Long-term cryopreservation of human oocytes does not increase embryonic aneuploidy

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Objective: To determine if long-term cryopreservation of human oocytes affects oocyte developmental competence, blastocyst euploidy, or live-birth rates.
Design: Retrospective cohort study.
Setting: University-based fertility center.
Patient(s): A total of 33 patients with cryopreserved oocytes underwent oocyte thaw, blastocyst culture, trophectoderm biopsy, and 24-chromosome preimplantation genetic screening (PGS) with array comparative genomic hybridization between December 2011 and July 2014; subjects were compared with 2:1 age-matched controls with fresh oocytes whose embryos underwent trophectoderm biopsy and PGS during the same period.
Intervention(s): None.
Main Outcome Measure(s): Rates of fertilization, blastulation, euploidy, implantation, and live birth.
Result(s): Thirty-three patients (mean age 36.2 ± 3.8 y) thawed 475 oocytes that had been cryopreserved for a median of 3.5 years. Compared with 66 age-matched controls who underwent in vitro fertilization and PGS with fresh oocytes, embryos derived from cryopreserved oocytes demonstrated compromised blastocyst formation (54.5% vs. 66.2%) despite no impairment in fertilization (72.8% vs. 73.2%). Results showed no difference in the number of euploid blastocysts (1.7 ± 1.9 vs. 2 ± 2.5), percentage of euploid blastocysts (44.5% vs. 47.6%), rate of implantation (65% vs. 65%), or rate of live birth and ongoing pregnancy (62.5% vs. 55%) after 24-chromosome PGS with cryopreserved or fresh oocytes.
Conclusion(s): Embryos derived from cryopreserved oocytes demonstrate impaired blastulation but equivalent rates of euploidy, implantation, and live birth compared with blastocysts derived from fresh oocytes, supporting the safety and efficacy of oocyte cryopreservation. (Fertil Steril® 2015;103:662–8. ©2015 by American Society for Reproductive Medicine.)
Key Words: Oocyte cryopreservation, fertility preservation, 24-chromosome preimplantation genetic screening, aneuploidy, blastocyst formation

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Oocyte cryopreservation (OC) is an effective means to preserve fertility in women at risk of medical- or age-related fertility loss (1–3). The oocyte is particularly vulnerable to physical injury during the cryopreservation process, owing to its large volume and high water content, and cryopreservation causes alterations to the oocyte, including zona pellucida thickening and premature cortical granule exocytosis (4). Concerns have been raised regarding the risk of meiotic spindle disruption and aneuploidy in cryopreserved oocytes, and conflicting animal and human studies have demonstrated slow cooling– and vitrification-induced damage to the meiotic spindle (5–10).

Early clinical OC outcomes have been reassuring (11), and an eloquent sibling-oocyte study by Forman et al. demonstrated that oocytes vitrified for a brief time (15 min) were at no greater risk of aneuploidy or impaired implantation. However, by definition, women seeking OC intend on long-term gamete storage. To our knowledge, no data have been reported regarding the risk of aneuploidy and likelihood of live birth after prolonged oocyte cryo-storage. Outcome data after long-term OC are

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critical, particularly as the demand for and utilization of OC technology continues to grow.

24-chromosome preimplantation genetic screening (PGS) has been shown to improve both neonatal and in vitro fertilization (IVF) outcomes (13–16), and in patients who have pursued 24-chromosome PGS using cryopreserved oocytes, PGS results can be used as an "assay" to assess the risk of meiotic spindle damage and aneuploidy after prolonged cryopreservation.

We sought to compare outcomes of patients who pursued 24-chromosome PGS using cryopreserved and thawed oocytes (OC-PGS) with outcomes using fresh oocytes (IVF-PGS). The aims were 2-fold: (1) to determine if blastocysts from cryopreserved oocytes have a greater likelihood of aneuploidy than blastocysts from fresh oocytes; and (2) to compare pregnancy potential of blastocysts derived from cryopreserved vs. fresh oocytes following trophectoderm biopsy and PGS.

MATERIALS AND METHODS

A retrospective analysis was performed of OC-PGS and IVF-PGS cycles completed at the Fertility Center at New York University (NYU) Langone Medical Center. Approval was obtained from the Institutional Review Board of the NYU School of Medicine. Patients were included if they had undergone either slow-cooling or vitrification of oocytes between 2006 and 2014 and subsequently presented between December 2011 and July 2014 for oocyte thaw, blastocyst culture, and trophectoderm biopsy with array comparative genomic hybridization (aCGH) for 24-chromosome embryonic aneuploidy screening.

In patients who underwent multiple oocyte retrieval procedures for OC, analysis was limited to oocytes obtained from the first procedure. All thaw-cycle data were analyzed for oocytes from the first retrieval procedure in order to appropriately compare outcomes with those in the control group of IVF patients for whom the biopsy results of all blastocysts are known. Five patients were included who had undergone polar body biopsy, oocyte vitrification, and subsequent thaw, blastocyst culture, trophectoderm biopsy, and rush day-6 embryo transfer (ET) under NYU Institutional Review Board Protocol 11-00395. All other ETs in this study were frozen ETs.

Data on OC and PGS were compared with 2:1 age-matched controls, randomly selected using the Excel (Microsoft) random-number generator, who underwent a first cycle of IVF-PGS with trophoectoderm biopsy and aCGH with fresh oocytes during the same time period. Patients were excluded if they underwent PGS for a history of translocation or used donor oocytes. Only patients in each group with ≥ 1 blastocyst available for biopsy were included in order to compare rates of aneuploidy.

Comparison parameters included: age at the time of oocyte retrieval; baseline serum follicle-stimulating hormone (FSH) and estradiol (E_2) levels; total units of gonadotropin (International Units [IU]) used; peak serum E_2 achieved on the day of ovulation trigger; total and number of mature (metaphase II [MII]) oocytes retrieved; 2-pronuclear (2PN) fertilization rate; blastocyst formation rate; total number of blastocysts biopsied; number of blastocysts biopsied on day 5, 6, and 7; and number of euploid and aneuploid embryos. The proportion of patients with no euploid embryos after biopsy was also reported.

Data pertinent to OC cycles were abstracted, including the number of oocytes thawed after vitrification and slow-freezing, the total number of oocytes surviving thaw, and the percentage of oocytes surviving thaw. In patients who underwent ET, additional parameters analyzed included number of embryos transferred, implantation rate, clinical pregnancy rate, and live birth and ongoing pregnancy rate. The 2PN fertilization rate was expressed in terms of oocytes exposed to sperm, and "usable blastocyst" formation rate was defined as the number of good-quality blastocysts available for biopsy per 2PN fertilization. Implantation rate was defined as the number of gestational sacs per total number of embryos transferred, and clinical pregnancy rate was defined as the number of pregnancies with fetal cardiac activity per ET procedure. Only outcomes from the primary ET were included in the analysis.

Ovarian Stimulation

Before initiation of treatment, menstrual day 2 or 3 serum E_2 and FSH levels were assessed. Patients with acceptable parameters (E_2 <75 and FSH <13.5) underwent controlled ovarian hyperstimulation using injectable gonadotropins (follitropin [Merck Serono]; and menotropins [Ferring Pharmaceuticals]), with luteinizing-hormone (LH) suppression achieved using either a gonadotropin-releasing hormone agonist or antagonist. Ovulation was triggered when ≥ 2 follicles reached ≥ 17 mm in diameter, and ultrasound-guided transvaginal oocyte retrieval was performed 34–36 hours later.

24-Chromosome Preimplantation Genetic Screening

Laser-assisted breaching of the zona pellucida was performed on day 3 (Saturn, Research Instruments Ltd). Embryos were assessed on days 5, 6, and rarely 7, and fully differentiated good-quality blastocysts were biopsied. The trophectoderm cells extruding from the expanded blastocyst were gently pulled using suction, and a laser was used at cell junctions to remove cells without disrupting the inner cell mass. Biopsied trophectoderm cells were transferred into polymerase chain reaction tubes and sent to the reference laboratory for 24-chromosome analysis using aCGH as previously described (13, 17). Following biopsy, blastocysts were vitrified to be replaced in subsequent frozen cycles, or in the case of a minority of patients, embryos underwent "rush" biopsy of a day-5 blastocyst followed by day-6 ET.

Oocyte Cryopreservation and Thawing and/or Warming

Oocyte cryopreservation and thawing and warming methods were performed according to those previously described by our group and are summarized below (18). Denuded oocytes noted to be metaphase II when evaluated 1.5 hours