Effect of vitrification on human oocytes: a metabolic profiling study

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Objective: To evaluate the effect of oocyte vitrification in the metabolomic profile of embryos developed from vitrified and fresh oocytes in our ovum donation program.
Design: Analysis of the metabolic profiles of spent culture medium samples corresponding to embryos developed from vitrified and fresh oocytes.
Setting: In vitro fertilization (IVF) unit/metabolomic facility.
Patient(s): Oocyte donors between the ages of 18 and 35 years.
Intervention(s): Metabolomic profile liquid chromatography coupled with mass spectrometry (LC-MS) of spent media samples.
Main Outcome Measure(s): Identification of spent media components and metabolites present and absent in vitrified and fresh day-3 embryos.
Result(s): We obtained a total of 190 spent media samples: vitrification group (65), fresh group (59), and a matched control media group (66). Multivariate data analysis was performed after global metabolomic and amino acid profiles did not reveal any statistically significant differences in day-3 embryos derived from fresh and vitrified oocytes, indicating that other metabolic differences between the samples (e.g., patient-to-patient variability, analytical variation) are greater than those between the vitrified and fresh sample groups. Univariate statistical analysis revealed a series of possible biomarkers, such as tryptophan, phenylalanine, and 2,5,7,8-tetramethyl-2-(2′-carboxyethyl)-6-hydroxychroman (alpha-CEHC), although only alpha-CEHC was statistically significant after correction for multiple testing.
Conclusion(s): Multivariate data analysis did not reveal statistically significant differences between the analyzed groups, suggesting that oocyte vitrification does not disturb embryonic metabolomic profiles. (Fertil Steril® 2013;99:565–72. ©2013 by American Society for Reproductive Medicine.)
Key Words: Embryo metabolism, LC-MS, metabolomic profile, vitrification

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Oocyte cryopreservation is one of the most fascinating challenges in assisted reproduction technology, and its addition to clinical practice has been a goal for many years. Fortunately, it is becoming evident that this goal has been recently achieved. There are several different types of human female gamete cryostorage used among subfertile and fertile women, including oncologic patients and women without medical problems who wish to delay their motherhood. Patients who are low responders, at risk of ovarian hyperstimulation syndrome, or for whom a semen sample is not available on the day of oocyte collection can also benefit from oocyte storage. This strategy is remarkably useful for ovum donation programs because no synchronization is needed between the donor and recipients, which makes it more flexible, easier, and safer as a procedure because a quarantine period can be applied before oocyte donation.

Vitrification has been revealed to be a very efficient method for oocyte cryopreservation (1); this physical phenomenon consists of the solidification of aqueous solutions without ice/crystal formation, facilitated by the use of high concentration cryoprotectants (CPA) coupled with very high cooling rates. Because of the toxicity of these substances, high CPA concentration has been a major drawback associated with vitrification; to counteract this effect, several devices and methodologies have been developed.

The availability of a vitrification method that can provide similar results to those achieved with the standard procedure used with fresh oocytes

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makes it possible to establish egg banks for ovum donation (2–5). A controlled clinical trial with a large sample size that involved the donation of vitrified and fresh oocytes failed to prove the superiority of using fresh oocytes in fertility treatments (3). Oocyte vitrification also has been applied successfully in infertile patients. A multicentric study has shown that the survival rate and positive clinical outcomes among the different centers involved was very consistent (6). Oocyte vitrification has also been useful in the management of low-responder patients (7).

Several reports provide evidence that healthy children are born when different vitrification approaches are employed (8, 9). These studies provide information about the survival, embryonic development, and clinical outcome of vitrified oocytes. However, there is lack of evidence in the literature regarding the affect these techniques have on early embryo metabolism.

A system has been developed within the metabolomics field for the quantitative/qualitative analysis of metabolites produced/secreted by an organism, tissue, or even a single cell; the system aims to obtain comprehensive metabolite profiles (10). Low-molecular-weight metabolites represent the final products of cellular metabolism and therefore reveal the response that biological systems have to a variety of genetic, nutritional, and environmental conditions (11). These noninvasive, quantitative techniques to study embryo metabolism, which use the spent conditioned media, are the focus of intense research to determine their value as predictors of embryo viability or pregnancy rates (12, 13), and even their ability to detect aneuploid embryos (14); however, prospective randomized trials have found no benefit to using these methods in predicting pregnancy (15). We used an untargeted metabolomic profiling approach based on liquid chromatography coupled to mass spectrometry (LC-MS and GC-MS) to identify the metabolic markers that could differentiate the embryos developed from vitrified oocytes compared with fresh oocytes from our ovum donation program.

MATERIALS AND METHODS

Patients

All donors included in our oocyte donation program were between the ages of 18 and 35 years and underwent a psychological evaluation. We had access to their complete medical history, which included current or past exposure to radiation or hazardous chemical substances, drug use, and reproductive history. Physical and gynecologic examinations were normal for all of the women, and there was no family history of hereditary or chromosomal diseases. All participants had a normal karyotype, and tested negative in a screening for sexually transmitted diseases. Donor ovarian stimulation was carried out as previously described elsewhere (16). In brief, all donors received a long protocol of down-regulation with daily doses of a gonadotropin-releasing hormone agonist (GnRH agonist, 0.1 mg of Decapeptyl; Ipsen Pharma). A transvaginal ultrasound was performed to ascertain ovarian quiescence on the first 3 days of menses and then controlled ovarian hyperstimulation (COH) was begun. The starting dose varied from 150 to 300 IU/day of recombinant follicle-stimulating hormone (recombinant FSH, Gonal-F; Serono; or Puregon; Organon) or highly purified human menopausal gonadotropin (hMG, Menopur; Ferring) for the first 2 to 5 days, according to age, body mass index (BMI), and response to previous ovarian stimulations. The dose was then adjusted to the ovarian response as determined by serum estradiol (E_2) levels and by ultrasound, performed at intervals of 2 to 3 days. Stimulation was performed out until the leading follicles were 18 mm in mean diameter. Recombinant human chorionic gonadotropin (hCG, Ovitrelle; Serono) was then administered, and ovarian puncture was performed 36 hours later. Anonymous donors were matched with their recipients according to phenotype and blood groups. All experiments were performed under the protocol approved by the Instituto Universitario IVI institutional review board.

Protocol for Oocyte Recipients

All recipients included in the study were thoroughly informed and completed written consent forms. They were given hormone therapy as previously described elsewhere (16). Increasing doses of E_2 valerate (Progynova; Schering Spain) were given as follows: 2 mg/day for the first 8 days of treatment, followed by 4 mg/day for the next 3 days, and then 6 mg/day. On day 15 or 16 of hormone therapy, the E_2 levels and endometrial thickness were determined. Recipients with residual ovarian function were first down-regulated with luteal phase GnRH-agonist administration. Micronized vaginal progesterone (P, 800 mg/day, Progeffik; Effik Laboratories) was started the day after oocyte donation.

All oocytes retrieved from a single donor were donated to a single compatible recipient. After ovum collection, oocytes were maintained in human tubal fluid (HTF) media. Denudation was performed enzymatically 2 hours after oocyte retrieval (Sage Inc.) in the case of vitrified oocytes. Fresh oocytes were denuded 4 hours after ovum collection. Only patients with elective embryo transfers on day-3 were included in the study.

Oocyte Vitrification

The Cryotop method for oocyte vitrification was used as previously described by Kuwayama et al. (17), with slight modifications. Oocytes were equilibrated in 7.5% (v/v) ethylene glycol (EG) 7.5% dimethyl sulfoxide (DMSO) in TCM199 medium with 20% synthetic serum substitute (SSS), referred to as equilibrium solution, at room temperature for 15 minutes. They were then placed into the vitrification solution containing 15% ethylene glycol, 15% DMSO, and 0.5 M sucrose. After 1 minute in this solution, oocytes were placed on the Cryotop strip (17) and immediately plunged into sterile liquid nitrogen (Ceralyn Online; Air Liquid France) (18). For warming, the Cryotop was taken out of liquid nitrogen and placed in 1.0 M sucrose in TCM199 20% SSS at 37°C. After 1 minute, oocytes were placed in 0.5 M sucrose in TCM199 20% SSS at room temperature for 3 minutes. Finally, oocytes were washed for 6 minutes in TCM199 20% SSS at room temperature before incubating the oocytes in HTF media for 2 hours before intracytoplasmic sperm injection (ICSI).