Pregnancies after Transfer of Vitrified Biopsied Blastocysts

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Objective  To show two cases describing the successful pregnancies after transplantation of vitrified biopsied embryos after preimplantation genetic diagnosis (PGD).

Methods  PGD by day 3 embryo biopsy was performed. Excess embryos were frozen using vitrification method. Four months later, the patient elected to undergo a frozen-thawed embryo transfer (FET) cycle.

Results  These two patients became pregnant and both of them delivered healthy PGD baby respectively.

Conclusion  This report shows that vitrification is an efficient and practical method for embryo cryopreservation during PGD. Frozen-thawed blastocysts that do survive obtained from vitrified D3 biopsied embryos are able to implant.

Key words: vitrification; biopsy; preimplantation genetic diagnosis (PGD)

Couples with infertility are at risk of carrying genetic disorders. Preimplantation genetic diagnosis (PGD) is regarded as a non-invasive prenatal diagnosis to improve live birth rates and can avoid termination of pregnancy in couples with a high risk of transmitting genetic disorders to their offsprings. PGD refers to the biopsy of one or two cells from human embryos, usually at the cleavage stage on day 3. First pregnancy after preconception diagnosis of translocations of maternal origin has been reported by Santiago Munne[1]. Most PGD transfers are currently performed at the blastocyst stage so as to allow extended time for diagnosis. Excess normal embryos are available for cryopreservation during PGD. In a study significantly fewer biopsied embryos survived intact and fewer cells survived in the non-intact embryos after conventional cryopreservation and thawing compared with embryos...
that were not biopsied[2].

In another study[3], a high survival rate and a successful pregnancy achieved with vitrification embryos that had been biopsied previously has been reported. Magli et al.[4] reported that biopsied and cryopreserved mouse 8-cell stage embryos had the same survival and implantation potential than non-biopsied embryos.

**Case report**

Case 1: A 29-year-old woman and her 30-year-old husband consulted to our center because of primary infertility. The husband was a carrier of balanced 46,XY,t(4;22) translocation. Karyotype analysis of female was normal. The husband’s seminal routine examination presented severe oligoasthenospermia according to World Health Organization criteria (WHO, 1999).

Case 2: A 28-year-old woman and her 35-year-old husband consulted to our center because of primary infertility. The male was a carrier of 47,XXY also known as Klinefelter syndrome. Karyotype analysis of female was normal. The husband’s seminal routine examination showed asthenozoospermia according to World Health Organization criteria (WHO, 1999).

Both patient 1 and patient 2 underwent exogenous gonadotropin stimulation with GnRH-agonist suppression using a GnRH long-protocol. The injection of hCG was given when at least 2 dominant follicles reached a diameter of 18 mm. Oocytes-cumulus complexes (OCCs) were retrieved using transvaginal ultrasound guidance 36 h after hCG injection. All MII oocytes underwent ICSI at 4 h post-retrieval and were individually cultured in cleavage medium. Per standard laboratory procedure, embryo morphologic characteristics (cell number, cellular fragmentation, and cell symmetry) were recorded by the embryologists on day 3.

The biopsy procedure was performed on day 3 after fertilization as described previously[5]. Embryos contained at least 5 blastomeres and not exceeding 30% fragmentation were biopsied. One blastomere containing a single nucleus was removed from the opening of each embryo and then the blastomere was fixed on a poly-lysine coated slide using dissolution medium (0.01 mol/L HCl, 0.1% Tween 20) to remove cytoplasm. The slide was washed by PBS and then dehydrated through an ethanol series.

Fluorescence *in situ* hybridization (FISH) process: for patient 1, two rounds of FISH were applied to single blastomeress[6] within 2 working days, 22q (Spectrum Orange, Vysis) and 4p (Spectrum Green, Vysis) telomere probes were simultaneously hybridised in a first FISH round, followed by a second hybridisation with a 4q telomere probes (Spectrum Orange, Vysis); for patient 2, a commercial probe mix containing chromosome centromere-specific probes for X (CEP X, SpectrumGreen,Vysis), Y (CEP Y, Spectrum Orange, Vysis) were used for sex diagnosis in one round of FISH within one working day.
At the same time, the biopsied embryos continued to cultivate to blastula or morula stage. For embryos at the blastocyst stage, grading usually takes into account the morphology of the inner cell mass (ICM) and trophectoderm (TE), as well as the degree of expansion of the blastocyst cavity[7].

Excess normal embryos were frozen using vitrification method. At first, the embryos were pre-equilibrated for 5 min in 0.75 mol/L ethylene glycol (EG; Sigma) and 0.75 mol/L 1,2-propanediol (PROH) at room temperature. Embryos were then placed for the final equilibration in 1.5 mol/L EG and 1.5 mol/L PROH with 0.5 mol/L sucrose for 60–90 s. In the meantime, 1–3 embryos were mounted on the tip of a Cryotop (Kitazato Ltd, Tokyo, Japan). Laser-pulse opening of the zona pellucida was performed before vitrified cryopreservation[8].

Thirty-six oocytes were aspirated from patient 1, of which 28 were mature MII; 23 oocytes were fertilized and 22 of them cleaved. Totally 14 embryos were biopsied, and 13 of them were biopsied successfully. Four embryos were diagnosed as normal. These 4 embryos continued to culture, and all reached to the blastocyst stage. Two hatching blastocysts were transferred into the uterus on day 5. The other two blastocysts (an expanding and a hatching blastocyst) were cryopreserved using vitrification.

Seventeen oocytes were aspirated from patient 2, of which 17 were mature MII; 15 oocytes were fertilized and 14 of them cleaved. Totally 8 embryos were biopsied and all of them were biopsied successfully. The XX or XY embryos were transplantable. Five embryos were diagnosed as normal, 4 of them reached to the morula on day 4 and 2 of them were transferred into the uterus. The other 2 morulas were frozen. The cryopreserved method was as same as patient 1.

In fresh cycle, patient 1 got ectopic pregnancy, patient 2 failed to get pregnancy.

Four months later, these two patients elected to undergo a FET cycle. All their frozen embryos were thawed. Urine β-hCG was determined on day 14 after embryo transfer, 35 d after embryo transfer, a singleton pregnancy was seen by transvaginal ultrasound scan both in patient 1 and patient 2. Both of them delivered healthy PGD baby respectively.

Discussion

Supernumerary normal good-quality embryos may be available after PGD. These embryos can be cryopreserved. Some recent studies have suggested that human embryo survival at different stages after biopsy and conventional cryopreservation is very poor[9]. Therefore, patients undergoing PGD should be informed that, currently, their chances of becoming pregnant with frozen-thawed biopsied embryos are rather low using conventional freezing method such as slow freezing.

However, vitrification can increase the survival rate of human biopsied embryos.
Vitrification has been proposed as an efficient method for cryopreservation of biopsied embryos, either at early cleavage stage\textsuperscript{[10]} or at the blastocyst stage\textsuperscript{[11]}. Recent studies also strongly showed that cryopreservation of human blastocysts using vitrification was superior to slowly freezing methods in spite of their biopsy status.

During vitrification, 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\textsuperscript{[12]}. Vitrification could be obtained by combining the high concentration cryoprotectant with high cooling and warming rates\textsuperscript{[13]}. A high survival rate and a successful pregnancy has been reported with vitrification after PGD\textsuperscript{[3]}. In a retrospective clinical study, the pregnancy rate and the implantation rate were the highest in cases where vitrified blastocysts that had previously undergone day 3 blastomere biopsy (37% and 36%) versus those where cryopreserved by conventional freezing (23% and 26%)\textsuperscript{[14]}. A study investigated the effect of vitrification on biopsied embryos at various developmental stages. Biopsied embryos vitrified at an advanced stage such as morula stage had as high survival rate as non-biopsied embryos. A significantly higher survival rate was observed in the biopsied blastocyst group compared with the non-biopsied group\textsuperscript{[15]}. This study has also revealed that vitrification is an efficient method for cryopreservation of blastocysts that had previously undergone day 3 blastomere biopsy. Day 3 biopsied embryos vitrified at an advanced stage or blastocyst stage is feasible.

The quality of an early embryo determines the quality of the blastocyst, and therefore the outcome of the blastocyst vitrification. In a study by Vanderzwalm et al.\textsuperscript{[16]}, vitrified blastocysts that originated from a cohort of early embryos with less than 30% fragmentation had survival, implantation and ongoing pregnancy rates of 73%, 32% and 19%, respectively. On the contrary, when the blastocyst came from embryos with 30%-50% fragmentation and/or unequally sized blastomeres, these rates decreased to 38%, 9% and 6%, respectively\textsuperscript{[16]}. In this study, the blastocysts in freezing-thawing cycle came from embryos with high embryology score which had 5%-20% fragmentation and approximately equally sized blastomeres thus increased the chance of embryo implantation.

Artificial shrinkage or collapse of blastocysts prior to vitrification is a relatively new approach to improve blastocyst viability, because applying artificial shrinkage or collapse of blastocysts prior to blastocyst vitrification allows better permeation of the cryoprotectants and better blastocoel dehydration\textsuperscript{[17]}. In this study, we used laser drilling as the artificial shrinkage method which can reduce blastocoelic cavity before vitrified cryopreservation. In concordance with these findings, we have demonstrated that laser drilling have a significantly positive impact on the blastocyst viability.

In summary, this study suggests that vitrification is an efficient and practical method for embryo cryopreservation during PGD. Laser drilling before vitrified cryopreservation is helpful and transplantation of frozen-thawed biopsied human embryos is safe and effective.
References


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Conference Information

88th Semi-Annual Meeting of Gynecologic Oncology Group (GOG)
February 6th to 9th California / San Diego Obstetrics / Gynecology, Oncology
Contact: GOG Administrative Office
Phone: 215-854-0770
E-mail: meeting_reg@gog.org
Website: http://www.gog.org/meetinginformation.html

Thromboprophylaxis in General Medicine and Obstetrics
February 6th United Kingdom / Leeds General Medicine, Obstetrics / Gynecology
Contact: Hartley Taylor Medical Communications Ltd
Phone: 011-44-15-6562-1967
E-mail: office@hartleytaylor.co.uk
Website: http://www.hartleytaylor.co.uk

18th Annual Winter Conference on Clinical Issues in OB / GYN
February 12th to 15th Hawaii / Big Island Obstetrics / Gynecology
Contact: Symposia Medicus
Phone: 800-327-3161 or 925-969-1789
Fax: 925-969-1795
Website: http://symposiamedicus.org/Conferences.aspx

37th Annual Advanced Ultrasound Seminar: Ob / Gyn
February 12th to 15th Florida / Orlando Obstetrics / Gynecology, Radiology / Imaging
Contact: American Institute of Ultrasound in Medicine
Phone: 800-638-5352 or 301-498-4100
Fax: 301-498-4450
E-mail: ddelanko@aium.org
Website: http://www.aium.org/cme/events.aspx

Asian American MultiSpecialty Summit VI: Laparoscopy & Minimally Invasive Surgery
February 12th to 15th Hawaii / Honolulu Obstetrics / Gynecology, Surgery, Urology
Contact: Society of Laparoendoscopic Surgeons
Phone: 305-665-9959
Fax: 305-667-4123
E-mail: info@sls.org
Website: http://www.sls.org/