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Original Article

Clinical outcomes of two different endometrial preparation methods for cryopreserved-thawed embryo transfer in patients with a normal menstrual cycle

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Aim: To compare the clinical outcomes of cryopreserved-thawed embryo transfer among patients with a normal menstrual cycle who had natural or hormone-replacement cycles.

Methods: From January 2004 to June 2006, cryopreserved embryos following conventional *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) were thawed and transferred in a total of 720 natural cycles and 136 hormone-replacement cycles.

Results: Cryopreserved-thawed embryo transfer in patients who had a natural or hormone-replacement cycle resulted in clinical pregnancy in 43.1% and 40.4%, respectively; a rate of miscarriage of 14.5% and 23.6%, respectively; and a rate of ongoing pregnancy and delivery of 36.5% and 30.9%, respectively. None of these differences were statistically significant.

Conclusions: Patients with a normal menstrual cycle who have natural or hormone-replacement cycles can be expected to have comparable clinical outcomes with cryopreserved-thawed embryo transfer. (Reprod Med Biol 2007; **6**: 53–57)

Key words: cryopreserved-thawed embryo transfer, hormone-replacement cycle, natural cycle.

INTRODUCTION

EVER SINCE TROUNSON *et al*.[1] reported the first childbirth after cryopreserved-thawed embryo transfer in 1983, this technique has become a very common and extremely important technique in assisted reproductive medicine. Cryopreservation of surplus embryos improves pregnancy outcomes per ovum collection cycle, and it is very useful to cryopreserve all embryos to avoid ovarian hyperstimulation syndrome (OHSS). Furthermore, cryopreserved-thawed embryo transfer is effective in patients in whom fresh embryo transfer in a stimulated cycle has been repeatedly unsuccessful or in patients with a thin endometrium.[2]

With recent improvements in assisted reproductive medicine, fewer embryos have to be transferred. Many more institutions are actively carrying out only single embryo transfer in certain cases. This increases the number of surplus embryos and the opportunity for cryopreservation. In fact, according to a European study in 2002, *in vitro* fertilization (IVF) was carried out in 122 634 cycles, intracytoplasmic sperm injection (ICSI) in 135 048 cycles, and cryopreserved-thawed embryo transfer in 57 162 cycles.[3]

Cryopreserved-thawed embryo transfer is an extremely effective and very safe technique for preventing the two major complications, OHSS and multiple pregnancy, that occur in assisted reproductive medicine. Cryopreserved-thawed embryo transfer is carried out in a natural, clomiphene, or hormone-replacement cycle. As a general rule, in patients with ovulation disorders, cryopreserved-thawed embryo transfer is carried out in either a clomiphene or hormone-replacement cycle. However, in patients with spontaneous ovulation and a normal menstrual cycle, cryopreserved-thawed embryo transfer can be carried out in either a natural or hormone-replacement cycle.

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Cryopreserved-thawed embryo transfer in a hormone-replacement cycle can be carried out with or without a GnRH agonist. Furthermore, in order to simplify the procedure, a GnRH agonist need not be given.

There have been no previous studies of patients with a normal menstrual cycle who had cryopreserved-thawed embryo transfer that compared a natural cycle and a hormone-replacement cycle without a GnRH agonist.

In the present study, the clinical outcomes for cryopreserved-thawed embryo transfer in patients with normal menstrual cycles were compared between natural and hormone-replacement cycles.

MATERIALS AND METHODS

FROM JANUARY 2004 to June 2006, cryopreserved-thawed embryo transfer was carried out in a total of 856 cycles in patients with normal 25–38-day menstrual cycles. Of these, thawed embryo transfer was carried out in a total of 136 hormone replacement cycles when it was difficult for patients to make visits to ascertain the ovulation date or if the patients requested a specific date for embryo transfer, and in a total of 720 natural cycles when no particular schedule adjustment was needed.

Patients in the present study were given sufficient information to provide written informed consent for the assisted reproductive technique.

Ovarian stimulation was carried out using a gonadotropin-releasing hormone agonist (buserelin acetate; Buserecure, Fujipharmaceutical, Tokyo, Japan) starting at the midluteal phase of the previous cycle (long protocol) or on day 2 of the cycle (short protocol), in combination with pure follicle stimulating hormone (FSH; Fertinom P, Serono, Tokyo, Japan) or human menopausal gonadotropin (hMG; Humegon, Japan Organon, Osaka, Japan) 150–300 IU/day. If the long or short protocol had been previously unsuccessful, then starting on day 3 of menstruation, 150–300 IU/day of FSH or hMG were given and when the average diameter of the leading follicle exceeded 14 mm, 0.25 mg of gonadotropin-releasing hormone antagonist (Cetrotide; Serono, Frenchs Forest, NSW, Australia) was given for 2–4 days. In patients with a past history of OHSS, in patients with polycystic ovary syndrome (PCOS), and in patients who wished to have minimal ovarian stimulation, 50–100 mg of clomiphene citrate was given for 5 days starting on day 3 of menstruation. Then, starting on day 5–8 of menstruation, 75–225 units of FSH or hMG were injected every second day. When the leading follicle reached a diameter of >18 mm, 5000–10000 IU of human chorionic gonadotropin (hCG; Profasi, Serono, Tokyo, Japan) were given, and oocyte retrieval was carried out 35–36 h later by vaginal ultrasound-guided follicle aspiration.

Patients underwent standard insemination or intracytoplasmic sperm injection (ICSI), as clinically appropriate, 4–6 h after oocyte retrieval. ICSI was carried out if: (i) insemination by conventional IVF would have been difficult because of severe oligozoospermia or asthenozoospermia; (ii) insemination by conventional IVF had failed in the past; or (iii) the fertilization rate was extremely poor.

Embryos were cultured in Quinn's Advantage medium (SAGE In-Vitro Fertilization, Trumbull, CT, USA) or Universal IVF Medium and BlastAssist System 1,2 (MediCult a/s, Jyllinge, Denmark) or early cleavage medium and Multiblast medium (Irvine Scientific, Santa Ana, CA, USA) in a 5% CO_2, 5% O_2 and 90% N_2 environment.

Surplus embryos following fresh embryo transfer, as well as embryos from patients in whom OHSS was expected, were cryopreserved by vitrification (Cryotop and Vitrification kit, Kitazato Supply, Shizuoka, Japan) at the cleavage stage 2 or 3 days after ovum collection, or at the blastocyst stage 5 or 6 days after ovum collection.

In patients who had undergone several previous unsuccessful transfers of high-quality embryos, assisted hatching was carried out with consent, particularly when cryopreserved embryo transfer was carried out.

Embryos were thawed using the Cryotop and Vitrification kit (Kitazato Supply, Shizuoka, Japan).

Embryos, in either the cleavage or blastocyst stage, were transferred to the uterus using a Phycon IVF Catheter (Fuji System, Tokyo, Japan) or an FS-ET Catheter (Kitazato Supply, Shizuoka, Japan) under ultrasound guidance.

When carrying out cryopreserved-thawed embryo transfer in a natural cycle, the embryos that were cryopreserved 3 days after ovum collection were thawed and transferred on day 3 of ovulation (day 0, the day of ovulation in a natural cycle), or the embryos in the blastocyst stage that were cryopreserved 5 or 6 days after ovum collection were thawed and transferred on day 5 of ovulation.

Cryopreserved-thawed embryos transferred in a hormone-replacement cycle were cryopreserved 3 days after ovum collection, then thawed and transferred on day 3 of hormone replacement (or the fourth day of luteal hormone replacement; day 0, day of luteal hormone replacement), or the embryos in the blastocyst stage that were cryopreserved 5 or 6 days after ovum collection were thawed and transferred on day 5 of

hormone replacement (or the sixth day of luteal hormone replacement).

For cryopreserved-thawed embryos transferred in a natural cycle, patients were instructed to visit the hospital a few days before the expected ovulation date, so that a transvaginal ultrasound could be carried out to ascertain follicle size, and urinary LH could be measured. Urinary LH was monitored for ovulation, and ovulation was facilitated by giving hCG. Transvaginal ultrasound was carried out to confirm the loss of follicles (end of ovulation). From day 2 of ovulation, chlormadinone acetate (2T, b.i.d., 10 days) was given in order to prevent implantation difficulties caused by corpus luteum insufficiency.

In patients who had cryopreserved-thawed embryo transfer in a hormone-replacement cycle, transdermally absorbed estradiol (Estrana, 0.72 mg/day; Hisamitsu Pharmaceutical, Tokyo, Japan) was given starting 1–3 days after the onset of menstruation; on the first day of menstruation, one sheet of transdermal estradiol was given and the number of sheets was increased every other day up to four sheets. After confirming that the thickness of the endometrium was at least 8 mm, the number of sheets was decreased to three and after embryo transfer, two sheets were given every other day. With respect to the luteal hormones, 222 mg of progesterone vaginal suppository were given twice daily every 12 h, and 125 mg of hydroxyprogesterone caproate (Proge Depot; Mochida Pharmaceutical, Tokyo, Japan) were injected intramuscularly once weekly until the 8th and 10th week of pregnancy. When it was difficult to insert a vaginal suppository, 50 mg of progesterone (medroxyprogesterone acetate; Fujipharma, Tokyo, Japan) were given daily intramuscularly until the 8th week of pregnancy, and then 125 mg of hydroxyprogesterone caproate was given until the 10th week of pregnancy.

Statistical analysis

In the present study, clinical pregnancy was defined as the presence of a fetal sac on transvaginal ultrasonography. The clinical pregnancy rate per embryo transfer was calculated as the number of cycles that were diagnosed as pregnant, divided by the number of transfers carried out. The ongoing pregnancy/delivery rate per oocyte retrieval was calculated as the number of ongoing pregnant and delivered cycles minus miscarried cycles, divided by the number of cycles that oocyte retrieval was carried out.

Statistical analysis was carried out using the χ^2-test and Student's *t*-test, with a significance level of 0.05.

RESULTS

AS SHOWN IN Table 1, the average age of the patients who had cryopreserved-thawed embryo transfer in

Table 1 Patients who had cryopreserved-thawed embryo transfer in a natural or hormone-replacement cycle

	Natural cycle	Hormone-replacement cycle
No. cycles	720	136
Age (years, mean ± SD)	34.7 ± 3.4	34.5 ± 3.6
Age range (years)	24–44	25–44
Average infertility period (months)	42.6 ± 30.7	39.5 ± 30.8
Mean number of embryos transferred	1.58 ± 0.57	1.52 ± 0.54
Number of transferred embryo (%)		
1	332 (46.1%)	68 (50.0%)
2	359 (49.9%)	65 (47.8%)
3	29 (4.0%)	3 (2.2%)
Cycles of cleavage stage and blastocyst stage (%)		
Cleavage stage	149 (20.7%)	29 (21.3%)
Blastocyst stage	571 (79.3%)	107 (78.7%)
Cycles of conventional IVF and ICSI cycles (%)		
Conventional IVF	412 (57.2%)	81 (59.6%)
ICSI	295 (41.0%)	54 (39.7%)
Conventional IVF and ICSI	13 (1.8%)	1 (0.7%)
Cycles involving assisted hatching (%)	568 (78.9%)	104 (76.5%)
Endometrial thickness (mm)	11.5 ± 3.7	10.9 ± 2.0

None of the differences between the two groups were statistically significant for any of the variables.
ICSI, intracytoplasmic sperm injection; IVF, *in vitro* fertilization.

Table 2 Comparison of the clinical outcomes for cryopreserved-thawed embryo transfer carried out in natural and hormone-replacement cycles

	Natural cycle	Hormone-replacement cycle
No. chemical pregnancies	371	73
Chemical pregnancy rate per transfer cycle	51.5%	53.7%
No. clinical pregnancies	310	55
Clinical pregnancy rate per transfer cycle	43.1%	40.4%
Implantation rate	29.5%	28.0%
No. miscarriages	45	13
Miscarriage rate	14.5%	23.6%
No. ectopic pregnancies	2	0
No. ongoing pregnancies and deliveries	263	42
Ongoing pregnancy/delivery rate per transfer cycle	36.5%	30.9%
No. twin pregnancies	23	3
Twin pregnancy rate	7.4%	5.5%

None of the differences between the two groups were statistically significant for any of the variables.

a total of 720 natural cycles and 136 hormone-replacement cycles was 34.7 and 34.5 years, respectively; this difference was not statistically significant. Also, there were no differences between the groups in: the average period of infertility; the proportions of embryo transfer cycles in the cleavage and blastocyst stages; the proportion of cycles involving assisted hatching in conventional IVF and ICSI; and the average number of transferred embryos or endometrial thickness.

Table 2 shows the clinical outcomes. For cryopreserved-thawed embryo transfer in natural and hormone-replacement cycles, the rate of pregnancy confirmed biochemically was 51.5% and 53.7%, respectively; the rate of clinical pregnancy was 43.1% and 40.4%, respectively; the rate of implantation was 29.5% and 28.0%; and the rate of ongoing pregnancy and delivery was 36.5% and 30.9%, respectively. None of these differences were statistically significant.

DISCUSSION

IT IS DIFFICULT to carry out cryopreserved-thawed embryo transfer in a natural cycle in patients with ovulation disorders. Thus, it is essential to transfer the embryo in a hormone-replacement cycle or, if the ovulation disorders are mild, in a clomiphene cycle. In contrast, cryopreserved-thawed embryo transfer in patients with a normal menstrual cycle is generally carried out in a natural or hormone-replacement cycle.

In both endometrial preparation methods, to achieve successful implantation after cryopreserved-thawed embryo transfer, synchronization of the embryo stage and endometrial date is extremely important. It is also necessary to match embryo stage and transfer date within the implantation or nidation window.[4]

For patients who are to receive a cryopreserved embryo transfer in a hormone-replacement cycle, once menstruation begins, it is possible to set the date for transfer to suit patient and hospital schedules. Thus, this approach is very convenient for both patients and medical staff. Furthermore, cryopreserved-thawed embryo transfer in a hormone-replacement cycle is unaffected by ovulation, and cancellation is extremely rare. However, it is necessary to continue to administer follicular and luteal hormones until the 8th to 12th week of pregnancy, which requires that patients make many visits to the hospital for the luteal hormone injections, which increases patients' stress. If luteal hormone suppositories are used, issues such as vaginal inflammation, vaginal bleeding and poor absorption need to be considered. Furthermore, estrogen patches can cause dermal symptoms. As well, long-term hormone therapy is expensive. Given all these issues, the amount of stress that patients endure after pregnancy is rather high.

In contrast, for cryopreserved embryo transfer in a natural cycle, it is only necessary to clearly identify the ovulation date. Thus, transvaginal ultrasound is carried out to monitor follicle size, and urinary LH and blood hormone levels are measured. As a consequence, patients must make many visits to the clinic prior to the expected date of ovulation. If favorable follicle development is not seen, then thawed embryo transfer is cancelled. However, if the ovulation date can be accurately ascertained, active therapy is not necessary after synchronizing the embryo stage and thawed embryo transfer, thus reducing patient stress.

A protocol that includes giving GnRH agonist from the cycle previous to thawed embryo transfer has been used when cryopreserved embryo transfer is carried out in a hormone-replacement cycle.[5–7] However, several studies have reported that no GnRH agonist is required.[8,9] The results of the present study suggest that the administration of a GnRH agonist is not required for cryopreserved-thawed embryo transfer in a hormone-replacement cycle.

According to Kamiya *et al.*,[10] the rate of pregnancy for 35–39 year-olds was 8.0% for cryopreserved-thawed embryo transfer in a natural cycle and 33.3% in a hormone-replacement cycle. In the present study, for all age groups, the rate of pregnancy was 15.0% for transfer in a natural cycle and 26.4% for transfer in a hormone-replacement cycle.

Schmidt *et al.*[11] studied a small number of patients and reported that the rate of pregnancy for cryopreserved-thawed embryo transfer in a natural cycle was 15% and in a hormone-replacement cycle using a GnRH agonist it was 29%.

Furthermore, Gelbaya *et al.*[12] documented that, among patients with a normal menstrual cycle, pregnancy outcomes after transfer were similar for natural and hormone-replacement cycles. As well, several studies[13–15] have reported no significant difference in pregnancy outcome for cryopreserved-thawed embryo transfer carried out in natural and hormone-replacement cycles.

In our hospital, there were no significant differences in the rates of pregnancy and miscarriage after cryopreserved-thawed embryo transfer in natural and hormone-replacement cycles. Thus, given the high stress level that patients must endure after embryo transfer, in institutions where embryo transfer can also be carried out on weekends and holidays, cryopreserved-thawed embryo transfer might also be carried out in a natural cycle if the patient has a normal menstrual cycle.

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