

Twin delivery following 12 years of human embryo cryopreservation: Case report

A.Revel¹, A.Safran, N.Laufer, A.Lewin, B.E.Reubinov and A.Simon

In Vitro Fertilization Unit, Department of Obstetrics and Gynecology, Hadassah University Hospital, Ein Kerem, PO Box 12000, Jerusalem 91120, Israel

¹To whom correspondence should be addressed. E-mail: Revel@md.huji.ac.il

It is uncertain how long IVF units can keep frozen embryos. Few data exist on success of embryo transfer for embryos that have been cryopreserved for many years. We report the delivery of healthy twins following the transfer of embryos cryopreserved for 12 years. To the best of our knowledge, this is the longest reported successful human embryo freezing.

Key words: assisted reproductive techniques/cryopreservation/frozen embryo/IVF/multiple pregnancy

Introduction

Cryopreservation of human embryos is a routine procedure in most assisted reproduction units. The use of embryo cryopreservation enables an improved cumulative success rate for IVF, decreasing the rate of multiple pregnancies and preventing ovarian hyperstimulation syndrome (OHSS). The transfer of frozen–thawed embryos can be performed after controlled endometrial preparation using oral estradiol (E₂) tablets from the first day of the cycle followed by a concomitant progesterone administration (Simon *et al.*, 1999). Alternatively, endometrial thickening is monitored by ultrasound in a natural cycle and the embryos are transferred after a spontaneous ovulation (Byrd, 2002).

The length of time that an embryo can remain frozen, and be successfully transferred, remains unknown.

Case report

A 39-year-old patient (Gravida₂, Para₂, Twins₁, Living Children₃) was admitted for frozen embryo transfer. The embryos had been frozen 12 years earlier, in 1990, following IVF for unexplained infertility. At that time, four fresh embryos (all were 6-cell, grade A) were transferred, resulting in a triplet pregnancy. The embryos were scored according to the grading system described by Veeck (1990). Following intrauterine fetal death of one of the three fetuses, a Caesarean section performed at 32 weeks gestation resulted in the delivery of two healthy children. A spontaneous pregnancy a few years later resulted in a singleton vaginal delivery.

As a result of the fresh IVF cycle performed in 1990, eight embryos (4–10 cells grade A, B) were cryopreserved 72 h after fertilization using 1,2-propanediol (PROH) as the cryoprotectant (Testart *et al.*, 1987). Briefly, the embryos were washed in enriched phosphate-buffered saline (PBS) medium containing 20% maternal serum and 0.4% human serum albumin (Sigma,

St Louis, MO, USA) before being transferred to the same medium also containing 1.5 mol/l PROH (Sigma). Following 15 min of incubation, the embryos were transferred to an ampoule containing 250 µl of enriched PBS medium containing 1.5 mol/l PROH and 0.1 mol/l sucrose. Freezing protocol included slow cooling at a rate of –20°C/min to –7°C, at which temperature manual seeding was performed. The embryos were then cooled at a rate of –0.3°C/min to –30°C, at which temperature the ampoule was plunged into liquid nitrogen (LN₂) for long-term storage. Our embryo bank consists of several Taylor–Wharton LN₂ containers (Theodore, AL) and the LN₂ level is continuously monitored and attached to an alarm system (Custom Biogenic Systems, Shelley Township, MI).

At the couple's request, and after written consent, being aware of the fact that cryopreservation technique was suboptimal 12 years ago, it was decided to transfer four embryos. Embryos were thawed using commercially available solutions (Irvine Scientific, Santa Ana, CA, USA). The embryos were incubated in a series of solutions containing decreasing PROH concentrations before being transferred to culture medium (Testart *et al.*, 1987). Embryos were maintained in culture until their transfer within 2 h. Full blastomere survival was noted in all four embryos.

The four thawed embryos were transferred using artificial endometrial preparation. This include daily oral micronized E₂ tablets from day 1 of the cycle for 2 weeks, followed by concomitant administration of micronized progesterone placed in the vagina as reported previously (Simon *et al.*, 1999). After 3 days of progesterone exposure, embryo transfer was performed. The embryos were transferred using a Casmed (Casmed International, Banstead, UK) catheter under sonographic guidance.

A triplet pregnancy resulted. Selective fetal reduction to twins was successfully performed at 13 weeks. During

pregnancy the patient was treated with β -methasone 12 mg from the 30th week. Caesarean section at 36 weeks resulted in the delivery of healthy twins weighing 2500 g each.

Discussion

Although frozen embryo transfers result in lower success rates than fresh embryo transfer (ASRM/SART Registry, 2002), this is usually related to the freezing and thawing process (Aytoz *et al.*, 1999). No effect on survival has been reported in the prolonged cryopreservation of human (Machtlinger *et al.*, 2002) or sheep (Fogarty *et al.*, 2000) embryos. The longest reported human cryopreservation resulting in delivery is 7 years (Ben-Ozer and Vermesh, 1999). To the best of our knowledge, we report the longest successful human embryo cryopreservation. The ampoule contained four embryos, as was customary at the time of cryopreservation. As all embryos survived cryopreservation and thawing, the decision to transfer all four embryos was made following concern for the extremely long cryopreservation period and the assumed lower embryo quality and suboptimal cryopreservation technique 12 years ago. The patient consented to the possible need for fetal reduction.

Good prognostic factors for the outcome of frozen-thawed cycles include age <40 years, a pregnancy having resulted from the initial embryo transfers, and the embryo grade and cleavage stage at the time of freezing (Karlstrom *et al.*, 1997). Implantation potential of frozen-thawed human embryos with full blastomere survival has been shown to be 3-fold higher than partially damaged embryos (Van den Abbeel *et al.*, 1997). Moreover, the quality of the embryos was found to be more important than the duration of the storage as predictor of implantation (Kondo *et al.*, 1996; Salat-Baroux *et al.*, 1996). No increase in congenital malformations was seen following frozen embryo transfer (Salat-Baroux *et al.*, 1996; Wennerholm *et al.*, 1997; Aytoz *et al.*, 1999). Post-thaw culture may allow selection of the embryos that continue to cleave *in vitro*, and thus improve implantation rate per transferred embryo (Van der Elst *et al.*, 1997; Ziebe *et al.*, 1998). Thus, although embryo cryopreservation may adversely affect embryo quality, it does not seem to have detrimental effects on the implantation or pregnancy potential of high-quality thawed embryos (Selick *et al.*, 1995).

The policy at our IVF unit is to store cryopreserved embryos for as long as requested by the couple. This report, of the longest cryopreservation period to be followed by successful pregnancy and delivery, confirms the finding that the duration of the storage does not appear to adversely affect the survival of frozen embryos.

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Submitted on July 22, 2003; accepted on September 29, 2003