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Effect of repeated cryopreservation on human embryo developmental potential

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KEY MESSAGE

Surplus surviving embryos may occasionally be available following frozen-thawed embryo transfer cycles. Repeated cryopreservation procedures should be considered in such cases. This study revealed that the transfer of twice-cryopreserved human embryos can result in acceptable clinical pregnancy outcomes.

A B S T R A C T

Repeated cryopreservation of surplus embryos from frozen-thawed cycles should occasionally be considered. The purpose of this retrospective cohort study was to evaluate the pregnancy and perinatal outcome of repeated cryopreservation by vitrification of human blastocysts derived from slowly frozen-thawed day 3 embryos. In total, 571 vitrified-warmed blastocyst transfer cycles were investigated. The vitrified-warmed blastocysts were derived from slowly frozen-thawed cleavage embryos (twice-cryopreserved group) or fresh embryos (control group) cultured to the blastocyst stage. Age, body mass index, endometrial thickness, blastocyst developmental rate and number of embryos transferred were not significantly different between twice-cryopreserved and control groups. Clinical pregnancy and implantation rates were also similar. Compared with controls, the miscarriage rate was significantly higher in the twice-cryopreserved group (33.93% versus 19.07%, $P = 0.017$). This resulted in a significantly lower live birth rate in the twice-cryopreserved group than in controls (29.13% versus 39.18, $P = 0.038$). No differences were observed in mean gestational age, birthweight and sex ratio of newborns between groups. In conclusion, acceptable clinical pregnancy outcomes may be expected from transfer of twice-cryopreserved human embryos. While the neonatal outcome is not affected, the correlation between the risk of higher pregnancy loss and repeated cryopreservation needs further investigation.

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Introduction

Since the tremendous progress in assisted reproductive technologies, embryo cryopreservation has become a widespread reliable procedure in IVF clinical treatment. A successful embryo cryopreservation method will increase the cumulative pregnancy rate in a single ovarian stimulation cycle while minimizing the risk of multiple pregnancies and ovarian stimulation. The first delivery with a cryopreserved embryo (Trounson and Mohr, 1983) used the slow freezing method. Ever since, the slow freezing protocol has become widely accepted for human embryo cryopreservation. The survival rate of the slow freezing protocol is very stable (65–80%). In our centre, one to three cleavage embryos have been preserved in one straw. In order to avoid the risk of multiple pregnancies, up to two embryos have been transferred per cycle in recent years. As a result, a surplus of surviving embryos might occasionally be available in frozen-thawed embryo transfer (FET) cycles. In addition, some patients request the thawing of more day 3 embryos and to pre-form a blastocyst culture and transfer for higher success rates. However, sometimes there are more than two blastocysts. Therefore, repeated cryopreservation procedures for surplus embryos should be considered. To our knowledge, limited data are available for FET outcome with twice-cryopreserved human embryos, and most are case reports (Baker et al., 1996; Check et al., 2001; Estes et al., 2003; Farhat et al., 2001; Murakami et al., 2011; Sills et al., 2009). Lierman et al. (2014) used spare poor-quality human embryos as material and indicated that there are no differences in terms of survival and re-expansion status between twice-cryopreserved and once-vitrified blastocysts. However, no data about the pregnancy and perinatal outcome were presented. The purpose of our study was to evaluate the pregnancy and perinatal outcome of repeated cryopreservation by vitrification of human blastocysts derived from slowly frozen-thawed day 3 embryos.

Materials and methods

Patients

The study was approved by the Ethics Committee of the Peking University Third Hospital on 26 February 2013 (reference number 201352013). All warmed blastocyst transfer cycles from January 2009 to December 2012 at a single fertility centre were retrospectively reviewed. Women receiving pre-implantation genetic diagnosis and pre-implantation genetic screening were excluded. With this defined cohort, two age- (at the time of transfer) and body mass index (BMI)-matched groups of patients were identified according to the transferred blastocyst origin: (i) vitrified-warmed blastocysts derived from slowly frozen-thawed cleavage embryos (twice-cryopreserved group, $n = 127$) and (ii) vitrified-warmed blastocysts derived from fresh embryos (once-vitrified = control group, $n = 444$). Non-medical staff blind to the results performed the matching. Closest matches by patient age and BMI were preferentially selected within the required ranges. One cycle per patient was included in the study.

Fresh cycles

All patients used long or short protocols for ovarian stimulation. Thirty-six hours after administration of human chorionic gonadotrophin (HCG), the oocytes were retrieved and fertilized using conventional IVF or intracytoplasmic sperm injection. Normal fertilization was assessed by the presence of two pronuclei and a second polar body at 16–18 h after insemination. The zygotes were cultured in cleavage medium (Vitrolife, Sweden) to day 3 and then transferred. Patients having more than five good-quality embryos on day 3 after transfer were selected for cleavage embryo cryopreservation using the slow freezing protocol. For those patients who did not meet these criteria, the remaining embryos were placed in extended culture media.

Freezing and thawing protocol of the day 3 embryos

The primary cryopreservation was performed with good-quality cleavage embryos (5–10 cell embryos with ≤30% fragmentation) using the slow freezing method. The freezing–thawing procedure was performed at room temperature as previously described (Zheng et al., 2008). Briefly, the embryos were placed in a 1.5 mol/l solution of 1,2-propanediol (PROH; Sigma Chemical Co., Sigma-Aldrich, St Louis, MO, USA) for 15 min, and then moved to 1.5 mol/l PROH + 0.1 mol/l sucrose for 15 min, and subsequently aspirated into the cryostraws. Cooling was carried out in a programmable planar freezer (Kryo 10 series; Planer PLC, Sunbury-on-Thames, UK) at a rate of −3 to −7°C/min, at which point seeding was induced manually. Cooling was then continued at rates of −0.3 to −30°C/min and −30 to −150°C/min before storage in liquid nitrogen until thawing.

On the day of FET, the embryos were thawed rapidly by removing the straws from liquid nitrogen, exposing them to air for 40 s, and immersing them in a water bath at 30°C for 1 min. Embryos were sequentially placed in water baths for 5 min at room temperature with decreasing PROH concentrations (1.0 mol/l, then 0.5 mol/l and finally 0.0 mol/l), while the sucrose concentration was kept constant (0.2 mol/l) to remove the cryoprotectant (Zheng et al., 2008). Embryos with less than 50% of blastomeres showing signs of damage were considered as surviving. Up to two surviving embryos were transferred to patients. Occasionally, the remaining surviving embryos were placed in extended culture media.

Blastocyst culture and vitrification/warming procedure

Any remaining day 3 good-quality embryos, regardless of whether in fresh or in FET cycles, were cultured for 2–4 days. From day 5 to 7, those embryos achieving the blastocyst stage were evaluated morphologically. Gardner's grading system was used to determine the developmental level. Blastocysts reaching the expanded or hatching stage, earning a score above grade CC (inner cell mass/trophectoderm) (Gardner and Schoolcraft, 1999) were cryopreserved by vitrification. Embryos achieving the expanded stage on day 5 were cryopreserved on day 5 and classified as day 5 blastocysts. Those progressing to the expanded blastocyst stage on day 6 were cryopreserved on day 6 and classified as day 6 blastocysts. Occasionally, there were several embryos cryopreserved on 7. The expanded blastocysts collapsed after artificial shrinkage and were vitrified and warmed as previously demonstrated (Chen et al., 2013). Briefly, the blastocysts were equilibrated in 7.5% (v/v) dimethyl sulphoxide (DMSO; Sigma Chemical Co., MO, USA) and 7.5% (v/v) ethylene glycol (EG; Sigma Chemical Co.) at 37°C for 2 min and placed in 15% DMSO, 15% EG and 0.65 mol/l sucrose for 30 s. During this period, one or two blastocysts were placed on the Cryotop strip (Kitazato, Fuji, Japan), which was then quickly plunged into liquid nitrogen. For warming, the Cryotop was quickly placed in 0.33 mol/l sucrose at 37°C. After 2 min, the blas-