SHORT COMMUNICATION

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Cryopreserved Sibling Oocytes and Intracytoplasmic Sperm Injection Rescue Unexpectedly Poor Fertilization in Conventional In Vitro Fertilization

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Purpose: To report a successful pregnancy from cryopreserved sibling oocytes and intracytoplasmic sperm injection (ICSI) for an infertile couple with an unexpectedly low fertilization rate in the fresh in vitro fertilization (IVF) cycle.

Methods: The woman had bilateral tubal obstruction and polycystic ovarian syndrome. The man had normal semen parameters. The couple underwent a cycle of controlled ovarian hyperstimulation in that 20 oocytes were retrieved. Twelve oocytes were conventionally inseminated and eight were cryopreserved using a slow freezing method. However, only one oocyte was fertilized, and no pregnancy was achieved. In the next cycle, the frozen oocytes were thawed and ICSI was performed.

Results: After thawing, seven oocytes (88%) survived and one was damaged. Six were at the metaphase II stage and were injected. Five (83%) achieved normal fertilization, and all of them cleaved (100%). After replacement of the embryos, a singleton pregnancy developed. A healthy female baby was delivered at term. Karyotyping revealed 46, XX.

Conclusions: In addition to well-known indications, cryopreservation of excess sibling oocytes for patients receiving IVF has a possible advantage of preventing unexpectedly low fertilization rate or fertilization failures.

KEY WORDS: Cryopreserved oocytes; intracytoplasmic sperm injection; unexpected fertilization failures.

INTRODUCTION

Oocyte cryopreservation achieved inferior results in the past, compared with embryo freezing. That was

mainly due to the low rates of survival, fertilization, and development. Cryopreservation may lead to the premature release of cortical granules, hardening of the zona, and depolymerization of meiotic spindles (1). In recent years, modifications of the slow-freezing method or vitrification have significantly improved the survival rate (2–6). Intracytoplasmic sperm injection (ICSI) is widely used as an effective technique to achieve fertilization for cryopreserved oocytes. After thawing, incubation for around 3 h followed by microinsemination may permit recovery of meiotic spindles and improve normal fertilization and cleavage (5,7–9).

Several authors have found that the survival, implantation, and pregnancy rates from thawed oocytes and embryos were similar (2,4,10). This suggests that oocyte freezing can be an efficient alternative to embryo freezing and applied on a routine basis (4). Women who have no spouse and fear the “biological clock” issue or are about to lose their ovarian function because of surgery, chemotherapy, or radiotherapy could save their oocytes for future fertility. For patients undergoing in vitro fertilization (IVF), freezing the excess oocytes could preclude ethical or religious issues arising from embryo cryopreservation. It could be used in countries that do not allow embryo freezing (11). In addition, the cryopreserved oocytes could be an important source for donations.

In 2001, we started our oocyte cryopreservation program using the slow-freezing protocol that was developed by Fabbri *et al.* (12). Recently, we experienced a successful pregnancy from cryopreserved oocytes and ICSI for an infertile couple for whom IVF of fresh sibling oocytes led to an extremely low fertilization rate. Here we proposed a possible supplementary advantage of cryopreservation of excess sibling oocytes for couples undergoing IVF in preventing failure from unexpectedly low fertilization rates or non-fertilization.

CASE HISTORY

This couple had suffered from infertility for 4 years. The 31-year-old woman was diagnosed as having bilateral tubal obstruction through hysterosalpingography

in December 2001. She had experienced irregular menstrual cycles and obesity since menarche. The basal body temperature chart exhibited a monophasic pattern, and the baseline hormonal profile revealed follicle-stimulating hormone (FSH), 4.8 mIU/mL; luteinizing hormone, 7.4 mIU/mL; estradiol, <20 pg/mL; and testosterone, 1.03 ng/mL. Ultrasonographic pictures of the bilateral ovaries were polycystic appearance with multiple small follicles. Polycystic ovarian syndrome was diagnosed accordingly.

The regular semen analysis of the man, aged 31 years, revealed normal findings with a concentration of 50×10^6 /mL, a motility of 56%, and normal morphology of 35%. In July 2002, the couple entered into our IVF program. The female received controlled ovarian hyperstimulation with a combination of a long protocol of gonadotropin-releasing hormone agonist (Supremon; buserelin acetate; Hoechst, Frankfurt, Germany), recombinant FSH (Puregon; Organon, Oss, the Netherlands) and human menopausal gonadotropin (HMG; Pergonal; Serono, Aubonne, Switzerland). The follicular sizes and serum estradiol levels were monitored to adjust the dosage of gonadotropin. When the leading follicles reached a mean diameter of 18 mm with a serum estradiol level of 5297 pg/mL, 10,000 IU of human chorionic gonadotropin (hCG; Profasi; Serono) was given. Oocyte retrieval was carried out 34 h later through the vagina under sonographic guidance and 20 oocytes were recovered. Intravenous albumin was given for prevention of ovarian hyperstimulation syndrome (13).

The couple decided to inseminate 12 fresh oocytes, and freeze eight oocytes for future use or possible donation to help the other infertile couple. The eight cumuli-denuded oocytes were cryopreserved using a slow method with the regime of 1.5 mol/L 1,2-propandiol with 0.3 mol/L sucrose (12). The 12 fresh oocytes were inseminated using conventional IVF techniques. The semen showed a sperm count of 50×10^6 /mL with 60% motility, and the morphological study revealed 43% with normal forms. However, only one oocyte was normally fertilized. A four-cell embryo was transferred into the uterus. Progesterone in oil (75 mg) intramuscularly as well as oral estradiol valerate (12 mg) were administered daily after transfer for a luteal support. Unfortunately, she did not get pregnancy at this cycle.

In the next cycle, she received hormonal replacement to prepare the endometrium with incremental dosages of oral estradiol valerate to 12 mg daily.

When the endometrial thickness was 12 mm with a serum estradiol level of 151 pg/mL, progesterone in oil (75 mg) was given intramuscularly daily. The eight cryopreserved oocytes were thawed using stepwise dilutions (12). Seven oocytes (88%) survived and one was damaged. For the surviving oocytes six were at the metaphase II stage and one was at metaphase I. Because of a previous history of low fertilization rate plus possible zona hardening of frozen-thawed oocytes, ICSI was chosen as the method to achieve fertilization. The timing of ICSI was 3 h after completion of thawing. Five normal zygotes (83%) were obtained, and five embryos (100%) cleaved on the next day. After discussion, the couple requested to have five embryos replaced into the uterus. The serum β -HCG level was 169 mIU/mL 14 days after transfer of the embryos. A singleton pregnancy developed, and a healthy female baby weighing 3632 g was delivered at 40 weeks of gestation in May 2003. Chromosomal analysis of umbilical cord blood, collected at delivery, revealed 46, XX.

DISCUSSION

Freezing surplus eggs for patients receiving IVF has provided a viable alternative to embryo cryopreservation with ethical or legal advantages (3). In addition, cryopreserved oocytes could be an important source to help other infertile couples with ovarian failure. This is especially significant in countries, such as Taiwan, where donation of oocytes is permitted, but not of embryos. Freezing oocytes also would allow for screening of infectious disease of the donor (4). Here we reported a successful pregnancy from cryopreserved oocytes and ICSI for a normozoospermic infertile couple who had very low fertilization rate using IVF of fresh sibling oocytes. In addition to the renowned benefits, cryopreservation of excess oocytes may have a possible advantage of preventing failure due to unexpected nonfertilization.

IVF or ICSI has helped many infertile couples to reproduce. ICSI is powerful for treating male-factor infertility that could not be assisted using conventional IVF. However, unexpected fertilization failures from IVF in couples with normal semen may occasionally occur (14,15). There are no absolute parameters or sperm function tests to predict fertilization outcome for apparently normal semen. The occult defect of the sperm or zona pellucida might lead to nonfertilization. Efforts to rescue nonfertilization by late ICSI resulted in a low conception rate. ICSI

usually can improve the fertilization rate during the next cycle, but that needs another ovarian stimulation and oocyte retrieval.

Some investigators attempted IVF–ICSI split on fresh sibling oocytes as the insemination method for normal semen to prevent fertilization failures (14,15). For couples with tubal or unexplained infertility, they found that the use of ICSI rescued 9–16% of cycles where IVF failed. Embryo freezing would be used for excess numbers in their strategy. Although some authors achieved results for oocyte cryopreservation comparable to embryo freezing; however, the case numbers were still small (2,4,10). At present, oocyte freezing is still not widely used in IVF centers (16). The additional benefits of cryopreservation of excess oocytes in preventing unexpected nonfertilization of IVF need further investigation.

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