—Case Report—

Successful Pregnancy Following Transfer of Frozen–thawed Day 7 Blastocysts Derived from Transported Oocytes

Taketo Inoue[1,2]*, Yoshiyuki Ono[1], Yukiko Yonezawa[1], Takuo Fujiwara[3], Ataru Nohara[3], Osamu Mimura[3], Junji Kishi[1] and Nobuyuki Emi[1]

[1]*Ono Ladies Clinic, Hyogo 675-1375, Japan*
[2]*Department of Biophysics, Kobe University Graduate School of Health Sciences, Hyogo 654-0142, Japan*
[3]*Asagiri Hospital, Hyogo 673-0852, Japan*

Abstract: **Purpose:** Pelvic adhesions inhibit follicular growth and ovum transport, causing female infertility. In this report, we describe successful ovum pick-up (OPU) during adhesiotomy in a woman with severe adhesions that prevented transvaginal OPU. Subsequently, *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) were attempted after transport of cumulus–oocyte complexes (COCs). **Materials and methods:** Clinical examination revealed a chocolate cyst on the patient's left ovary. Her right ovary was adherent to the posterior part of the uterus. COCs were harvested during adhesiotomy and stored at 37°C in a 1.5-ml tube containing Sperm Washing Medium. The tube was transported to our clinic using hot gel bags and a portable infant incubator. Immediately upon arrival, IVF and ICSI were performed. On day 7, two ICSI-derived blastocysts were cryopreserved. Two months later, these blastocysts were transferred to the patient's uterus. **Results:** The patient conceived, and a normal, healthy boy was born. **Conclusions:** In summary, we performed OPU during adhesiotomy, and COCs were transported to the infertility clinic on the same day; subsequently, the patient was successfully impregnated using the transported oocytes.

Key words: Blastocyst, Cumulus-oocyte complex, Intracytoplasmic sperm injection, Pelvic adhesion, Transportation

Introduction

Pelvic adhesions are often observed in patients with a history of endometriosis [1], pelvic infection [2] or pelvic surgery [3]. Periovular adhesions inhibit follicular growth, obstruct the oviducts and hinder ovum passage, consequently causing female infertility. Particularly, performance of transvaginal ovum pick-up (OPU) is difficult in women with severe adhesions. Moreover, when OPU is performed transvaginally, the success of pregnancy following assisted reproductive technology depends on the range and severity of periovular adhesions [4]. In patients with severe adhesions, OPU is performed during or after adhesiotomy.

Some small, independent obstetrics and gynecology clinics such as ours do not have the equipment for adhesiotomy. Under these circumstances, transport of oocytes from hospitals to infertility clinics is necessary when OPU is performed during adhesiotomy. In 1986, successful pregnancy was achieved following transportation of oocytes at body temperature [5]. Thereafter, several study groups reported the efficacy of short-term transportation of oocytes [6, 7] and embryos [8, 9] using portable incubators or multiblock heaters maintained at 37°C.

In this report, we describe the case of a woman with severe adhesions due to endometriosis, in whom transvaginal OPU was unsuccessful. We performed OPU during adhesiotomy: cumulus–oocyte complexes (COC) were transported on the same day, and intracytoplasmic sperm injection (ICSI) was performed. Subsequently, she conceived by transport ICSI, and a healthy baby was born. In this report, we introduce our method of COC transportation.

Preliminary examination

We investigated whether the temperature of the medium was stable using hot gel bags and a portable infant incubator. The duration of transport from aspirating the

Received: June 25, 2013
Accepted: August 27, 2013
*To whom correspondence should be addressed.
e-mail: taketoein@yahoo.co.jp

first follicle to the arrival of COCs at our clinic was estimated to be 3–4 h. When only hot gel bags were used for thermal management, their temperature remained stable for 40 min at 37°C. However, after 40 min, their temperature decreased by 2°C every 1 h for 3 h. However, use of a portable infant incubator maintained the temperature of the hot gel bags at 37°C for over 8 h. Accordingly, the temperature of the medium was sufficiently stable using our method.

Materials and Methods

Patient

A 38 year-old woman and her 41 year-old husband consulted our clinic after *in vitro* fertilization (IVF) was recommended by another hospital. They had undergone infertility treatment for 8 months at a previous hospital and had been infertile for 4 years. Clinical examination of the woman revealed a chocolate cyst on her left ovary. In addition, her right ovary was adherent to the posterior part of the uterus. Abnormal tubal patency was not evident on hysterosalpingography. Her husband exhibited an average number of motile spermatozoa of 53 × 10^6 spermatozoa/ml, and their motility rate was 49.5%. Artificial insemination was performed three times at our clinic without success.

During ovum stimulation for artificial insemination, we investigated whether transvaginal OPU was possible and determined that transvaginal OPU was unfeasible because of severe adhesions. Consequently, we attempted to remove the adhesions by laparoscopic surgery. However, we changed the surgical procedure to laparotomy: exclusion of the intestine was difficult because of the amount of visceral fat, combined with hemorrhage from the vicinity of an ovarian cystoma on the left side. As mentioned in her operative notes regarding the left ovary, a thumbprint-sized ovarian cyst was found adherent to the posterior part of the broad ligament of the uterus on its left posterior aspect, and the cystic contents had spilled in the form of a brown liquid. The right ovary was located at the right posterior aspect of the uterus, and the fallopian tube was adherent to the posterior aspect of the uterus and to the pouch of Douglas. Adhesion to the intestinal tract was not observed. Her ovaries were repositioned by laparotomy. After recovery, transvaginal OPU was attempted four times without success. IVF was suspended; we ceased infertility treatment and attempted artificial insemination three times and timed intercourse once.

In the next IVF cycle, one year after the initial consultation, we determined during ovum stimulation that transvaginal OPU was again unfeasible because of adhesions (Fig. 1). Accordingly, we had no choice but to attempt OPU by laparotomy at Asagiri Hospital, Hyogo, Japan, and subsequently transported the COCs obtained from the hospital to our clinic. Informed consent was obtained to conduct OPU by laparotomy and transport COCs. All the procedures were performed in accordance with the *Declaration of Helsinki*. Because we intended this to be a case report, the procedure was not referred to the institutional review board of Ono Ladies Clinic, Hyogo, Japan.

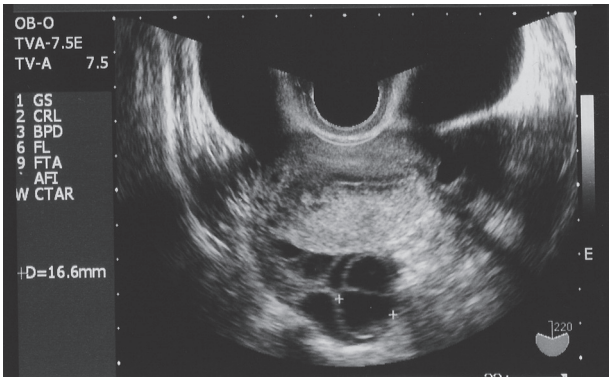

Fig. 1. The repositioned ovary after laparotomy. Some mature follicles were observed in the repositioned ovary on day 8 of the patient's menstrual cycle during the short protocol; however, transvaginal ovum pick-up was unfeasible because of severe adhesions.

Ovum stimulation, ovum pick-up and oocyte transport

For ovarian stimulation, the patient was treated with the gonadotropin-releasing hormone analogue buserelin acetate (Fuji Pharmaceutical Co., Ltd., Tokyo, Japan) and human menopausal gonadotropin (ASKA Pharmaceutical Co., Ltd., Tokyo, Japan) using the short protocol. When at least two follicles reached a diameter of 18–20 mm (determined by transvaginal ultrasound), 10000 IU of human chorionic gonadotropin (hCG; Fuji Pharmaceutical Co., Ltd., Tokyo, Japan) was administered. OPU was performed by laparotomy 35 h after hCG injection.

Six COCs were obtained. COCs were washed four times in 1 ml Sperm Washing Medium (Irvine Scientific, Santa Ana, CA, USA) with N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES). Subsequently, COCs were stored in a 1.5-ml graduated microcentrifuge tube (Quality Scientific Plastics, San Diego, CA, USA) containing 300 μl Sperm Washing Medium at 37°C. The volume of the Sperm Washing Medium was then increased to 1 ml. The 1.5-ml tube was placed into a light-resistant bag. The light-resistant bag was placed between hot gel

bags (Hakugen Co., Ltd., Tokyo, Japan), which were in turn placed in a portable infant incubator (Portable Couveuse ACDC; Medtech, Tokyo, Japan). The hot gel bags were warmed using a microwave before initiating laparotomy and transport. The portable infant incubator was transported from the hospital to our clinic in about 1.5 h. The duration for which COCs were soaked in the Sperm Washing Medium, from aspiration of the first follicle to transfer into Universal IVF Medium (MediCult A/S, Jyllinge, Denmark) at our clinic, was 2.5 h.

In vitro fertilization, intracytoplasmic sperm injection and cryopreservation

Immediately upon arrival, COCs were washed eight times with 1 ml Universal IVF Medium (MediCult A/S). Thereafter, COCs were cultured in 1 ml Universal IVF Medium for 15 min. COCs were then washed four times with 1 ml Universal IVF Medium. Three COCs were inseminated using IVF. The other three COCs were freed from cumulus cells using 0.025% hyaluronidase (Sigma–Aldrich Corp., St. Louis, MO, USA) dissolved in Universal IVF Medium. Two metaphase II (MII) oocytes and one germinal vesicle oocyte were obtained and cultured for ≥30 min. Subsequently, the oocytes were inseminated using piezo-assisted ICSI in Universal IVF Medium. The following morning, fertilization was confirmed on the basis of the presence and number of pronuclei and polar bodies.

Using IVF, no fertilized eggs were obtained on day 1. Accordingly, the three unfertilized eggs underwent rescue ICSI, and three fertilized eggs were obtained. Using ICSI, two fertilized eggs were obtained. Eventually, five fertilized eggs were cultured in Continuouos Single Culture™ medium (Irvine Scientific), including 10% serum substitute supplement (Irvine Scientific), until day 7. Two blastocysts (one blastocyst from ICSI; one blastocyst from rescue ICSI) remained on day 7 and were cryopreserved using the Cryotop technique, as described by Kuwayama [10].

Embryo transfer

Two months later, endometrial stimulation for embryo transfer [11, 12] was performed four days before blastocyst transfer. Two blastocysts were thawed as per the procedure described by Kuwayama [10], except that thawing was performed at room temperature. Two frozen–thawed day 7 blastocysts were transferred into the patient's day 5 uterus [13].

For luteal support, 2 mg chlormadinone acetate (Lutoral; Shionogi & Co., Ltd., Osaka, Japan) was administered three times daily from beginning on the day of ovulation; moreover, 0.72-mg transdermal estradiol patches (Estrana® Tape; Hisamitsu Pharmaceutical Co., Inc., Tokyo, Japan) were applied to the abdomen from the beginning of menstruation for 10 weeks, and a 200-mg progesterone vaginal suppository was administered once daily from ovulation to check the gestational sac (GS). The progesterone vaginal suppositories were prepared by dissolving progesterone (Sigma-Aldrich Corp.) and Macrogol 6000 (Nikko Chemicals Co., Ltd., Tokyo, Japan) in Macrogol 400 (Yoshida Pharmaceutical Co., Ltd., Tokyo, Japan). Subsequently, the patient was injected with 125 mg 17α-hydroxyprogesterone caproate once every five days for seven weeks after assessing GS.

Results

Pregnancy was confirmed by positive urine hCG test 10 days after blastocyst transfer, and the GS and fetal heartbeat were observed by transvaginal ultrasound at 16 and 21 days after embryo transfer, respectively. No structural abnormalities were evident on ultrasound during routine pregnancy checkups. A normal baby boy (3196 g) was delivered by cesarean section at 38 weeks of gestation, and he was healthy when examined during the second and fourth weeks after birth.

Discussion

We achieved successful pregnancy after ICSI using transported oocytes harvested during adhesiotomy. In previous reports, transportable incubators [6, 8, 14] and multiblock heaters [7] were used to transport human oocytes. In this report, a 1.5-ml tube containing COCs was stored between hot gel bags for thermal management, and they were placed like this in a portable infant incubator to prevent a decrease in temperature during transport. Our preliminary examination revealed that the temperature of the hot gel bags was maintained at 37 °C for over 8 h by using the portable infant incubator. Regarding the duration of transport, in this case, a take-home baby was achieved after a transport duration of 2.5 h from harvesting of the first follicles to their arrival at our clinic. Accordingly, the temperature remained sufficiently stable using our method. Several studies [5–8, 14, 15] have reported oocyte transport durations of 30–225 min. Buckett *et al*. [6] reported oocyte transport by airplane lasting 225 min. Although the case report demonstrated the presence of two fetal heartbeats, the report did not detail whether either fetus resulted in a take-home baby. Moreover, Takanashi *et al*. [14] recommended that the oocyte transport time should not exceed 2 h. Although

multiblock heaters and portable incubators are better suited for temperature control during transportation of COCs, we successfully transported COCs using hot gel bags and a portable infant incubator.

It was necessary to use a medium including HEPES for COC transportation because our method did not allow sufficient carbon dioxide control. Morgia *et al*. [16] reported that the number of degenerated oocytes, triploid embryos and degree of embryo fragmentation significantly increased with use of microinjection medium containing HEPES during ICSI. Accordingly, the use of a portable CO_2 incubator might be more suitable for COC transportation. However, we did not have a portable CO_2 incubator, and purchasing an incubator would have increased the treatment cost. As a compromise, a medium including HEPES was used for transportation.

Takanashi *et al*. [14] reported lower pregnancy rates following ICSI using oocytes transported for short (<60 min; 16.7%) and long (60–120 min; 17.3%) durations than using nontransported oocytes (35.2%). Conversely, Alfonsín *et al*. [7] reported no significant difference in the pregnancy rates between transport IVF (20.1%), transport ICSI (23.9%) and conventional IVF (22%). Moreover, in their report, seven of almost 5300 COCs were lost during transport (0.13%). With regard to oocyte cryopreservation, Rienzi *et al*. [17] reported that the pregnancy rate was not significantly different between ICSI using frozen–thawed MII oocytes and that using untreated MII oocytes. The survival rate of frozen–thawed MII oocytes was 96.7%. Accordingly, compared with the cryopreserved oocytes, we used a higher number of oocytes transported by incubator. Furthermore, with regard to the short-term transportation of oocytes, storage at 37°C might be better than at −196°C.

We demonstrated successful pregnancy following transfer of frozen–thawed day 7 blastocysts. In cases of late-developing embryos such as day 6 and day 7 blastocysts derived by rescue ICSI of embryos, pregnancy rates were improved by performing frozen–thawed embryo transfer for synchronization of embryo development with that of the endometrium [13, 18]. Chen and Kattera [19] reported a significantly higher implantation rate following fresh embryo transfer using rescue ICSI at 6 h post insemination than at 22 h post insemination. Moreover, the implantation rate was significantly lower in the fresh day 6 blastocyst transfer group than in the fresh day 5 blastocyst transfer group [20, 21]. In these studies, the decline in implantation rates 22 h post insemination using rescue ICSI and after fresh day 6 blastocyst transfer may be the result of the asynchroncity of embryo development with that of the endometrium. Accordingly, in this case report, two frozen-thawed day 7 blastocysts were transferred into the patient's day 5 uterus.

Some small, independent obstetrics and gynecology clinics such as ours do not have the equipment for adhesiotomy. In such clinics, OPU is performed after adhesiotomy; however, in rare cases, transvaginal OPU is unfeasible despite adhesiotomy. In such cases, further surgery may be required, consequently increasing the physical, economic and temporal burdens on the patient. We suggest that these burdens could be reduced if OPU is performed during adhesiotomy and the COCs obtained are immediately transported to an infertility clinic.

References

1) Somigliana, E. Benaglia, L. Vigano' P. Candiani, M. Vercellini, P. and Fedele, L. (2011): Surgical measures for endometriosis-related infertility: a plea for research. Placenta., 32, S238–S242.
2) Kobayashi, Y. Takeuchi, H. Kitade, M. Kikuchi, I. Sato, Y. and Kinoshita, K. (2006): Pathological study of Fitz-Hugh-Curtis syndrome evaluated from fallopian tube damage. J Obstet Gynaecol Res., 32, 280–285.
3) Davey, AK. and Maher, PJ. (2007): Surgical adhesions: a timely update, a great challenge for the future.: J Minim Invasive Gynecol., 14, 15–22.
4) Nagata, Y. Honjou, K. Shindou, M. and Shirakawa, K. (1997): Ransou yutyaku hakurijyutu no yuuyousei ni kannsuru kenntou–yutyakuhakurijyutu zenngo no IVF-ET tiryou seiseki niyoru kaiseki. Sanfujinka maikuro sajari gakkai zasshi., 10, 33–38. (in Japanese)
5) Jansen, CA. van Beek, JJ. Verhoeff, A. Alberda, AT. and Zeilmaker, GH. (1986): In-vitro fertilisation and embryo transfer with transport of oocytes. Lancet., 327, 676.
6) Buckett, WM. Fisch, P. Dean, NL. Biljan, MM. and Tan, SL. (1999): In vitro fertilization and intracytoplasmic sperm injection pregnancies after successful transport of oocytes by airplane. Fertil Steril., 71, 753–755.
7) Alfonsín, AE. Amato, AR. Arrighi, A. Blaquier, JA. Cogorno, M. Feldman, ES. Gonzalez Echeverría, F. Horton, M. Della Vecchia, DL. and Millas, N. (1998): Transport in vitro fertilization and intracytoplasmic sperm injection: results of a collaborative trial. Fertil Steril., 69, 466–470.
8) Anjum, ZK. Abdo, K. Horne, G. Brison, DR. Abdulla, U. and Lieberman, BA. (2003): Transport of embryos resulting from intracytoplasmic sperm injection, but not oocytes, adversely affects implantation. Fertil Steril., 80, 1529–1531.
9) Takanashi, Y. Abe, Y. and Kubo, H. (2005): Clinical study of transport fresh embryo frozen-thawed embryo transfer. J Mamm Ova Res., 22, 170–177.
10) Kuwayama, M. (2007): Highly efficient vitrification for cryopreservation of human oocytes and embryos: the Cryotop method. Theriogenology., 67, 73–80.
11) Goto, S. Kadowaki, T. Hashimoto, H. Kokeguchi, S. and Shiotani, M. (2007): Stimulation of endometrium embryo transfer (SEET): injection of embryo culture supernatant

into the uterine cavity before blastocyst transfer can improve implantation and pregnancy rates. Fertil Steril., 88, 1339–1343.

12) Goto, S. Kadowaki, T. Hashimoto, H. Kokeguchi, S. and Shiotani, M. (2009): Stimulation of endometrium embryo transfer can improve implantation and pregnancy rates for patients undergoing assisted reproductive technology for the first time with a high-grade blastocyst. Fertil Steril., 92, 1264–1268.
13) Hiraoka, K. Hiraoka, K. Miyazaki, M. Fukunaga, E. Horiuchi, T. Kusuda, T. Okano, S. Kinutani, M. and Kinutani, K. (2009): Perinatal outcomes following transfer of human blastocysts vitrified at day 5, 6 and 7. J Exp Clin Assist Reprod., 6, 4.
14) 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.
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) Morgia, F. Torti, M. Montigiani, M. Piscitelli, C. Giallonardo, A. Schimberni, M. Giannini, P. and Sbracia, M. (2006): Use of a medium buffered with N-hydroxyethylpiperazine-N-ethanesulfonate (HEPES) in intracytoplasmic sperm injection procedures is detrimental to the outcome of in vitro fertilization. Fertil Steril., 85, 1415–1419.
17) Rienzi, L. Romano, S. Albricci, L. Maggiulli, R. Capalbo, A. Baroni, E. Colamaria, S. Sapienza, F. and Ubaldi, F. (2010): Embryo development of fresh 'versus' vitrified metaphase II oocytes after ICSI: a prospective randomized sibling-oocyte study. Hum Reprod., 25, 66–73.
18) Sermondade, N. Hugues, JN. Cedrin-Durnerin, I. Poncelet, C. Benzacken, B. Lévy, R. and Sifer, C. (2010): Should all embryos from day 1 rescue intracytoplasmic sperm injection be transferred during frozen-thawed cycles? Fertil Steril., 94, 1157–1158.
19) Chen, C. and Kattera, S. (2003): Rescue ICSI of oocytes that failed to extrude the second polar body 6 h post-insemination in conventional IVF. Hum Reprod., 18, 2118–2121.
20) Shapiro, BS. Richter, KS. Harris, DC. Daneshmand, ST. (2001): A comparison of day 5 and day 6 blastocyst transfers. Fertil Steril., 75, 1126–1130.
21) Barrenetxea, G. López de Larruzea, A. Ganzabal, T. Jiménez, R. Carbonero, K. Mandiola, M. (2005): Blastocyst culture after repeated failure of cleavage-stage embryo transfers: a comparison of day 5 and day 6 transfers. Fertil Steril., 83, 49–53.