J. Mamm. Ova Res. Vol. 22, 170–177, 2005

Clinical Study of Transport Fresh Embryo Frozen-thawed Embryo Transfer

Yasuhiro Takanashi[1]*, Yuji Abe[1] and Harumi Kubo[1]

[1]The First Department of Obstetrics and Gynecology, Toho University School of Medicine, 6-11-1 Omori-nishi, Ota-ku, Tokyo 143-8541, Japan

Abstract: *We evaluated the clinical efficacy of the transport fresh embryo frozen-thawed embryo transfer method, whereby fresh embryos are transported from the satellite center for cryopreservation at the main ART center. In the Transport group (T group), surplus embryos from the satellite center were transported to the main ART center for frozen-thawed embryo transfer in 28 cycles in 15 patients. In the Center group (C group), oocytes were collected for frozen-thawed embryo transfer at the main ART center in 256 cycles in 165 patients. The slow freezing method was used. No significant differences were seen between groups in rates of embryo viability, embryo transfer, pregnancy, IVF embryo viability, ICSI embryo viability, pronuclear phase embryo viability, and cleavage phase embryo viability, or the numbers of transferred embryos. The transport fresh embryo frozen-thawed embryo transfer method is suitable for clinical application because there were no adverse effects from either transport or freeze/thawing.*

Key words: *Blastocyst implantation, Frozen pronuclear phase embryo, Frozen-thawed embryo transfer, Transport ICSI, Transport IVF*

Introduction

The field of human reproductive medicine has seen rapid progress since the first success of *in vitro* fertilization and embryo transfer (IVF-ET). The development and introduction of a variety of related technologies has contributed greatly to the treatment of hitherto insurmountable infertility. This raises, however, the question of what is the most appropriate way of delivering the latest reproductive medical techniques to patients. Such considerations of patient convenience led to the development of the transport system, whereby oocytes are transported between satellite clinics and a central assisted reproductive technology (ART) institution for treatment [1, 2]. The results of an independent basic study by the authors have demonstrated the efficacy and safety of this method, including transport of oocytes and embryos. We went on to develop the Round Trip method, improving on the previous One Way method, and began to apply the new method in clinical practice in 1995 [3–5].

Cryopreservation of embryos has become an essential part of ART treatments today, as increasing emphasis has been placed on improving patient amenity, and avoiding the problems of multiple pregnancies, low pregnancy rates per oocyte retrieval, increased severity of ovarian hyperstimulation syndrome (OHSS), and excessive physical, emotional and financial burdens on patients. Cryopreservation is a difficult proposition for most small facilities with small patient numbers. The traditional slow freezing method requires an expensive program freezer, large quantities of liquid nitrogen, and long operation times. The vitrification method requires a considerable level of experience, as the conditions for the time of exposure of embryos to the vitrification solution, and the processing temperatures, are very strict, and at present it is unrealistic from the cost performance point of view. With these considerations in mind, we made further modifications to the transport system for fresh embryo transfers, devising the transport fresh embryo frozen-thawed embryo transfer method, in which fresh embryos are transported from satellite clinics to the main ART center for cryopreservation. In this study, we investigated the clinical efficacy of this system.

Received: May 17, 2005
Accepted: July 6, 2005
*To whom correspondence should be addressed.
e-mail: jbl-ya@nifty.com

Materials and Methods

Informed consent was obtained from all patients who attended the satellite facilities and the main ART center from May 1998 to December 2003. The satellite center is located in Kisarazu City in Chiba Prefecture, making the transport distance some 26 km as the crow flies, usually taking 60 min travel time. All procedures at the satellite center were performed by the same personnel, using the same equipment and techniques.

We analyzed 28 cycles in 15 patients in the Transport group (T group), for whom the satellite center performed all procedures from ovarian stimulation to insemination or intracytoplasmic sperm injection (ICSI), with surplus embryos transported to the main ART center for frozen-thawed embryo transfer, and 256 cycles in 165 patients in the Center group (C group), for whom the main ART center performed all procedures from ovarian stimulation to insemination or intracytoplasmic sperm injection (ICSI), with surplus embryos used for frozen-thawed embryo transfer. All frozen-thawed embryo transfers were performed at the main ART center.

The ovarian stimulation technique [6] used for the C group was the 7-day fixed schedule regimen (7-day schedule method). Briefly, administration of a gonadotropin-releasing hormone agonist (GnRH-a; buserelin acetate, Suprecur, Aventis Pharma, Tokyo, Japan), 900 μg/day, was commenced on days 3–5 of the luteal phase of the previous cycle, and after withdrawal bleeding human menopausal gonadotropin (hMG; Humegon, Japan Organon, Osaka, Japan), 300 IU/day, was administered for 7 days, starting 9 days prior to the scheduled oocyte retrieval day. On the eighth day after the commencement of hMG, if two or more ovarian follicles at least 16 mm in diameter were confirmed, then GnRH-a was ceased and a single dose of human chorionic gonadotrophin (hCG; Gonatropin, Mochida, Tokyo, Japan) 10,000 units was administered 36 h prior to oocyte collection. Oocyte retrieval was performed under transvaginal ultrasonic guidance using modified neuroleptanalgesia (NLA) anesthesia. For IVF cases, after preculture of 4–6 h, the oocytes were inseminated with 10×10^4/mL of sperm with favorable motility using the swim-up method. For ICSI cases, after removal of the granulosa cell layer of the precultured oocytes using 80 IU/mL hyaluronidase, they were placed together with the sperm in 10% polyvinyl pyrrolidone solution and ICSI was performed. Embryo transfers were performed under transabdominal ultrasonic guidance using an Edwards-Wallace embryo replacement catheter (REF1816, Portex Ltd, Wallace Division, Kent, UK).

Surplus embryos at the satellite center were placed in HEPES buffered human tubal fluid (modified HTF, Irvine Scientific) with 30% synthetic serum substitute (SSS, Irvine Scientific, Santa Anna, USA) added, drawn up into cryopreservation plastic straws (ZA-475; IMV Technologies, L'Aigle Cedex, France), and transported to the main ART center in a portable incubator (PI-4, Nippon Medical & Chemical Instruments, Osaka, Japan). The transported embryos ranged from pronuclear to blastocyst stages.

On arrival at the main ART center, the surplus embryos were first washed with HTF + 20% SSS, to which 1.5 M propandiol was added and then 0.1 M sucrose in two steps to produce the freezing solution. They were then slowly frozen using a program freezer (Cryoembryo-HP, Air Water, Osaka, Japan). The thawing process involved exposing the embryos to a solution initially containing 0.1 M sucrose and 1.0 M propandiol, and progressively reducing the concentration of these cryopreservatives. After washing in culture fluid, embryos were cultured further, then assessed for viability and growth potential, and used in embryo transfers. If the post-thawing blastomere viability rate was 100%, and there was at least one embryo with Veeck classification [7] of 2 or better, then up to two embryos were transferred, otherwise the number was up to three. For embryos in the blastocyst stage, if at least one embryo was undamaged post-thawing, then in general one embryo was transferred, otherwise the number was up to two.

In the initial T group, there were a small number of cases to whom the prenatal course was not confirmed. Therefore, the pregnancy judgement was done by hCG positivity at the time. This is being improved now.

Ovarian stimulation cycle therapy was used for frozen-thawed embryo transfers as for fresh embryo transfers. When a pregnancy was confirmed, luteal support therapy was continued until the 10th week of gestation.

Analyses

Comparisons were made between groups in terms of age and the number of transferred embryos using Student's t-test. Comparisons of rates of blastomere viability, embryo viability, embryo transfer, pregnancy, miscarriage, and ectopic pregnancy, were made using Fisher's test. Intergroup comparisons were made of C vs T, C-IVF vs T-IVF, C-ICSI vs T-ICSI, cryopreserved pronuclear stage embryos in C group (C-PS) vs — in T

Table 1. Clinical results of frozen-thawed embryo transfer (May 1998–December 2003)

	C group	T group
Age (mean ± SD)	33.8 ± 4.0[a]	31.6 ± 5.1[b]
No. patients	165	15
Thawed cycles	256	28
Total no. thawed embryos	946	114
Blastomere survival rate (%) <50%	43.9 (415/946)	37.7 (43/114)
50%~<100%	7.8 (74/946)	9.6 (11/114)
100%	48.3 (457/946)	52.6 (60/114)
Embryo survival rate (%) (blastomere survival rate ≥ 50%)	56.1 (531/946)	62.3 (71/114)
No. transfer cycles	174	23
No. transferred embryos (mean ± SD)	2.0 ± 0.6	2.0 ± 0.5
Embryo transfer rate (%) (/thawed cycles)	68.0 (174/256)	82.1 (23/28)
Pregnancy rate (%) (/patient)	21.2 (35/165)[c]	53.3 (8/15)[d]
(/transfer)	20.1 (35/174)	34.8 (8/23)
(/thaw cycle)	13.7 (35/256)[e]	28.6 (8/28)[f]
Miscarriage rate (%)	31.4 (11/35)	50.0 (4/8)
Ectopic pregnancy rate (%)	2.9 (1/35)	0 (0/8)

e-f, $p<0.05$, a-b, c-d, $p<0.01$. C group: Center group; T group: Transport group.

group (T-PS), cryopreserved cleavage stage embryos in C group (C-CS) vs — in T group (T-CS), blastocyst transfer in C group (C-BT) vs — in T group (T-BT), C-IVF vs C-ICSI, T-IVF vs T-ICSI, C-PS vs C-CS, and T-PS vs T-CS. $P<0.05$ was considered statistically significant.

Results

Embryo transport frozen-thawed embryo transfer

In 256 thaw cycles in 165 patients in the C group (average age 33.8 ± 4.0 yr), <50% of blastomeres were viable in 415 out of 946 instances (43.9%), 50%~<100% in 74 out of 946 (7.8%), and 100% in 457 out of 946 (48.3%), giving an embryo viability rate (= 50% blastomere viability rate) of 56.1% (531/946). The number of transferred embryos was 2.0 ± 0.6; the embryo transfer rate was 68.0% (174/256); the pregnancy rate was 21.2% (35/165) per patient, 20.1% (35/174) per transfer, and 13.7% (35/256) per thaw cycle; the miscarriage rate was 31.4% (11/35); and the ectopic pregnancy rate was 2.9% (1/35).

In 28 cycles in 15 patients in the T group (average age 31.6 ± 5.1 yr), <50% of blastomeres were viable in 43 out of 114 instances (37.7%), 50%~<100% in 11 out of 114 (9.6%), and 100% in 60 out of 114 (52.6%) giving an embryo viability rate of 62.3% (71/114). The number of transferred embryos was 2.0 ± 0.5; the embryo transfer rate was 82.1% (23/28); the pregnancy rate was 53.3% (8/15) per patient, 34.8% (8/23) per transfer, and 28.6% (8/28) per thaw cycle; the miscarriage rate was 50.0% (4/8); and the ectopic pregnancy rate was 0% (0/8).

No significant differences were seen between groups in any parameters, with the exception of age, and pregnancy rates per patient and per thaw cycle (Table 1).

Embryo viability following freeze-thaw in IVF/ICSI embryos

In 38 thaw cycles in the C-IVF group (IVF derived embryos in the C group), <50% of blastomeres were viable in 65 out of 169 instances (38.5%), 50%~<100% in 13 out of 169 (7.7%), and 100% in 91 out of 169 (53.8%), giving an embryo viability rate (= 50% blastomere viability rate) of 61.5% (104/169). In 5 cycles in the T-IVF group, <50% of blastomeres were viable in 8 out of 25 instances (32.0%), 50%~<100% in 3 out of 25 (12.0%), and 100% in 14 out of 25 (56.0%), giving an embryo viability rate of 68.0% (17/25). In 168 thaw cycles in the C-ICSI group (ICSI derived embryos in the C group), the blastomere viability rate was <50% in 250 out of 620 instances (40.3%), 50%~<100% in 63 out of 620 (10.2%), and 100% in 307 out of 620 (49.5%), whereas the embryo viability rate was 59.7% (370/620). In 22 cycles in the T-ICSI group, <50% of blastomeres were viable in 33 out of instances (38.8%), 50%~<100% in 7 out of 85 (8.2%), and 100% in 45 out of 85 (52.9%), whereas the embryo viability rate was 61.2% (52/85). No significant differences were seen

Table 2. Embryo survival rates following freeze-thawing according to IVF/ICSI (May 1998–December 2003)

	C-IVF group	T-IVF group	C-ICSI group	T-ICSI group
No. thaw cycles	38	5	168	22
Blastomere survival rate (%) <50%	38.5 (65/169)	32.0 (8/25)	40.3 (250/620)	38.8 (33/85)
50%~<100%	7.7 (13/169)	12.0 (3/25)	10.2 (63/620)	8.2 (7/85)
100%	53.8 (91/169)	56.0 (14/25)	49.5 (307/620)	52.9 (45/85)
Embryo survival rate (%) (blastomere survival rate ≥ 50%)	61.5 (104/169)	68.0 (17/25)	59.7 (370/620)	61.2 (52/85)

C-IVF group: embryos derived by IVF in the C group; T-IVF group: embryos derived by IVF in the T group. C-ICSI group: embryos derived by ICSI in the C group; T-ICSI group: embryos derived by ICSI in the T group.

between IVF and ICSI groups in any parameters. Patients who underwent both IVF and ICSI in the same cycle were excluded (Table 2).

Pronuclear/cleavage phase embryo transport frozen-thawed embryo transfer

In 20 cycles in 12 patients in the C-PS group (cryopreservation of pronuclear phase embryos, average age 34.0 ± 3.2 yr), <50% of blastomeres were viable in 13 out of 65 instances (20.0%), 50%~<100% in 0 out of 65 (0%), and 100% in 52 out of 65 (80.0%), giving an embryo viability rate (= 50% blastomere viability rate) of 80.0% (52/65). The number of transferred embryos was 1.9 ± 0.4; the embryo transfer rate was 85.0% (17/20); the pregnancy rate was 41.7% (5/12) per patient, 29.4% (5/17) per transfer, and 25.0% (5/20) per thaw cycle; the miscarriage rate was 20.0% (1/5); and the ectopic pregnancy rate was 0% (0/5). In 6 cycles in 3 patients in the T-PS group (average age 29.0 ± 0.6 yr), <50% of blastomeres were viable in 3 out of 16 instances (18.8%), 50%~<100% in 0 out of 16 (0%), and 100% in 13 out of 16 (81.3%), giving an embryo viability rate of 81.3% (13/16). The number of transferred embryos was 2.0 ± 0; the embryo transfer rate was 100% (6/6); the pregnancy rate was 66.7% (2/3) per patient, 33.3% (2/6) per transfer, and 33.3% (2/6) per thaw cycle; the miscarriage rate was 0% (0/2); and the ectopic pregnancy rate was 0% (0/2).

In 236 cycles in 162 patients in the C-CS group (cryopreservation of cleavage phase embryos, average age 33.8 ± 4.1 yr), <50% of blastomeres were viable in 402 out of 881 instances (45.6%), 50%~<100% in 74 out of 881 (8.4%), and 100% in 405 out of 881 (46.0%), giving an embryo viability rate of 54.4% (479/881). The number of transferred embryos was 2.0 ± 0.6; the embryo transfer rate was 66.5% (157/236); the pregnancy rate was 18.5% (30/162) per patient, 19.1% (30/157) per transfer, and 12.7% (30/236) per thaw cycle; the miscarriage rate was 33.3% (10/30); and the ectopic pregnancy rate was 3.3% (1/30). In 22 cycles in 14 patients in the T-CS group (average age 32.4 ± 5.7 yr), <50% of blastomeres were viable in 40 out of 98 instances (40.8%), 50%~<100% in 11 out of 98 (11.2%), and 100% in 47 out of 98 (48.0%), giving an embryo viability rate of 59.2% (58/98). The number of transferred embryos was 2.1 ± 0.6; the embryo transfer rate was 77.3% (17/22); the pregnancy rate was 42.9% (6/14) per patient, 35.3% (6/17) per transfer, and 27.3% (6/22) per thaw cycle; the miscarriage rate was 66.7% (4/6); and the ectopic pregnancy rate was 0% (0/6). The high miscarriage rate in the T-CS group was thought to be due to individual factors related to recurrent pregnancy loss, as 3 of the 4 miscarriages were the same patient.

No significant differences were seen between groups in any parameter, with the exception of age for pronuclear phase cryopreservation, and pregnancy rates per patient for cleavage phase cryopreservation. A significant difference was seen in the C group in embryo viability rates between pronuclear and cleavage phase cryopreservation (Table 3).

Pregnancy rates, miscarriage and ectopic pregnancy rates following frozen-thawed blastocyst transfer

In the C-BT group (blastocyst transfer), the pregnancy rate was 40.0% (10/25) per transfer, the miscarriage rate was 40.0% (4/10), and the ectopic pregnancy rate was 10.0% (1/10). In the T-BT group, the pregnancy rate was 50.0% (2/4) per transfer, the miscarriage rate was 0% (0/2), and the ectopic pregnancy rate was 0% (0/2). The cryopreserved embryos ranged from the 4-cell to the blastocyst (Table 4).

Discussion

Favorable results have been reported from western countries with one-way transport ART, entailing the transport of oocytes from satellite facilities to the central

Table 3. Frozen-thawed embryo transfer of pronuclear and cleavage phase embryos (May 1998–December 2003)

	C-PS group	T-PS group	C-CS group	T-CS group
Age (mean ± SD)	34.0 ± 3.2[a]	29.0 ± 0.6[b]	33.8 ± 4.1	32.4 ± 5.7[c]
No. patients	12	3	162	14
No. thaw cycles	20	6	236	22
Total no. thawed embryos	65	16	881	98
Blastomere survival rate (%) <50%	20.0 (13/65)[d]	18.8 (3/16)	45.6 (402/881)[e]	40.8 (40/98)
50%~<100%	0 (0/65)[f]	0 (0/16)	8.4 (74/881)[g]	11.2 (11/98)
100%	80.0 (52/65)[h]	81.3 (13/16)[i]	46.0 (405/881)[j]	48.0 (47/98)[k]
Embryo survival rate (%) (blastomere survival rate ≥ 50%)	80.0 (52/65)[l]	81.3 (13/16)	54.4 (479/881)[m]	59.2 (58/98)
No. transfer cycles	17	6	157	17
No. transferred embryos (mean ± SD)	1.9 ± 0.4	2.0 ± 0	2.0 ± 0.6	2.1 ± 0.6
Embryo transfer rate (%) (/thaw cycle)	85.0 (17/20)	100 (6/6)	66.5 (157/236)	77.3 (17/22)
Pregnancy rate (%) (/patient)	41.7 (5/12)	66.7 (2/3)	18.5 (30/162)[n]	42.9 (6/14)[o]
(/transfer)	29.4 (5/17)	33.3 (2/6)	19.1 (30/157)	35.3 (6/17)
(/thaw cycle)	25.0 (5/20)	33.3 (2/6)	12.7 (30/236)	27.3 (6/22)
Miscarriage rate (%)	20.0 (1/5)	0 (0/2)	33.3 (10/30)	66.7 (4/6)
Ectopic pregnancy rate (%)	0 (0/5)	0 (0/2)	3.3 (1/30)	0 (0/6)

b-c, f-g, i-k, n-o, $p<0.05$, a-b, d-e, h-j, l-m, $p<0.0001$. C-PS group: cryopreserved pronuclear stage embryos in C group; T-PS group: cryopreserved pronuclear stage embryos in T group. C-CS group: cryopreserved cleavage stage embryos in C group; T-CS group: cryopreserved cleavage stage embryos in T group.

Table 4. Pregnancy rates, miscarriage rates and ectopic pregnancy rates following frozen-thawed blastocyst transfer (May 1998–December 2003)

	C-BT group	T-BT group
Pregnancy rate (/transfer) (%)	40.0 (10/25)	50.0 (2/4)
Miscarriage rate (%)	40.0 (4/10)	0 (0/2)
Ectopic pregnancy rate (%)	10.0 (1/10)	0 (0/2)

C-BT group: Blastocyst transfer in C group; T-BT group: Blastocyst transfer in T group.

ART center, where they are utilized in fresh embryo transfers [8–14]. There have been reports of embryo quality being affected by transport ICSI [15], however, our own study showed a decline in pregnancy rates [16]. This suggests the possibility of some factor that affects ICSI, but not IVF. Hardarson *et al.* found that in ICSI, the spindle in MII oocytes is not always adjacent to the first polar body, so the denuding procedure may cause displacement (mean deviation of spindle from first polar body: 41.7°) [17]. Shaking or some other physical factor is the likely cause, so the transport process itself may well cause increased displacement, and affect the clinical results. If this is the case, the position of the spindle should be confirmed before performing ICSI.

In recent years, widespread uptake of ICSI has seen a rapid increase in the number of institutions offering this service, making cryopreservation of surplus embryos essential. With the aim of resolving some of the concerns raised over transport ICSI, we developed a new method, whereby IVF or ICSI is performed at a satellite center, and surplus fresh embryos are transported to the main ART center, where they are used in frozen-thawed embryo transfers. As this method involves dual stresses to the embryos, both transport and freeze-thaw, we investigated the possible effects on rates of embryo viability, embryo transfer and pregnancy.

In our previous comparison of frozen-thawed embryo transfers, derived from earlier transport ART methods (oocyte transport) and derived from embryo transport (as in the T group in this study), with a non-transport group (as in the C group in this study), we found no differences between groups in terms of age, embryo damage following freeze-thaw, number of embryos transferred, or pregnancy rates. We concluded that transport of oocytes or embryos does not influence the clinical results of frozen-thawed embryo transfers [18]. Reexamination of patients undergoing embryo transport frozen-thawed embryo transfers did reveal significant differences in age, and pregnancy rates per patient and per thaw cycle, however, the age difference was thought to be due to the high proportion of repeaters with refractory infertility in the C group. Pregnancy rates were in fact higher in the T group, reflecting lower levels of age factors and severe male infertility than in the C group. Bias of the same sort affecting embryo

viability rates cannot be excluded, but no significant difference was seen between C and T groups, and this was thought to rule out any influence from embryo transport.

Our earlier study did not take into account the different fertilization processes, or the stage of development of the transported embryos. In this study we divided embryos into IVF vs ICSI, and pronuclear vs cleavage stage, as well as transport vs non-transport, for our comparisons. The results showed no significant differences in embryo viability rates between C-IVF and T-IVF, or C-ICSI and T-ICSI. This tends to rule out any effect from the above mentioned dual stresses on embryos derived from either IVF, that follows a similar fertilization process to that *in vivo*, or from ICSI, that uses an artificial fertilization process. As there were also no significant differences seen in embryo viability rates between C-IVF and C-ICSI, or T-IVF and T-ICSI, the ability to endure the stress of freeze-thaw did not vary between IVF and ICSI embryos, irrespective of transport.

No significant differences were seen in embryo viability rates between C-PS and T-PS, or C-CS and T-CS, so transport did not affect embryos in either the pronuclear or cleavage stages. Comparison of C-PS and C-CS embryos confirmed the superiority of cryopreserved pronuclear stage embryos. No significant difference was seen between the T-PS and T-CS groups, but patient numbers were small, and favorable results were achieved with cryopreserved pronuclear stage embryos even after transport. The reason that cryopreserved pronuclear phase embryos in the transport group were more common in younger patients was thought to be that more oocytes were harvested, producing more embryos. A similar trend was not seen in the non-transport group, however. This may have been due to the higher incidence of severe male infertility, in particular cases requiring testicular sperm extraction (TESE)-ICSI, so the number of embryos was affected by male factors rather than the number of oocytes. Excluding cases where all embryos go into cryopreservation due to OHSS or poor endometrial development, because pronuclear phase embryo cryopreservation occurs on Day 1, it is difficult to perform fresh embryo transfers in the oocytes collection cycle if embryo numbers are insufficient. When a reasonable number of embryos has been produced, the best course is to transport some pronuclear stage embryos to the main ART center for cryopreservation, and culture some early embryos at the satellite facility for use in embryo transfers. Any surplus embryos are then transported to the main ART center as cleavage phase embryos, including blastocysts, for cryopreservation.

High implantation rates have been reported for blastocyst transfers, raising the possibility of single embryo transfers [19–24]. We achieved favorable results in the transport group in our study, although the patient numbers were extremely small. An unresolved question is whether embryos should be transported at the pronuclear or cleavage stage (Day 2–3 embryos), cryopreserved, and transferred at the blastocyst stage, or transported, cryopreserved, and transferred at the blastocyst stage. Transport at the pronuclear stage is regarded as superior from the point of view of embryo viability, but the rate of progression to the blastocyst stage must also be considered. Embryo viability following transport and cryopreservation at the blastocyst stage is another question requiring further study. The vitrification method is most appropriate for cryopreservation following transport at the blastocyst stage, so consideration of the stress to the embryos makes transport at the pronuclear stage more appropriate if technically feasible. Rienzi *et al.* compared Day 3 and Day 5 fresh embryo transfers, with combined evaluation at the pronuclear and cleavage stages, in patients where at least eight 2 pronuclei embryos had been obtained using ICSI, finding no difference in the rates of pregnancy, clinical pregnancy, delivery, implantation, or birth [25]. Similar results were achieved for cryopreserved embryo transfers. Day 3 embryo transfers were, however, superior in terms of pregnancy, clinical pregnancy, and delivery rates per oocyte retrieval. The greatest drawback to embryo transport frozen-thawed embryo transfer is the stress it places on the embryo; so all possible steps should be taken to reduce this stress. This means that the *in vitro* incubation period should not be prolonged any more than is absolutely necessary.

In choosing the method of cryopreservation of transported embryos, in consideration of potential cytotoxicity from high levels of cryoprotectant agents (CPAs) associated with the vitrification method, we opted for the slow freezing method. Superior rates of embryo viability, implantation, and clinical pregnancy have been reported for human embryos cryopreserved after slow freezing with ethylene glycol (EG) than for vitrification using 1,2-propanediol (PROH) as the CPA [26]. Modified HTF is used as the transport culture fluid, but it is preferable to use sequential culture fluids suited to the stage of the transported embryos. Further study is needed into the development of culture fluids

containing HEPES buffer, etc., that stabilize the pH during transport, and the use of piezo-ICSI, which is said to provide superior oocyte viability [27]. It is also preferable to transfer embryos without damaged blastomeres, although there have been reports of improved implantation rates after laser-assisted removal of necrotic blastomeres from partially damaged embryos prior to transfer [28, 29].

In this study, we found no adverse effects on rates of embryo viability, embryo transfer, or pregnancy, so we consider the embryo transport frozen-thawed embryo transfer method to be clinically applicable. The field of transport ART has recently seen a variety of new developments, with different applications departing significantly from the original objectives. These are noted in earlier reports by the authors as well as Langley *et al.* of embryo biopsies and genetic analysis [30], a report of transport of testicular tissue in addition to oocytes and testicular tissue [31], and transport of genetic materials by car and train, as well as by air as dictated by long distances or time restrictions [30, 32].

In our case, we are considering enlargement of our transport area to overcome regional differences in infertility treatments. Our hospital is located very close to a major airport, and we plan to meet the demand for services from isolated islands such as Izu Oshima and Hachijojima using the air postal service, although we will need to make arrangements in advance to avoid X-ray screening. A number of variations such as these are possible to meet patient needs, and these will be applicable to almost all forms of ART. We should also be able to satisfy any future patient demands.

References

1) Jansen, C.A.M., van Beek, J.J., Verhoeff, A., Alberda, A.T. and Zeilmaker, G.H. (1986): *In vitro* fertilization and embryo transfer with transport of oocytes. Lancet, 1, 676.
2) van Beek, J.J., Jansen, C.A.M., Verhoeff, A., Alberda, A.T. and Zeilmaker, G.H. (1986): 'Transport'in vitro fertilization and embryo transfer. Hum. Reprod., 1 (Suppl 1), 28.
3) Nakano, H., Abe, Y., Ikenaga, H., Sasabe, Y., Kubo, H. and Hirakawa, S. (1996): The effect of transport conditions on the development of mouse embryos: a basic study of transport ART methods. Jpn. J. Fertil. Steril., 41, 325–333 (in Japanese).
4) Abe, Y., Nakano, H., Hashida, E., Sasabe, Y., Kubo, H. and Hirakawa, S. (1997): Assisted reproduction technology using the transport method. Obstet. Gynecol. Pract., 46, 1127–1132 (in Japanese).
5) Abe, Y. and Kubo, H. (1999): Transport ART. In: Comprehensive Handbook of Women's Medicine Vol. 16: Assisted Reproductive Technology (Kubo, H., ed.), pp. 211–251, Nakayama Shoten, Tokyo (in Japanese).
6) Abe, Y., Ikenaga, H., Nakano, H., Sasabe, Y., Katagiri, Y., Masaki, K., Yano, Y., Morita, M., Kubo, H. and Hirakawa, S. (1995): Results of an ART program using fixed timing for oocyte retrieval. Jpn. J. Fertil. Steril., 40, 211–251 (in Japanese).
7) Veeck, L.L. (1991): Atlas of the Human Oocyte and Early Conceptus, Vol. 2, Williams & Wilkins, Baltimore.
8) Alfonsin, A.E., Amato, A.R., Arrighi, A., Blaquier, J.A., Cogorno, M., Feldman, E.S., Echeverria, F.G., Horton, M., Vecchia, D.L.D. and Millas, N. (1998): Transport in vitro fertilization and intracytoplasmic sperm injection: results of a collaborative trial. Fertil. Steril., 69, 466–470.
9) De Sutter, P., Dozortsev, D., Verhoeff, A., Coetsier, T., Jansen, C.A.M., van Os, H.C. and Dhont, M. (1996): Transport intracytoplasmic sperm injection (ICSI):a cost effective alternative. J. Assist. Reprod. Genet., 13, 234–237.
10) Roest, J., Verhoeff, A., Coetsier, T., De Sutter, P., Dozortsev, D., Dhont, M. and Zeilmaker, G.H. (1995): Oocytes--will travel? Fertil. Steril., 63, 682.
11) Roest, J., Verhoeff, A., van Lent, M., Huisman, G.J. and Zeilmaker, G.H. (1995): Results of decentralized *in-vitro* fertilization treatment with transport and satellite clinics. Hum. Reprod., 10, 563–567.
12) Booker, M.W. (1993): Transport in-vitro fertilization and embryo transfer. Br. J. Hosp. Med., 50, 369–370.
13) Verhoeff, A., Logmans, A., Leerentveld, R.A., Huisman, G.J. and Zeilmaker, G.H. (1992): Transport IVF and satellite transport IVF: one laboratory and several clinics, results of 860 ovum pick-ups. Hum. Reprod., 7, 160–161.
14) Zarutskie, P.W., Kuzan, F.B., Moore, D.E. and Soules, M.R. (1988): An *in vitro* fertilization program using satellite physicians. Obstet. Gynecol., 72, 929–934.
15) Coetsier, T., Verhoeff, A., De Sutter, P., Roest, J. and Dhont, M. (1997): Transport-*in-vitro* fertilization/intracellular sperm injection: a prospective randomized study. Hum. Reprod., 12, 1654–1656.
16) Takanashi, Y., Abe, Y., Shibui, Y., Hanaoka, K., Takeshita, N., Masaki, K. and Kubo, H. (2004): Effect of oocyte transportation time on the clinical results of transport in vitro fertilization/intracytoplasmic sperm injection-embryo transfer. Reprod. Med. Biol., 3, 123–131.
17) Hardarson, T.H., Lundin, K. and Hamberger, L. (2000): The position of the metaphase spindle cannot be predicted by the location of the first polar body in the human oocyte. Hum. Reprod., 15, 1372–1376.
18) Takanashi, Y. (2004): Clinical evaluation of frozen/thawed embryo transfer following transport of oocytes and embryos. Reprod. Med. Biol., 3, 1–8.
19) Karaki, R.Z., Samarraie, S.S., Younis, N.A., Lahloub, T.M. and Ibrahim, M.H. (2002): Blastocyst culture and transfer: a step toward improved in vitro fertilization outcome. Fertil Steril., 77, 114–118.
20) Gardner, D.K., Lane, M., Stevens, J., Schlenker, T. and Schoolcraft, W.B. (2000): Blastocyst score affects

implantation and pregnancy outcome: towards a single blastocyst transfer. Fertil. Steril., 73, 1155–1158.

21) Schoolcraft, W.B., Gardner, D.K., Lane, M., Schlenker, T., Hamilton, F. and Meldrum, D.R. (1999): Blastocyst culture and transfer: analysis of results and parameters affecting outcome in two *in vitro* fertilization programs. Fertil. Steril., 72, 604–609.

22) Cruz, J.R., Dubey, A.K., Patel, J., Peak, D., Hartog, B. and Gindoff, P.R. (1999): Is blastocyst transfer useful as an alternative treatment for patients with multiple *in vitro* fertilization failures? Fertil. Steril., 72, 218–220.

23) Damario, M.A., Phy, J.L. and Tummon, I.S. (2002): Successful elective single blastocyst transfer in a patient with prior repetitive high-order multiple gestations. J. Assist. Reprod. Genet., 19, 205–208.

24) Frattarelli, J.L., Leondires, M.P., McKeeby, J.L., Miller, B.T. and Segars, J.H. (2003): Blastocyst transfer decreases multiple pregnancy rates in *in vitro* fertilization cycles: a randomized controlled trial. Fertil. Steril., 79, 228–230.

25) Rienzi, L., Ubaldi, F., Iacobelli, M., Ferrero, S., Minasi, M.G., Martinez, F., Tesarik, J. and Greco, E. (2002): Day 3 embryo transfer with combined evaluation at the pronuclear and cleavage stages compares favourably with day 5 blastocyst transfer. Hum. Reprod., 17, 1852–1855.

26) Chi, H.J., Koo, J.J., Kim, M.Y., Joo, J.Y., Chang, S.S. and Chung, K.S. (2002): Cryopreservation of human embryos using ethylene glycol in controlled slow freezing. Hum. Reprod., 17, 2146–2151.

27) Yanagita, K., Kuretake, S. and Sato, A. (2002): In vitro fertilization and microinsemination. In: Pictorial ART Manual (Mori, T., Kubo, H. and Okamura, H., eds.), pp. 94–101, Nagai Shoten, Tokyo.

28) Guerif, F., Bidault, R., Cadoret, V., Couet, M.L., Lansac, J. and Royere, D. (2002): Parameters guiding selection of best embryos for transfer after cryopreservation: a reappraisal. Hum. Reprod., 17, 1321–1326.

29) Rienzi, L., Nagy, Z.P., Ubaldi, F., Iacobelli, M., Anniballo, R., Tesarik, J. and Greco, E. (2002): Laser-assisted removal of necrotic blastomeres from cryopreserved embryos that were partially damaged. Fertil. Steril., 77, 1196–1201.

30) Langley, M., Marek, D., Cieslak, J., Masciangelo, C., Doody, K.M. and Doody, K.J. (2001): Successful Day 5 embryo transfer and pregnancies resulting after transport of embryos by air for biopsy and genetic analysis. J. Assist. Reprod. Genet., 18, 330–335.

31) Scholtes, M.C.W., van Hoogstraten, D.G., Schmoutziguer, A. and Zeilmaker, G.H. (1999): Extraction of testicular sperm from previously cryopreserved tissue in couples with or without transport of oocytes and testicular tissue. Fertil. Steril., 72, 785–791.

32) Buckett, W.M., Fisch, P., Dean, N.L., Biljan, M.M. and Tan, S.L. (1999): *In vitro* fertilization and intracytoplasmic sperm injection pregnancies after successful transport of oocytes by airplane. Fertil. Steril., 71, 753–755.