Effect of oocyte vitrification on embryo quality: time-lapse analysis and morphokinetic evaluation

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Objective: To analyze whether oocyte vitrification may affect subsequent embryo development from a morphokinetic standpoint by means of time-lapse imaging.
Design: Observational cohort study.
Setting: University-affiliated private IVF center.
Patient(s): Ovum donation cycles conducted with the use of vitrified (n = 631 cycles; n = 3,794 embryos) or fresh oocytes (n = 1,359 cycles; n = 9,935 embryos) over 2 years.
Interventions(s): None.
Main Outcome Measure(s): Embryo development was analyzed in a time-lapse imaging incubator. The studied variables included time to 2 cells (t2), 3 cells (t3), 4 cells (t4), 5 cells (t5), morula (tM), and cavitated, early, and hatching blastocyst (tB, tEB, tHB) as well as 2nd cell cycle duration (cc2 = t3 − t2). All of the embryos were classified according to the hierarchic tree model currently used for embryo selection. The analyzed variables were compared with the use of analysis of variance or chi-square and included 95% confidence intervals (CIs).
Result(s): The embryos that originated from vitrified oocytes showed a delay of ~1 hour from the first division to 2 cells (t2) to the time of blastulation (tB). The embryos that originated from vitrified oocytes showed a delay of ~1 hour from the 1st division to 2 cells (t2) to the time of blastulation (tB) ($P<.05$). The proportions of embryos allocated to categories A–E in the hierarchical tree were similar between groups. No differences in implantation rates between the fresh (51.3% [95% CI 47.1%–55.7%]) and vitrified (46.4% [95% CI 38.4%–54.4%]) groups were found.
Conclusion(s): The embryo quality of vitrified oocytes was not impaired: cc2, quality according to our hierarchic morphokinetic model, and implantation rates were similar between fresh and vitrified oocytes. However, morphokinetic differences were observed from t2 to tB. Our main study limitation was the retrospective nature of the analysis, although a large database was studied. (Fertil Steril® 2017;108:491–7. ©2017 by American Society for Reproductive Medicine.)
Key Words: Vitrification, morphokinetics, time-lapse, oocyte, embryo

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The possibility of successful female gamete cryopreservation is a remarkable milestone in contemporary assisted reproductive technology. Ovum donation programs have been major beneficiaries of the establishment of efficient egg-banking. Oocyte cryostorage results are very useful for overcoming the most common drawbacks involved with the use of fresh donations, such as synchronization between donors and recipients and long waiting lists, and make the process safer, given the possibility of saving the quarantine period. Egg banking has been possible thanks to vitrification providing high success rates (1, 2). In the past decade, the use of donors' vitrified oocytes has gradually increased, and it can be stated that egg banking in ovum donation programs is currently a frequent approach (2). Given the positive results, oocyte cryopreservation has been conveyed to other applications, mainly for women who wish to preserve fertility (3).

The suitability of oocyte vitrification has been established thanks to the evaluation of survival rates, embryo development parameters, and implantation, pregnancy, and live birth rates. These outcomes have been typically compared between fresh and vitrified oocytes or between slow freezing and vitrification. Similar embryo development

Received March 6, 2017; revised June 16, 2017; accepted June 20, 2017.
A.Cobo has nothing to disclose. A.Coello has nothing to disclose. J.R. has nothing to disclose. J.S. has nothing to disclose. J.M.d.l.S. has nothing to disclose. M.M. has nothing to disclose.
Supported by the Spanish Ministry of Economy and Competitiveness (PI14/00523) through an Instituto de Salud Carlos III program and by a grant from Fertility Innovation, Merck Serono.
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Fertility and Sterility® Vol. 108, No. 3, September 2017 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2017.06.024

has been shown in embryos that originate from fresh versus vitrified oocytes in a sibling cohort study (4), and the clinical validation of using vitrified oocytes for egg donation has been demonstrated in a large randomized controlled clinical trial (5). Similar obstetrical and perinatal outcomes of the babies conceived with the use of vitrified versus fresh oocytes has been recently demonstrated in a large study that involved more than 2,000 infants (6).

Classically, embryo quality evaluations have been based on morphologic criteria, which involves conventional static observations that are linked to specific time points and inter-observer variations. Embryo development is a dynamic process, and several critical stages can go unnoticed with the use of traditional morphologic assessments. As previously demonstrated, the time-lapse imaging of cell division kinetics offers a far more accurate analysis of embryo development and provides detailed information of the dynamic morphology in each cell-division step (7, 8). Relevant parameters, such as multinucleation and fragmentation, critical abnormal cleavage patterns, and morphokinetics can (9) be seen or evaluated only with the use of time-lapse technology (10–14).

Very little is known about the morphokinetics of the embryos generated from vitrified oocytes. Vitrification consists in the solidification of an aqueous solution in the absence of ice crystals. During the process, cells are subjected to high osmotic stress and most intracellular water content is replaced with permeable cryoprotectants. On warming, cells rehydrate and cryoprotectants are removed. Whether all of these physicochemical changes cause any alteration in embryo morphokinetics is still not well known. The only report on this topic has been attempted with the use of the embryo kinetics of fresh and sibling vitrified-warmed oocytes, limited to a 44-hour time-lapse analysis on 168 fertilized sibling vitrified-warmed oocytes (15).

Although no differences in clinical outcomes and embryo morphology have been observed in previous studies that have compared fresh and vitrified oocytes (4, 5), the time-lapse imaging of embryos from vitrified oocytes can help to elucidate whether vitrification can cause subcellular effects that are able to alter cell division dynamics.

The purpose of the present study was to evaluate the effect of oocyte vitrification on the morphokinetic parameters of embryos generated after the vitrification and warming procedures compared with embryos developed from fresh oocytes in ovum donation cycles, with the use of the largest sample size reported to date and covering all embryo development stages.

MATERIALS AND METHODS

Study Design and Population

This was an observational 2-year cohort study approved by the Institutional Review Board that governs the clinical use of IVF procedures for research at the Instituto Valenciano de Infertilidad (ref. 1511-VLC-062-AC, 111/11/2015). The study included 1,359 ovum donation (OD) cycles (n = 9,936 embryos) conducted with the use of fresh oocytes, and 631 OD cycles (n = 3,794 embryos) carried out with the use of vitrified oocytes.

Protocol for Donors

All of the involved donors fulfilled our inclusion criteria. The controlled ovarian stimulation (COS) protocols used in our center for donors are described elsewhere (1). In the GnRH agonist protocols, triggering was performed with the use of 250 mg recombinant hCG (rhCG; Ovitrelle; Serono). Alternatively, the flexible GnRH antagonist protocol was used as follows: COS was initiated on day 2–3 after bleeding with the use of 150 or 225 IU/d recombinant FSH (Gonal-F, Merck-Serono; or Puregon, MSD), combined with 75 IU/d hMG (Menopur; Ferring Pharmaceuticals). Doses were adjusted to ovarian response. Daily doses of 0.25 mg GnRH antagonist (ganirelix [Orgalutran; MSD] or cetrorelix [Cetrotide, Merck Serono]) were started when a follicle measuring >14 mm was observed. A single dose of the GnRH agonist (0.1 mg triptorelin; Decapeptyl; Ipsen Pharma) was administered to trigger final oocyte maturation when at least three follicles measuring >17.5 mm or one follicle measuring >20 mm was observed. In some cases, triggering was performed with the use of 250 mg rhCG. Transvaginal oocyte retrieval was conducted 36 hours later. The retrieved metaphase II (MII) oocytes were vitrified 2 hours after ovum pick-up. Anonymous donors were matched with their recipients according to phenotype and blood groups, and the assignment of fresh or vitrified donations depended on the availability of couples' suitable oocytes and/or patients' preferences. Informed consent was obtained in all cases.

Endometrial Preparation for Oocyte Recipients

As described elsewhere (1), women with ovarian function were first down-regulated in the luteal phase with the use of a single dose of GnRH agonist depot (3.75 mg Decapeptyl, Ipsen Pharm; or 3.75 mg Gonapeptyl Ferring). After menses, all subjects received oral E_2 valerate (EV; 6 mg/d Progynova; Schering). Approximately 10–15 days after initiating EV, serum E_2 and P levels and endometrial thickness were measured. Administration of micronized P (800 mg/d vaginally; Progeffik; Effik Laboratories) was initiated on the day after oocyte donation. If pregnancy was achieved, administration of EV and P was maintained until gestation week 12.

Oocyte Vitrification Protocol

Oocytes were denuded 2 hours after oocyte retrieval. Fresh oocytes were denuded and microinjected 4 hours after oocyte retrieval. The MII oocytes were vitrified immediately after the nuclear maturity evaluation, by checking for the presence of the first polar body. All of the materials and tools for vitrification were obtained from Kitazato. The Cryotop method followed for oocyte vitrification has been described elsewhere (1, 16, 17). Specifically, after 12 minutes of stepwise equilibration in a mixture of 15% (v/v) ethylene glycol and dimethylsulfoxide in buffer media supplemented with hydroxypropyl cellulose (17), oocytes were exposed to a vitrification solution by maintaining the same mixture of