transfer on day 17 or 18;
- Cleavage stage embryo: thaw and replace on day 17 or 18;
- Blastocysts: thaw and replace on day 19 or 20.

If pregnancy is established, continue the HRT with 8 mg estradiol valerate and the higher dose of progesterone supplement daily until day 77 after ET. Gradually, withdraw the drugs with monitoring of blood P4 (progesterone) levels.

Aim of the study

The study was conducted to find out the optimal protocol for endometrial preparation, particularly for FET, to increase the take-home baby rate in infertile population undergoing ART.
Materials and methods

Our approach of FET

Patient selection

In the period between January 2006 and December 2010, 270 patients between 28 and 40 years of age who have bilateral tubal blocks were included in the study for FET. All these patients had either previously unsuccessful single IVF attempt or postponed ET due to threat of ovarian hyperstimulation syndrome (OHSS) or poor endometrial development in fresh cycle. Informed consent was obtained from the participants for these procedures.

The patients were allocated to three methods of endometrial preparation: 100 patients were subjected to FET after preparing the endometrium by down-regulated followed by hormone replacement therapy and FET (DR HRT FET). These patients had irregular cycles and relatively high FSH levels and were older, that is, more than 35 years of age. A total of 55 patients had natural cycle FET. The patients allocated in the natural cycle are of younger age group and had a previous evidence of spontaneous ovulation with regular cycles. The factors for infertility were tubal block or typical unexplained infertility. The remaining 115 patients were allocated to endometrial preparation with letrozole and hCG. The patients allocated in the letrozole group had either minimum ovulation dysfunction or inadequate luteal phase. Most of them had postponed ET due to the threat of OHSS or poor endometrial development in the treatment cycle.

Embryo cryopreservation following original treatment cycle

Embryos were cryopreserved by slow freezing method using Embryo Freeze Kit from SAGE In-vitro Fertilization Inc., USA, in a programmable freezer (Freeze Control, Model CL-863 from Cryologic, Australia), PETG clear rigid embryo straws (Cryo Bio System, IMU, France), properly labeled with the patient couple’s names, embryo numbers, cell stage, day of culture, and date of freezing, were used for loading embryos before subjecting to cryopreservation, following which straws were stored in liquid nitrogen (N₂).

Endometrial preparation in down-regulated cycle

Cycle is down-regulated with leuprolide acetate (LA), and subsequently, the endometrium is prepared by administration of estradiol valerate. Luteal suppression is accomplished using 1.0 mg GnRHα from day 21 of the previous cycle. This dosage was reduced to 0.5 mg, starting on the predetermined day 1 of the cycle and maintained until the starting day of progesterone. When the patient stayed far or there was difficulty in taking daily dose, LA depot (3.75 mg, S/C) was administered on day 21 of the cycle. Down-regulation was confirmed by an endometrial thickness of <5 mm and inactive ovaries at US and, sometimes, seeing the LH and E₂ levels. Estradiol valerate was administered at 2 mg. BD daily dose started from day 1 or day 2 of the cycle, increasing as required after monitoring for EM starting from D5/D6, until the ET reaches a minimum of 8 mm with clear triluteal phase. The patients showing poor endometrial development were excluded from the study.

Endometrial preparation in the natural cycle

The whole procedure is performed as described in the present practice.

Our procedure of endometrial preparation beyond present practice (using letrozole)

A third procedure for endometrial development for FET was performed using letrozole for ovarian stimulation. This was used in patients with infrequent ovulation and irregular cycles; letrozole 5 mg daily in two divided doses was used to induce ovulation. Ovulation and EM monitoring was continued from day 7 of the cycle. hCG was administered with the largest follicle being of 18 mm in diameter and EM more than 7 mm. These patients were abstained from having any sexual intercourse. Ovulation was confirmed by US monitoring, and ET was performed after 48 h of proven ovulation.

Embryo thawing

Thawing was performed by taking out the respective straw from a liquid N₂ can, keeping in air for 30 min, and then putting into water bath at 30°C for 1 min, following which the embryos were treated serially in a thawing solution of embryo thawing kit from SAGE In-vitro Fertilization Inc., USA, next washed in an equilibrated cleavage medium from SAGE, and kept in culture for 1 h before transfer. Embryos were transferred using K-JETS 6019 catheter from Cook Int. (Australia), under US guidance.

Embryo transfer

A minimum of two and maximum of three frozen embryos were transferred in all these cases. The average number of embryos transferred was 2.6±0.6. All of them were cleaving stage embryos, frozen after 48 h of incubation in treatment cycle. All of them were of Grade 1-2 embryos, and any embryo less than Grade 2 was discarded from the study. ET was performed under US guidance, and no anesthesia was given.

The entire experimental protocol was performed according to the ethical guidelines of the Institutional Ethical Committee and the Indian Council of Medical Research, New Delhi, India.

Results

The pregnancy rate (clinical and biochemical) or live birth rate in our series is presented in Table 1. It was observed that the pregnancy rate (clinical and biochemical) and live
birth rate are maximal in the letrozole-ovulation induction (OI) group (p=0.03). The pregnancy rate is less in the natural cycle than the down-regulated cycle, but the take-home baby rates are almost similar. This indicates that the abortion rate is minimum in natural cycle FET.

The success rates mentioned in the literature are so many. A Cochrane review entitled “Cycle Regimens for Frozen-Thawed Embryo Transfer” showed a comparison between DR HRT FET and clomiphene FET. This comparison was identified in one RCT including 104 women. The clinical pregnancy rate was noted to be higher in the down-regulated hormone replacement therapy frozen embryo transfer (DR HRT FET) group. Although we used letrozole instead of clomiphene citrate (CC), our result is much better and statistically, significantly higher. The same Cochrane review performed a comparison between estrogen-progesterone FET and DR HRT FET, which did not find a significant success rate. A similar comparison of four studies compared DR HRT FET and estrogen-progesterone supplemented FET. The meta-analysis did not show any statistically significant difference in the success rates in the two groups. A similar study presented by the Bourn Hall group did not find any statistically significant difference in the pregnancy rate of natural cycle FET and DR HRT FET, although the patients were assigned to these two groups according to different criteria [15].

Discussion

The uniqueness of embryo cryopreservation either in the pronuclear, cleavage, or blastocyst stage is to allow multiple transfer cycles from single oocyte retrieval and thereby reducing the overall cost of fertility treatment. The techniques for embryo cryopreservation include slow freezing and rapid freezing or vitrification. ET is accomplished by brief exposure to air and warm water followed by rehydration [16]. Although the pregnancy rates for freeze/thaw transfer (FET) cycles using the two cryopreservation methods are similar, vitrification is associated with a higher post-thaw embryo survival (93% vs. 76% with slow freezing) [17]. In fact, outcomes are reassuring for slow freezing but are more limited for the newer technique of vitrification [18]. The result of FET as compared with its fresh counterpart shows a lower pregnancy rate, which may be due to embryo selection or the availability of next best embryos in FET, because the best groups are utilized for fresh transfer.

There are certain studies where, in the non-down-regulated cycles, exogenous estrogen supplementation is continued for endometrial preparation during FET. The pregnancy and live birth rate outcome is found to be similar in our observational study, which corresponds to several other similar investigations. But in the case of letrozole-stimulated cycle as we have performed, the take-home baby rate is higher than the other two protocols, although the clinical pregnancy rate is comparable with that of their counterparts. This is probably due to better endometrial preparation, leading to less implantation failure and early embryonic loss. Letrozole does not stimulate the supraphysiologic estradiol level, as it happens with the CC or COH cycle, which may be harmful to the embryos as well as the endometrium. Moreover, there is no hormonal supplementation that may lead to untimed ovulation [19, 20]. Untimed ovulation as it happens with non-down-regulated cycles appears to be the principal cause of less pregnancy rate after embryo replacement [21, 22]. Untimed ovulation as it happens with non-down-regulated cycles appears to be the principal cause of less pregnancy rate after embryo replacement [23]. Another factor for getting a higher pregnancy rate in the letrozole group is the use of hCG in OI. LH receptors are localized in the endometrium [23, 24], indicating that the endometrium is a target organ for LH. Unlike previous suggestions that LH can only indirectly affect the endometrium through ovarian steroid hormone production [2], recent studies have linked exposure to LH with various regulatory changes pertinent to the morphological and functional proliferation and differentiation of endometrial glands and stroma, mainly via activation of the adenylate cyclase and phospholipase C pathways and increasing the local synthesis of steroid hormones [25–29]. Because these changes are normally synchronized to

<table>
<thead>
<tr>
<th>Patients</th>
<th>Biochemical pregnancies</th>
<th>Clinical pregnancies</th>
<th>Live birth rates</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR HRT FET</td>
<td>100</td>
<td>44 (44%)</td>
<td>32 (32%)</td>
<td>24 (24%)</td>
</tr>
<tr>
<td>Natural cycle FET</td>
<td>55</td>
<td>15 (27%)</td>
<td>14 (27%)</td>
<td>11 (20%)</td>
</tr>
<tr>
<td>Letrozole-OI induced FET</td>
<td>115</td>
<td>61 (53%)</td>
<td>48 (41.7%)</td>
<td>38 (33%)</td>
</tr>
</tbody>
</table>
follicular development in the natural cycle [30], it is possible that the administered hCG, which acts like LH, may induce favorable changes in the endometrium by proper decidualization, making it more receptive for implanting the embryos.

OI with letrozole not only helps in fertility promotion in anovulatory women but also is of immense importance for preparing the endometrium ideally to create a proper implantation bed for frozen embryos following transfer in ART program.

Conflict of interest statement

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