

CASE REPORT

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## Live Birth from Previously Vitrified Oocytes, After Trophoctoderm Biopsy, Revitrification, and Transfer of a Euploid Blastocyst

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**Abstract:** Our objective is to describe a successful live birth from oocyte vitrification followed by thaw, fertilization, blastocyst culture, trophoctoderm biopsy, vitrification, and subsequent thaw. Fifteen mature oocytes were frozen from a patient with uterine factor infertility. Thirteen oocytes survived the thaw, and five underwent trophoctoderm biopsy and were refrozen. Three euploid embryos were obtained. A single euploid embryo was transferred in the second thaw cycle to a known recipient leading to the delivery of a normal male infant. This case report is proof of the concept that preimplantation screening and diagnosis is an option for fertility preservation patients.

**Keywords:** fertility preservation, oocyte cryopreservation, preimplantation genetic diagnosis, trophoctoderm biopsy, array comparative genomic hybridization, embryo biopsy

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## Introduction

The use of oocyte freezing for fertility preservation is becoming more widely practiced.<sup>1</sup> Although there is evidence that the success rates of oocyte cryopreservation and subsequent thaw cycles appear to approach that of a fresh in vitro fertilization (IVF) cycle (coupled with reports of numerous live births), oocyte cryopreservation is still not considered mainstream, but has become a more accepted option for cancer patients.<sup>2</sup>

Despite the controversy surrounding embryo biopsy for aneuploidy assessment (preimplantation genetic screening [PGS]), recent advances such as trophectoderm biopsy and array comparative genomic hybridization (a-CGH) have provided more support for its continued and expanded use. As more patients are choosing oocyte cryopreservation for fertility preservation, and as more evidence accumulates that aneuploidy screening lowers miscarriage rates and improves implantation rates, it is likely the combination of oocyte cryopreservation and PGS will be utilized in the future.<sup>3,4</sup> We report a successful live birth from previously vitrified oocytes from a known donor, which were thawed, fertilized, cultured to blastocyst, underwent trophectoderm biopsy, and refrozen awaiting analysis of ploidy by a-CGH. After transfer of a thawed euploid blastocyst, a normal male infant was delivered.

## Methods

A 31-year-old with a history of two miscarriages requested PGS due to our decreased loss rates in patients with recurrent pregnancy loss utilizing PGS compared to those not using PGS.<sup>3,4</sup> The patient was in a same-sex relationship and had failed several intrauterine inseminations and two IVF cycles using donor sperm (donor karyotype known to be 46, XY) in the past. One miscarriage occurred after an insemination (chromosomes not tested) and one after an IVF cycle, which was found to be 45, X. The patient was found to have uterine lining issues, including Asherman's syndrome, but due to her schedule, she requested to undergo ovarian stimulation with oocyte retrieval and cryopreserve her oocytes. At the time, we felt that our oocyte thaw pregnancy rates were greater than or equal to our frozen embryo transfer pregnancy rates, and after much discussion, the patient felt most comfortable

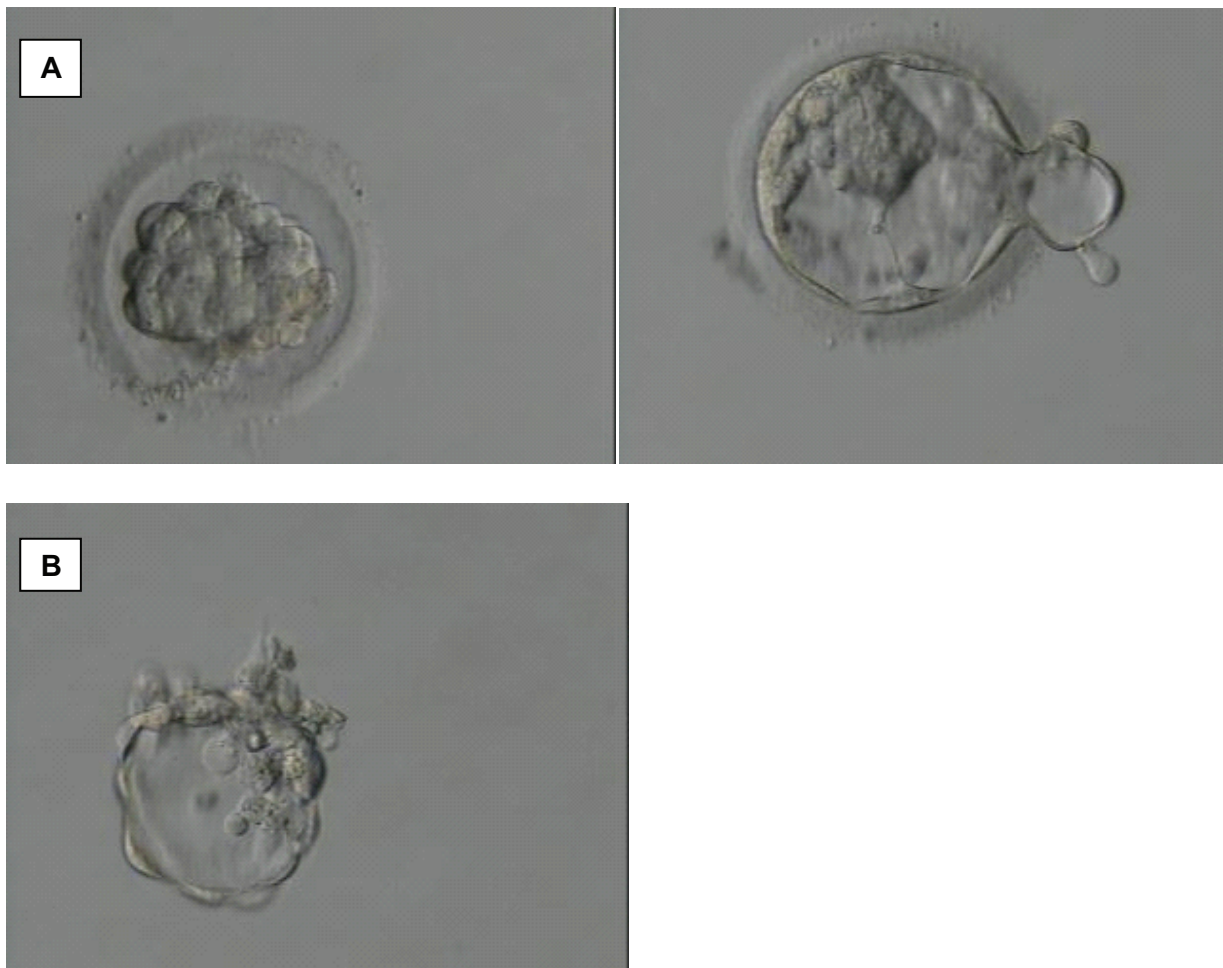
cryopreserving her oocytes over embryos with donor sperm. Her plan was to address her endometrial lining issues when her schedule permitted, which would then be followed by fertilization, embryo biopsy, and an embryo transfer. Ovarian stimulation for her third cycle was achieved by a combination of injectable recombinant follicle-stimulating hormone (Follistim; Organon, Orange, NJ, USA) and human menopausal gonadotropin (Menopur; Ferring, Suffern, NY, USA). An ovulation trigger of human chorionic gonadotropin (10,000 IU) was administered when her two lead follicles were 18 mm, and an ultrasound-guided transvaginal oocyte retrieval was performed 34–36 hours later.

## Oocyte cryopreservation

Eighteen oocytes were retrieved (15M2, 3MI). Following harvest, oocytes were frozen by both vitrification (n = 11) and slow freeze (n = 7) by our previously published methods, and thawed by the same methods.<sup>1,5</sup> The oocytes were thawed 2 months later and placed in human tubal fluid media (Irvine Scientific, Irvine, CA, USA), supplemented with 6% Plasmanate (5% USP plasma protein fraction [human]; Bayer, Elkhart, IN, USA), and overlaid with Sage mineral oil (Cooper Surgical Co., Trumbull, CT, USA). Thirteen mature oocytes survived the thaw (12/15 M2 frozen and one MI developed into an M2 upon thaw). Intracytoplasmic sperm injection was performed using donor sperm on all 13 oocytes. Fertilization was documented 18 hours after insemination by the presence of two pronuclei (2PN); this patient had twelve 2PNs (seven from vitrification and five from slow freeze), leading to a 92% fertilization rate.

## Blastocyst biopsy

On day 3, a hole was made in the zona pellucida using a Cronus laser (Research Instruments, Falmouth, UK) on a Nikon inverted TE-2000 microscope (Nikon Corporation, Tokyo, Japan) using Cronus software to allow hatching in all twelve embryos. Resultant embryos were cultured for 5 days, but the embryos were all morulas or cleavage-stage embryos and not suitable on day 5 for biopsy; therefore, they were cultured to day 6. On day 6, five (three from vitrification and two from slow-freeze) of her embryos underwent trophectoderm biopsy and were vitrified a second time. Vitrification was performed according to the



**Figure 1.** Embryos transferred in first (A) and second transfer (B).

Kuwayama vitrification method with CryoLocks and using an Irvine Scientific vitrification system, which contains dimethyl sulfoxide and ethylene glycol.<sup>6</sup> The biopsied cells were placed in Eppendorf tubes, frozen in dry ice, and then transported to ReproGenetics for PGS analysis once all of the biopsies were performed. This analysis was performed using the method described in Gutiérrez-Mateo et al<sup>7</sup> without modification. Three of the blastocysts were found to be euploid (all from vitrification).

## Results

Overall, the patient underwent three operative hysteroscopies with lysis of adhesions followed by uterine balloon catheter by three different skilled physicians without improvement in her uterine lining. Ultimately, the patient decided to transfer the euploid embryos into her partner who had a normal lining. The thaw cycle utilized sequential oral estradiol supplemented

by intramuscular progesterone after the endometrial stripe achieved a ring pattern and was 11 mm in the first thaw cycle and 9 mm in the second thaw cycle. An appropriately timed ultrasound guided embryo transfer was performed of euploid blastocysts. In the first embryo transfer cycle, two euploid embryos (3–5Bb, 6Bc) were transferred, but the cycle was unsuccessful. The second attempt with a single euploid embryo (3–5Bc) resulted in a pregnancy. Embryo images can be found in Figure 1. The couple went on to deliver a viable 8-pound, normal male infant (confirming a-CGH results) at 38 weeks gestation via normal spontaneous vaginal delivery.

## Discussion

As more oocytes are frozen for fertility preservation before cancer treatment or electively, there will be increasing instances where preimplantation genetic diagnosis (PGD) or PGS will be indicated or



requested after thawing. As more recessive genes are identified, more patients will be candidates for single gene PGD. In addition, as more cancer-causing genes are identified, cancer survivors who utilized fertility preservation will request PGD to avoid inheritance of these genes. The evolving literature on 24 chromosome screening already shows that the miscarriage rate is lower and the implantation rate is higher than IVF cycles without PGS (and equivalent to donor oocyte unscreened embryos), suggesting the possibility of routine aneuploidy assessment prior to embryo transfer.<sup>4,8,9</sup> In either case, more patients will electively request the utilization of these techniques for their previously cryopreserved oocytes. This case report demonstrates proof of a concept that it is possible to perform PGD or PGS on embryos derived from previously cryopreserved oocytes.

### Author Contributions

Conceived and designed the experiments: ASB, JG, AA, HL, EA, MCW, SM. Analyzed the data: JG, ASB, BHW, AA. Wrote the first draft of the manuscript: JG, BHW. Contributed to the writing of the manuscript: ASB, AA. Agree with manuscript results and conclusions: JG, BHW, HL, EA, MCW, AA, SM, ASB. Jointly developed the structure and arguments for the paper: BHW, JG, ASB. Made critical revisions and approved final version: ASB, JG. All authors reviewed and approved of the final manuscript.

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