Birth after transfer of frozen-thawed vitrified biopsied blastocysts

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Abstract *Purpose:* To present a case describing the birth of a healthy female after the replacement of vitrified biopsied embryos after Preimplantation Genetic Diagnosis.

Method: A descriptive case report of a single patient.

Results: Our patient carrier of an X-linked disease became pregnant and as a result a healthy girl was born.

Conclusions: This report shows that blastocysts obtained from biopsied embryos can be successfully cryopreserved by a simple, secure and low-cost vitrification method using a Hemi-straw support.

Keywords Biopsied human blastocyst · Birth · Vitrification

Introduction

Preimplantation genetic diagnosis (PGD) allows the selection of normal embryos for transfer in couples at risk of affected conceptions. Since the first case described in 1990 [1], PGD has become a routine procedure not only for couples at high genetic risk but also as a way of improving IVF results in terms of ongoing pregnancy and implantation rates. An efficient cryopreservation method for the biopsied embryos is needed in cases where surplus normal embryos are obtained. The results achieved with conventional cryopreservation protocols of biopsied embryos at different stages of development have shown low survival and implantation rates [2, 3]. Most PGD transfers are currently performed at

the blastocyst stage to allow extended time for diagnosis. As a consequence, there is a need for an optimised method to cryopreserve blastocysts with an open zona pellucida (ZP).

Vitrification has been used successfully to cryopreserve embryos from species that do not survive with the slow cooling method and it has been shown to be effective in cryopreserving human blastocysts with intact ZP [4–8]. Data regarding the successful outcome of vitrified blastocysts that were at the hatching stage or completely hatched are limited [9]. A high survival rate and a successful pregnancy has been reported with vitrification after PGD [10].

Case report

A 36-year-old woman and her 35-year-old husband consulted our clinic because of primary infertility. The husband's semen characteristics presented low motility according to World Health Organization criteria (WHO, 1999).

The woman was a carrier of Duchenne's muscular dystrophy. PGD for sex selection was recommended to avoid the birth of affected offspring.

The patient was treated using a GnRH long-protocol and stimulation with recombinant FSH (Gonal-F; Serono Laboratories, Geneva, Switzerland). Mature follicles were aspirated by ultrasound guided puncture 36 h after administration of 10,000 IU of human chorionic gonadotrophin (HCG) (Profasi 10000; Serono Laboratories, Geneva, Switzerland). Twenty-eight oocytes were recovered, of which 18 were mature metaphase II (MII), 2 were metaphase I, 7 were prophase germinal vesicle and 1 was a fractured zona pellucida.

Gamete and embryo culture was performed according to standard protocols used in the IVF laboratory of the Reproductive Medicine Service of the Institut Universitari Dexeus.

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Intracytoplasmic sperm injection (ICSI) was used with all the metaphase II oocytes. Sixteen of the 18 mature oocytes were fertilized. Embryos were biopsied on day 3 in commercial Ca- and Mg-free medium (embryo biopsy medium; Vitrolife, Gothenburg, Sweden) using laser technology, as previously described [11]. Blastomeres were assessed for the presence of a single nucleus prior to biopsy. Two blastomeres were removed when the embryo presented more than six cells. Blastomeres were fixed on slides according to Tarkowski's method with minor modifications. Slides were dehydrated in ethanol series.

A homemade probe mix containing chromosome centromere-specific probes for X (CEP X, Spectrum Orange, Vysis), Y (CEP Y, Spectrum Aqua, Vysis) and 18 (CEP 18, Spectrum Green, Vysis) were used for sex diagnosis, as previously described.

Six embryos were diagnosed as normal females. Four of the six female embryos reached the blastocyst stage. Two blastocysts were transferred into the uterus on day 5, but did not implant. Two blastocysts (an early and a hatching blastocyst) were frozen using a modified vitrification method previously described by Reed and coworkers [6], and loaded in an open pulled straw (Hemi-Straw method) [8].

Three months after the fresh transfer, a frozen blastocyst replacement cycle was planned. The patient received endometrial preparation with previous pituitary suppression with a GnRH agonist (Decapeptyl 3.75 mg; Ipsen Pharma S.A, Barcelona, Spain). Endometrial preparation with estrogen was initiated with 6 mg/day of estradiol valerate (Progynova, Schering España S. A., Madrid, Spain) for 13 days. Progesterone (Utrogestan, Laboratorios Seid, Barcelona, Spain) was added at 200 mg/day, five days before embryo transfer, and continued until the 11th week if the pregnancy was established.

The early blastocyst partially survived the warming process but no re-expansion was observed after overnight culture. The hatching blastocyst survived completely and expanded after culture. Both embryos were transferred under ultrasound guidance using a Wallace catheter (SIMS Portex Ltd, Kent, UK), as usually performed in our centre. Serum beta-HCG was determined on day 9 after embryo transfer and the pregnancy was confirmed at six weeks of amenorrhea by ultrasound, showing a single ongoing pregnancy. Amniocentesis was performed at 15 weeks (normal female karyotype) and pregnancy ended at 38 weeks by elective caesarean section. A healthy female was born, weighing 2,830 g and measuring 47 cm in length.

Discussion

Culture and transfer at the blastocyst stage in PGD programs allow a longer time for diagnosis and the selection of embryos with a higher developmental potential. Therefore, an

efficient method of blastocyst freezing is needed to optimize the results and avoid multiple pregnancies in PGD.

To our knowledge this case report describes the first birth after ultra-rapid vitrification of embryos biopsied for PGD. With this method, blastocysts are cryopreserved in a very small volume of cryoprotectant solution (1 μ l), thus increasing the cooling and thawing rate. Rapid dehydration and freezing (less than 1 min) could be beneficial in order to avoid a complete collapse of the blastocoelic cavity and the formation of ice crystals, enabling a faster recovery after thawing.

Alteration of the zona pellucida due to blastomere biopsy before freezing is detrimental to embryo viability during slow freezing. Several recent studies have shown that human embryo survival at different stages after biopsy and conventional cryopreservation is extremely poor [2, 3]. Vitrification has been proposed as an efficient method for vitrification of biopsied embryos, either at early cleavage stages [12] or at the blastocyst stage [9, 10]. In the latest results reported by Zech, it would seem that the survival rate of ZP-opened blastocysts is related to the degree of expansion, with higher survival rates for expanded blastocysts. In our case, the pregnancy has probably resulted following implantation of the expanded blastocyst, cryopreserved at the hatching stage.

In conclusion, this report shows that blastocysts obtained from biopsied embryos can be successfully cryopreserved by a simple, secure and low-cost vitrification method using a Hemi-straw support.

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