Birth of a healthy male infant after transfer of vitrified-warmed blastocysts derived from intracytoplasmic sperm injection with vitrified-warmed oocytes and frozen-thawed spermatozoa

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Abstract

Purpose To report a successful delivery of a healthy baby after transfer of vitrified-warmed blastocysts derived from intracytoplasmic sperm injection (ICSI) with vitrified-warmed oocytes and frozen-thawed sperm.

Methods A female patient and her husband with non-obstructive azoospermia received a transfer of vitrified-warmed blastocysts from vitrified-warmed oocytes and frozen-thawed sperm. The main outcome measures were fertilization, pregnancy and birth.

Results Nine oocytes were matured and vitrified. When the vitrified oocytes were warmed, six survived with good quality morphology. Using ICSI, frozen-thawed sperm was injected into the six warmed oocytes that survived, and the fertilization rate was 100%. The zygotes were cultured, and five of six early embryos became blastocysts. One of them was transferred, but pregnancy was not achieved. The second time around, two vitrified-warmed blastocysts were transferred resulting in pregnancy, and a healthy boy was delivered.

Conclusions This is a rare case of a successful birth using a vitrified-warmed blastocyst grown after ICSI with a vitrified-warmed oocyte and frozen-thawed sperm.

Capsule This is a rare case of a live birth following transfer of two vitrified-warmed blastocysts produced by ICSI using vitrified-warmed oocytes and frozen-thawed sperm.

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Introduction

Assisted reproductive technology (ART) has been advancing rapidly, and patients with clinically difficult characteristics can be successfully treated. Unfertilized oocyte cryopreservation, one of the advancing techniques of ART, has been established, since the first oocyte cryopreservation succeeded [1] in 1986. The freezing method was based on a slow cooling/rapid thawing procedure using propanediol (PROH) or dimethylsulphoxide (DMSO) and sucrose as cryoprotectants. The survival rate after thawing still needed to be improved.

Recently, the freezing method has been changing from slow cooling cryopreservation to ultra rapid cryopreservation, namely, vitrification. This technique assures a high survival rate [2–4] and is being used for cryopreservation of oocytes and embryos as a clinical application more and more in Japan. The freezing application has also helped cancer patients who wish to preserve their fertility before chemotherapy [5, 6].

In the present study, we report a case of a live birth following transfer of two vitrified-warmed blastocysts produced by ICSI using vitrified-warmed oocytes and frozen-thawed donor sperm.

Case report

The patient was a 35-year-old woman. Her husband had been diagnosed with non-obstructive azoospermia.

Testicular sperm extraction from the husband's bilateral testes was attempted, but no sperm was obtained. Nine mature oocytes were vitrified. After five months, the couple decided to pursue treatment using donor sperm. Consequently, after transfer of a single fresh blastocyst from a vitrified-warmed mature oocyte and frozen-thawed donor sperm, a healthy male baby was born on December 1st, 2004 [3]. In August, 2007, the couple visited our clinic again wishing for a second baby. On September 28th 2007, the patient underwent controlled ovarian hyperstimulation by a gonadotropin-releasing hormone (GnRH) agonist long protocol (Nafarelil, Fuji Pharma., Japan), and nine of the 17 retrieved oocytes were matured. The couple requested to use sperm of the same donor as the previous time, but it was no longer available, because the sperm had been used for other patients. The oocytes were vitrified, and two months later, the couple decided to pursue treatment using sperm from a different donor.

The same techniques were used for vitrification and warming as in the first trial of treatment [3].

Six of nine vitrified oocytes retained good quality morphological aspects, and ICSI was performed with six surviving oocytes using frozen-thawed donor sperm. All six oocytes were fertilized, and five of the six embryos developed into blastocysts. A single fresh blastocyst evaluated as grade 2 by Gardner's criteria was transferred, and four surplus blastocysts were vitrified, but pregnancy was not achieved. Consequently, on December 27th, 2007, two vitrified-warmed blastocysts (Gardner's criteria; 3BB and 4BB) were transferred to the patient, and a gestational sac with fetal heart movements was observed by ultrasonography at seven weeks of gestation. Finally, this resulted in a birth of a normal healthy male (46, XY), weighing 3388 g at 40 weeks of gestation by spontaneous delivery on September 9th, 2008.

Discussion

Although the first frozen-egg pregnancy was achieved by Chen in 1986 [1], this application was not adopted widely for clinical assistance, because survival and pregnancy rates were comparatively low. This freezing method was based on a slow cooling/rapid thawing procedure using propane-diol (PROH) and sucrose as cryoprotectants, in line with the conditions originally developed for embryo freezing [7]. Borini et al [8] reported pregnancies and births after oocyte cryopreservation with 68 patients. Since then, several other pregnancies have been reported worldwide [9–11]. The safety of this cryopreservation has now been established in the human reproductive field. This method has improved survival and pregnancy rates using oocyte cryopreservation with sodium and sucrose concentration [12, 13]; however,

we generally achieve better survival and pregnancy rates in vitrification compared to those of slow cooling/rapid thawing cryopreservation [2–4].

In other reports, Setti et al. [14] and Debra et al. [10] reported a live birth following transfer of a cryopreserved embryo generated from a cryopreserved oocyte and cryopreserved sperm, and Chen et al. [15] described a normal birth from cryopreserved embryos after ICSI of frozen sperm into vitrified human oocytes. In this case, the oocyte was vitrified, and embryos were frozen using slow cryopreservation. Also, Montag et al [16] reported a birth of a healthy girl after double cryopreservation of human oocytes at metaphase II and pronuclear stages. Therefore, it has been suggested that it is safe to use frozen-thawed or vitrified-warmed oocytes, sperm and embryos for patients with severe diseases.

In conclusion, to the best of our knowledge, this is a rare case of a successful delivery after transfer of vitrified-warmed blastocysts derived from ICSI with vitrified-warmed oocytes and frozen-thawed spermatozoa. However, further study on the safety of freezing, especially vitrification is needed.

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