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ORIGINAL PAPER

Natural cycle cryo-thaw transfer may improve pregnancy outcome

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Abstract *Objective*: To compare natural vs. hormone replacement treatment (HRT) for cryo-thaw embryo transfer cycles in patients with frozen embryos from previous ART. *Design and Settings*: Retrospective chart review of 164 patients (242 cycles) who underwent natural or HRT cryo-thaw embryo transfer between January 2002 and April 2005. *Main Outcome Measures*: Pregnancy rates per transfer in natural and HRT cryo-thaw cycles. *Results*: The pregnancy rate per transfer was higher with natural cycles (36.76% vs. 22.99%, $P = 0.0298$). There was no statistical difference in mean age, endometrial thickness, and average embryo quality in successful cycles. Mean endometrial thickness prior to transfer was greater in natural cycles (9.95 vs. 8.89 mm, $P < 0.001$). Mean serum estradiol levels were higher in the HRT group prior to transfer (526.1 vs. 103.8 pg/ml, $P < 0.001$), and were found to be lower in women who achieved pregnancy (337.1 vs. 433.3 pg/ml, $P = 0.0136$). *Conclusion*: Hormone replacement in preparation for cryo-thaw transfer of embryos was found to be associated with decreased pregnancy rates in comparison to natural cycle cryo-thaw transfer. Greater endometrial thickness was achieved with lower serum estradiol levels in patients undergoing natural cycles, suggesting that higher estradiol levels during HRT cycle may interfere with the window of implantation.

Keywords Cryo-thaw cycle · Natural · HRT

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Introduction

Cryo-thaw cycles with embryo transfer have become one of many assisted reproductive techniques widely used in this country and around the world. It involves the process of cryopreservation of embryos obtained during in-vitro fertilization (IVF) cycles. According to the 2003 CDC report, frozen embryos were used in approximately 14% of all assisted reproductive technology (ART) cycles performed in 2003 (17,517 cycles) [1].

Multiple factors influence the success rate of cryo-thaw cycles, including age of the patient at time of cryopreservation, cause of infertility, grade of the embryos being transfered, level of estradiol and endometrial thickness at time of transfer. It was suggested that the age of the patient above 40 years at a time transfer is a negative prognostic factor in cryo-thaw transfer [2]. Tubal disease as an etiology of infertility was also reported as a negative factor in cryo-thaw embryo transfer [2]. Endometrial thickness at a time of transfer greater than 9 mm was reported to be a positive prognostic factor for embryo implantation in in-vitro fertilization (IVF) [3, 4]. One of the debated topics in the field of embryo transfer is whether hormone replacement therapy with exogenous estrogen prior to transfer improves pregnancy rates as opposed to cryo-thaw transfer in natural cycles. Different IVF practices around the world are utilizing different approaches with regards to endometrial preparation with gonadotropins/GnRH agonists and exogenous estrogens for "controlled" cryo-thaw embryo transfer. Several reports suggest that there is no difference in implantation and pregnancy rates between natural and estrogen prepared cycles [5–7].

This report describes an examination of cryo-thaw data of several years duration in order to establish whether there was any correlation between age of the patient, embryo grade, level of estradiol, endometrial thickness immediately prior to transfer, estrogen administration before embryo transfer, and pregnancy rates. This study was designed to assess whether cryo-thaw embryo transfers performed during recipients' natural menstrual cycle were as successful as those performed in a hormone replacement treatment (HRT) cycle.

Methods

Charts of one hundred and twenty three patients who underwent cryo-thaw embryo transfer at Long Island IVF between January 2002 and April 2005 were reviewed. Approval from Institutional Review Boards at both Mather Hospital and North Shore-LIJ Medical Center were obtained before chart review was performed. Multiple parameters were collected including age, cause of infertility, day of embryo freezing, number of embryos frozen, number of embryos transferred, embryo grade at time of freezing and time of transfer, endometrial thickness and estradiol (E_2) level at time of transfer, method of endometrial preparation, and outcome with regard to establishing pregnancy.

Patient who underwent egg-donation cycles were excluded from our study. Embryos were frozen on day 3 after fertilization. For an embryo to be selected for cryo-thaw transfer, it must have satisfied the parameters of having 4 or more cells and scored a grade 3 or higher. The gradation system used was based on the fragmentation percentage of the embryo, with grade 1 equal to or less than 10% fragmentation, and grade 5 being equal to or more than 50% fragmentation. Average Embryo Score (AES) was calculated as the Combined Embryo Score (CES) divided by a total number of embryos transferred. The CES was calculated by multiplying the number of cells in the embryo by the grade, where the grade was assigned numerical values from 1 to 5. The value of grades was reversed for statistical calculations, with grade 1 weighing 5 points and grade 5 weighing 1 point. Embryos were assigned an AES both before freezing and just prior to transfer.

During the freezing process, embryos were cooled at room temperature for 5 min, then transferred into 1.5 M PROP/mHTF (LifeGlobal®, The Art Media Company, Canada) with 10% S.S.S. (Irvine Scientific, CA, USA) for 10 min, followed by transfer into 1.5 M PROP/mHTF/0.2 M Sucrose (Life Global) with 10% S.S.S. (Irvine) for 10 min. Finally, the embryos were transferred into 2.0 ml vials (Corning Inc., Corning, NY) containing 0.25 ml of 1.5 M PROP/mHTF/0.2 M Sucrose. Vials were loaded into the freezing machine (Planer PLC, Middlesex, United Kingdom) at 20°C, and cooled at a rate of 2°C/min to minus 7°C, held for 5 min at minus 7°C, seeded and subsequently cooled at a rate of 0.3°C to minus 35°C, followed by a "plunge" into liquid nitrogen.

Thawing was initiated the day prior to transfer. Vials were thawed at room temperature for 45 s, and then transferred into a 34°C water bath for 2.5 min. Embryos were then removed from the vials and transferred into 1.0 M PROP/0.2 M Sucrose/mHTF (Life Global) plus 10% S.S.S. (Irvine) for 5 min. Embryos were further thawed using 0.5 M PROP/0.2 M Sucrose/mHTF (Life Global) plus 10% S.S.S. (Irvine) for 5 min, followed by 0.2 M Sucrose/mHTF (Life Global) plus 10% S.S.S. (Irvine) for 10 min, followed by exposure to mHTF with 10% S.S.S for 5 min at room temperature, and finishing by warming up to 37°C for 5 min. Embryos were rinsed and cultured overnight using Multiblast (Irvine) with 15% S.S.S at 37°C with 5% CO_2.

Assisted hatching was performed on all embryos transferred using acid tyrods. Transfer was performed using a Wallace catheter by one of the board certified reproductive specialists of a large private infertility practice, utilizing the IVF Laboratory at John T. Mather Memorial Hospital.

The protocol for estrogen/progesterone replacement used in HRT cryo-thaw cycles was as follows: oral micronized estradiol, 2 mg, two times daily, was introduced on cycle day 2, and patient returned on cycle day 13 for transvaginal ultrasound measurement of endometrial thickness. If endometrial lining measured 8 mm or greater, progesterone (P_4) 50 mg in oil was administered via IM injection. Transfer of thawed embryos was performed 3 days later.

During natural cryo-thaw cycles, recipients were monitored for evidence of LH surge and dominant follicle collapse by serum testing and transvaginal ultrasound, respectively. Thawed embryos were transferred 3 days after collapse, and vaginal progesterone supplementation, 200 mg capsules four times daily, was started on the day after embryo transfer. No exogenous stimulation, e.g. clomiphene citrate, of either HRT or natural cry-thaw cycle was utilized.

Positive clinical pregnancy was defined as having documented fetal heart beat on transvaginal ultrasound by 7 weeks gestation. Patients with documented intrauterine pregnancy were referred back to their obstetricians for routine care. Negative pregnancy was defined as a level of beta-HCG below 5 mIU/ml.

Variables were analyzed using GraphPad Prism version 4.03 for Windows (GraphPad Software, San Diego, California USA). The chi-square test was used to compare pregnancy rates, and the unpaired t-test with a two-tailed P value ($P < 0.05$, CI 95%) was used to compare mean values for various other parameters.

Table 1 Comparison between HRT and natural cycles

	HRT	Natural cycle
Number of cycles	174	68
Number of pregnancies (pregnancy rate)	41 (22.99 %)*	25 (36.76 %)*
Mean no of embryos transferred	2.73 ± 0.08	2.86 ± 0.09
Mean age (years)	37.19 ± 0.38	35.46 ± 0.42
Mean AES at freezing	25.15 ± 0.56*	22.63 ± 0.79*
Mean AES at transfer	26.39 ± 0.57	25.88 ± 0.75
Mean endometrial thickness (mm)	8.89 ± 0.14*	9.95 ± 0.26*
Mean E_2 level (pg/ml)	526.1 ± 16.90*	103.8 ± 6.75*

*P-value < 0.05, CI - 95%.

Values expressed as Mean ± SER.

Results

Two hundred and forty two cryo-thaw cycles were analyzed. The data are summarized in Table 1 and Table 2. Variables between natural and HRT cryo-thaw cycles are recorded in Table 1. Variables between cryo-thaw cycles that achieved pregnancy and those that did not are summarized in Table 2.

We found that pregnancy rate per transfer was significantly higher in the natural cycle group as compared to the HRT group, 36.76% vs. 22.99%, respectively (P value 0.0298, 95% CI 1.42 – 26.13). There was no statistically significant difference between the mean ages of women who become pregnant vs. those who did not (36.46 vs. 38.49), or the mean ages of women in the HRT group vs. the natural cycle group (37.19 vs. 35.46).

There was no difference in the mean number of embryos transferred between the natural and the HRT groups. A modest statistically significant difference existed between the mean number of transferred embryos in pregnant and non-pregnant groups, 2.97 vs. 2.70, P value 0.04 (Table 2). No statistically significant differences were observed in the Average Embryo Score (AES) at transfer in the HRT and the natural cycle group, however a difference was noted in AES at freezing between the HRT and the natural cycle groups (25.15 vs. 22.63, P value 0.02), with higher embryo scores in the HRT group (Table 1). There was no statistical difference between AES at freezing or transfer in pregnant women and women who did not become pregnant (Table 2).

Table 2 Comparison between cycles that achieved pregnancy vs. cycles that did not

	Pregnant	Not pregnant
Number of cycles	65	177
Mean no of embryos transferred	2.97 ±0.11*	2.70 ± 0.07*
Mean Age (years)	36.46 ± 0.67	38.49 ± 1.71
Mean AES at freezing	25.42 ± 0.87	24.08 ± 0.55
Mean AES at transfer	27.59 ± 0.86	25.76 ± 0.55
Mean endometrial thickness (mm)	9.32 ± 0.29	9.83 ± 0.72
Mean E_2 level (pg/ml)	337.1 ± 29.86*	433.3 ± 20.72*

*P-value < 0.05, CI - 95%.

Values expressed as Mean ± SER.

Interestingly, mean endometrial thickness was noted to be significantly greater in the natural cycle group compared to the HRT group, 9.95 mm vs. 8.89 mm, $P < 0.001$, (Table 1), yet no statistical difference was observed in endometrial thickness between pregnant and non-pregnant patients (Table 2).

As expected, mean E_2 level was higher in the HRT group than in the natural cycle group (526.1 pg/ml vs. 103.8 pg/ml). Mean E_2 level was found to be significantly lower in women who achieved pregnancy than in those who did not (337.1 pg/ml vs. 433.3, $P = 0.0136$).

Discussion

Multiple reports have suggested that neither implantation rate nor pregnancy rate is affected by estrogen replacement therapy in cryo-thaw cycles [6, 8, 9]. HRT cycles are those in which exogenous estrogen and progesterone are administered in order to control and optimize the recipient's hormonal and endometrial environment in preparation for the transfer of the cryo-thawed embryos. Currently, controversy exists as to whether HRT cycles offer any benefit over transfers performed during natural cycles. Some authors have shown that in women with normal cycle length pregnancy rates are higher in natural cycles than in estrogen prepared cryo-thaw cycles [10]. One study did demonstrate a significant benefit of hormone replacement over natural cycle cryo-thaw transfer in women with oligomenorrhea [11]. However, in regularly menstruating women undergoing cryo-thaw transfer, natural cycle transfer appears to be the method of choice.

Research has shown that temporal characteristics of the endometrium such as the formation of uterodomes (pinopods)—markers of endometrial receptivity—become out-of-phase when measured in normally menstruating females who were placed on exogenous HRT [12]. An adult mouse model with progesterone-treated delayed implantation has shown that the window of uterine receptivity, the "nidation window," for implantation in IVF/ET cycles closes much faster at a higher level of E_2 (3.0–25.0 ng) compared to low E_2 levels (3.0 ng) [13]. This suggests that uterine gene expression responsible for blastocyst implantation is maintained at a lower estrogen concentration and becomes refractory at higher levels of circulating E_2. Our study supports this theory by demonstrating, contrary to previously published results, that higher pregnancy rates per transfer can be achieved with natural cycles in which E_2 levels are lower.

Endometrial thickness has been reported to be a positive prognostic factor in cryo-thaw embryo transfers, with higher success rates when endometrial thickness is greater than 8 mm at a time of transfer [8, 14]. Other studies suggest that an endometrial thickness of 10 mm or greater at the time of transfer achieves the best results [10, 15]. Although we did not demonstrate any difference in endometrial thickness between patients who became pregnant and those who did not, the average endometrial thickness at the time of embryo transfer was close to 10 mm in women who underwent the natural cycle protocol. This supports the hypothesis that better results are achieved with an endometrial thickness of 10 mm or greater. Thus, higher doses of estrogen replacement may be required to achieve an adequate endometrial thickness in HRT cycles, while at the same time compromising what seems to be a better pregnancy outcome with lower E_2 levels during cryo-thaw cycles. Further research is required to find the optimal dosing of estrogen in HRT cryo-thaw cycles.

Maternal age at the time of IVF/cryo-thaw cycle is an important predictor of success, with highest live birth rates reported in women aged 26–30 [16]. For women under 34 years of age, the chance of successful pregnancy outcome after a single IFV cycle is reported to be about 52%, with a linear decline in the live birth rate from ages 35 to 44. An age-dependent rise in the frequency of miscarriage is also evident, from 10.5% for women under 35 years to 16.1% for those 35–39 years, and 42.9% for those over 40 years [17]. In our study, we found no difference between the average age of women who became pregnant with the cryo-thaw cycle and whose who did not. We failed to demonstrate any statistically significant difference in the average age of women within either the HRT or the natural cycle groups. We have to acknowledge, however, that patients placed on HRT protocol were in part selected by age in anticipation of ovulatory dysfunction. Some HRT patients elected HRT for the convenience.

Several weaknesses exist within the study. Although maternal age may be a significant predictor of success, the age of the embryo itself remains an unexplored variable. Conceivably, embryos from younger women are healthier and once implanted would develop with the same success rate in both young and old recipients. This may explain why no statistical difference was demonstrated between the average age of women who became pregnant and those who did not. The progestin preparation and mode of administration, clearly different for HRT and natural cycles in our study, may affect the results, as demonstrated by Miles et al [18]. In addition, although the pool of patients was large enough to achieve statistical significance, the study would be stronger with a larger sample, especially in the natural cycle group. We do not have enough information to provide the statistics about delivery rates, which is the ultimate endpoint of fertility treatment. That is in part due to the specificity of fertility practice and the fact that all patients who conceive using ART go back to their respective obstetricians for routine care and delivery. Finally, the study was limited by the retrospective nature of chart review, a method with its own intrinsic bias.

Conclusion

Higher pregnancy rates per transfer were observed in recipients who underwent natural cryo-thaw cycles than in HRT cycles. These patients had lower estradiol levels and greater endometrial thicknesses than did the HRT cycle recipients. These results may support the theory that the window of uterine receptivity in ART cycles closes earlier at higher endogenous estrogen level, limiting the time for the transferred embryos to implant successfully. Our study also suggests that better endometrial thickness (close to10 mm) is achieved in natural cryo-thaw cycles. Future randomized prospective trials of natural vs. HRT cryo-thaw cycles would be useful in confirming these observational findings.

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