Cryopreserved human blastocysts after vitrification result in excellent implantation and clinical pregnancy rates

We conducted a retrospective review of all frozen ETs from May 2002 to October 2007 using our new vitrification technique in order to determine its efficacy. We found high pregnancy (70.5%; 79/112) and implantation (40.6%; 110/271) rates with the use of this new method. (Fertil Steril® 2009;92:2062-4, ©2009 by American Society for Reproductive Medicine.)

With the increased use of blastocyst culture as a routine protocol in clinical IVF, cryopreservation of surplus blastocysts has become an important part of assisted reproductive technology (1). In our previous study, the vitrification protocol showed promising results for both oocytes and blastocysts (2–5). The vitrification procedure uses an increased concentration of cryoprotectants along with faster cooling rates for preventing the formation of intracellular ice crystals. As a result, the injuries caused by ice crystals, as observed during the slow-freezing procedure, can be avoided. However, chemical toxicity caused by the high concentration of cryoprotectant agents remains one of the potential drawbacks of this technique (6, 7).

The purpose of this study was to determine the efficiency of our new vitrification technique. From May 2002 to October 2007, a total of 113 frozen ET cycles were performed on 102 patients. Clinical data from these patients were compared with the results of our previous study (Table 1). For obtaining blastocysts, embryo culture was carried out in sequential media (G1 and G2 series, Vitrolife, Inglewood, CO) at 37°C in a humidified atmosphere of 6.5% CO2, 5% O2, and 88.5% N2. Two of the most expanded blastocysts with big and tight inner cell masses among the given cohort were transferred on day 5 after oocyte retrieval. Surplus blastocysts, regardless of quality, were frozen on day 5, 6, or 7.

The solutions for equilibration, vitrification, and warming were prepared using a base solution of human tubal fluid with HEPES (SAGE IVF, Pasadena, CA) plus 20% HSA (SAGE IVF). The equilibration solution was made by adding 1.5 mol/L ethylene glycol (EG; Sigma Chemical Co., St. Louis, MO) to the base solution. The vitrification solution was made by adding 5.5 mol/L EG and 1.0 mol/L sucrose (Sigma) to the base solution. The warming solutions were prepared with 1.0, 0.5, 0.25, 0.125, and 0 mol/L sucrose in the base solution. The equilibration solution was prewarmed on the stage at 37°C, and the vitrification solution was kept at room temperature. Supernumerary blastocysts were placed into 1.0 mL of the prewarmed equilibration solution, and the solution was moved out of the stage to room temperature for 5 minutes. The blastocysts were then immersed in the vitrification solution for 20 seconds at room temperature. One to four blastocysts, depending on the quality, were loaded onto an electron microscope (EM) gold grid (Tedpella, Redding, CA) using a fine pipette, and excess vitrification solution was removed with the underlying paper. The grid was immediately plunged into a small container filled with liquid nitrogen (LN2) and then placed in a pre-cooled customized grid holder. The holder was capped with a plastic goblet to secure the grid inside the holder before being placed in an LN2 storage tank. This process was carried out under LN2.

Warming was performed around 4 p.m. a day before ET was scheduled to allow sufficient time to assess the viability and quality of the blastocysts. The EM grid holding the embryos was taken out of the holder using fine forceps under LN2 and then immediately transferred sequentially into Falcon two-well culture dishes (Beckton Dickinson, Franklin Lakes, NJ) containing 1.0 mL of warming solutions of 1.0, 0.5, 0.25, 0.125, and 0 mol/L of sucrose at intervals of 2.5 minutes at 37°C. The embryos were detached from the grid by pipetting after the final warming step. The blastocysts were then placed into preequilibrated 0.5 mL G2 medium. Embryos were assessed for viability and quality the next day around 10 a.m. Assisted hatching was performed using acid Tyrode’s solution (Irvine Scientific, Santa Ana, CA) at least 1 hour before ET. Embryos were transferred approximately 18–20 hours after warming. Biochemical pregnancy was assessed by measuring serum β-hCG level 9 days after ET. Implantation and clinical pregnancy were confirmed by the presence of a gestational sac(s) and fetal cardiac activity approximately 4–5 weeks after ET.

Binomial data analysis was done using the generalized linear model procedure (PROC-GLM) with analysis of variance using the SAS program (SAS Institute, Cary, NC). When significance of the main effect was detected in each experimental parameter, the treatment effects were compared by the least-squares method. P<.05 was considered statistically significant.
A total of 475 vitrified blastocysts were warmed, and 332 (69.9%) survived for 112 frozen ETs. One patient who had one vitrified blastocyst failed to undergo ET due to a nonsurviving embryo. Two hundred seventy-one blastocysts were transferred to 101 patients in 112 cycles. The mean number (±SD) of blastocysts transferred per cycle was 2.4 ± 0.8. Of 271 transferred blastocysts, 110 (40.6%) had a successful implantation confirmed approximately 4 weeks after the ET. Of the 112 transfers, 79 resulted in clinical pregnancy; the pregnancy rate was 69.9% per warmed cycle and 70.5% per ET cycle. These results were significantly higher than those of our previous report (Table 1). Between November 2003 and October 2008, 53 patients delivered 70 babies (34 boys and 36 girls), including 17 sets of twins. All babies are currently reported to be normal. Data on the delivery information were not available for seven patients as they were from overseas. There were 16 miscarriages (20.3%) during the study period, 13 singletons and three sets of twins. Two patients underwent therapeutic abortion after amniocentesis revealed trisomy 18 and 21.

Our speculation on the poor survival of blastocysts in a previous study was confirmed by the prior published report that the permeating property differs not only with the cryoprotectants used but also between oocytes and embryos (species and developmental stages) (8). In addition, the permeation of cryoprotectant is largely influenced by the temperature; cryoprotectant permeates faster at a higher temperature (9, 10). This observation led to the changes in our protocol with respect to the exposure temperature and duration of the vitrification process. The longer exposure time at lower temperatures might have allowed thorough equilibration and vitrification with less toxicity to the blastocysts (9–13). Although the survival rate (approximately 70%) seemed still somewhat lower than that of other published groups (over 80%) (14–17), the pregnancy (approximately 70%) and implantation (approximately 40%) rates were higher than those of others (pregnancy and implantation rates of ~50% and ~20%, respectively). This lower survival rate could be due to initial embryo quality and different survival evaluating methods. In this study, all of surplus blastocysts were cryopreserved regardless of their quality on day 5, 6, or 7. Also blastocysts were warmed a day before ET to allow enough time for better evaluation of embryo viability. Thus, this allowed more time for unhealthy embryos to drop out. Considering the high pregnancy and implantation rates of this study, it seems reasonable to expect that once the blastocysts survived vitrification, they have the comparable potential to develop further as fresh blastocysts.

In conclusion, in this study, we have shown that blastocysts vitrified using EG, sucrose, and an EM gold grid retained good developmental competency after warming. Furthermore, overnight culture after warming enables the selection of better quality blastocysts. To further improve clinical results, more studies are needed to develop cryopreservation protocols for increased embryo survival and better screening methods to identify developmental competence after warming.

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REFERENCES


