Outcome of cryotransfer of embryos developed from vitrified oocytes: double vitrification has no impact on delivery rates

Ana Cobo, Ph.D., Damià Castellò, Ph.D., Begoña Vallejo, M.L.T., Carmela Albert, Ph.D., José María de los Santos, Ph.D., and José Remohí, M.D.

IVI-Valencia, Institut Universitari IVI, Valencia, Spain

Objective: Evaluate the outcome of cryotransfer of embryos developed from vitrified oocytes.
Design: Retrospective cohort study.
Setting: Private university-affiliated IVF center.
Patient(s): Women undergoing warming cycles in which vitrified embryos were developed from vitrified or fresh oocytes.
Intervention(s): Vitrification by the Cryotop open device.
Main Outcome Measure(s): Delivery rate (DR) per warming cycle.
Result(s): A total of 471 warming cycles of 796 vitrified embryos developed from vitrified oocytes (group 1) and 2,629 warming cycles of 4,394 vitrified embryos derived from fresh oocytes (group 2) were evaluated. Overall survival rates were 97.2% [95% confidence interval [CI] 95.9%–98.6%] vs. 95.7% [95% CI 94.9–96.4], respectively. DRs per warming cycle were 33.8% (group 1) and 30.9% (group 2). Double vitrification had no effect on DR (odds ratio [OR] 0.877, 95% CI 0.712–1.080). Confounding factors (ovum donation or autologous cycles; day-3 or blastocyst embryo transfer [ET]; natural or hormonal replacement therapy for ET; single or double ET; previous cycles, number of oocytes, doses of gonadotropins and E_2 levels on the day of hCG) did not modify the effect of double vitrification on DR (OR 0.872, 95% CI 0.702–1.084).
Conclusion(s): Vitrification at early cleavage or blastocyst stage of embryos obtained from previously vitrified oocytes has no effect on DR/warming cycle. (Fertil Steril® 2013;99: 1623–30. ©2013 by American Society for Reproductive Medicine.)
Key Words: Delivery rate, oocyte vitrification, revitrification, survival rate, embryo vitrification, cryopreservation

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/coboa-cryopreservation-embryo-vitrification/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

The role of cryopreservation in human assisted reproduction technologies (ART) has become more relevant owing to a range of clinical, ethical, and legal considerations. The overall efficiency of a stimulated cycle may depend on the ability to cryopreserve both gametes and embryos. Embryo cryopreservation by vitrification has been carried out successfully using different types of devices and methodologies at the early cleavage stage (1–4) or blastocyst stage (5–9). This methodology allows the cytoplasm to solidify in the absence of ice, thereby avoiding the detrimental effects of chilling injury (10).

After years of representing a serious challenge to ART, the efficiency of oocyte cryopreservation has been increasingly documented in recent times (11–17). Specifically, oocyte vitrification has emerged as a highly efficient approach in terms of survival rates in comparison to slow freezing procedures (18). Moreover, fertilization rates and clinical outcome following oocyte vitrification have been shown to be similar to those of matched fresh oocytes, as reported in recently published prospective randomized studies of different patient populations (13, 19).

The great scope of the vitrification technology used in ovum donation programs was demonstrated by our group in a controlled clinical trial in which the ongoing pregnancy per treatment

Received September 21, 2012; revised January 14, 2013; accepted January 15, 2013; published online February 14, 2013.
A.C. has nothing to disclose. D.C. has nothing to disclose. B.V. has nothing to disclose. C.A. has nothing to disclose. J.M.d.l.S. has nothing to disclose. J.R. has nothing to disclose.
Reprint requests: Ana Cobo, Ph.D., IVI-Valencia, Institut Universitari IVI, Plaza de la Policía Local 3, 46015 Valencia, Spain (E-mail: ana.cobo@ivi.es).

Fertility and Sterility® Vol. 99, No. 6, May 2013 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2013.01.106

was similar among patients receiving fresh or vitrified cryostored oocytes (11). In the same study, the potential of vitrified oocytes to develop into competent embryos was also confirmed by evaluation of cleavage rates and morphologic assessment of the embryos that subsequently developed. The high proportion of competent embryos obtained from vitrified oocytes has led to the availability of surplus embryos that can be cryopreserved a second time after the transfer of "fresh" embryos. Anecdotal case reports have been published of human pregnancies achieved after cryotransfer of refrozen embryos (20–24). In addition, little, if anything, is known about the transfer of vitrified embryos developed from vitrified oocytes.

The aim of the present study was to evaluate the outcome of cryotransfer of embryos developed from vitrified oocytes in ovum donation cycles and own-oocyte cycles of patients undergoing IVF-ET. To the best of our knowledge this is the first report of a large series of cryotransfers of vitrified embryos derived from vitrified oocytes.

MATERIALS AND METHODS

This is a retrospective cohort study of warmed embryos developed from vitrified oocytes which underwent two rounds of vitrification: first as oocytes, and later as embryos. Institutional Review Board approval was obtained. The computerized data included in this report were obtained from 471 warming cycles of 796 vitrified embryos (group 1) and 2,629 warming cycles of 4,394 vitrified embryos derived from fresh oocytes which were used as control samples (group 2). The cycles took place from January 2007 to June 2010 and were performed consecutively during this period of time; no exclusion criteria were applied. Groups 1 and 2 included ovum donation cycles (n = 414 and 1,315, respectively), own-oocyte cycles (n = 57 and 1,314), and day-3 (n = 218 and 1,536) and blastocyst warming cycles (n = 253 and 1,093).

Surplus embryos were cyropreserved depending on their morphologic quality. Embryos were classified morphologically according to the criteria of the Spanish Embryologist Society, Asociación para el Estudio de la Biología de la Reproducción (ASEBIR) (25), with slight modifications. A summary of the ASEBIR classification system can be found in the Istanbul Consensus Workshop document on embryo assessment published by Alpha–Scientists in Reproductive Medicine (26, 27). In short, a type A embryo (with optimum implantation potential) showed <10% of focal fragments, no multinucleation, absence of vacuoles, and no irregularities in the zona pellucida (ZP) and consisted of 7–8 cells on day 3 (after having 4 cells on day 2). Day-3 type B embryos were defined as those having 7–8 cells (coming from a 4-cell day-2 embryo) with 11%–25% fragmentation or those with 9 cells developed from a 2-cell embryo with >25% fragmentation and a maximum of one multinucleated cell and with the same parameters for vacuoles and ZP evaluation as for type A. Embryos defined as type A or B on day 3 were selected for vitrification. Some suboptimal embryos were maintained in extended culture and were vitrified only if they developed into good-quality blastocysts.

ASEBIR grading for blastocysts is based on the assessment of inner cell mass (ICM) and trophoectoderm appearance, as proposed by Gardner et al. (28). Type A ICM was well defined, had a compact appearance, and was made up of many cells (1,900–3,800 μm^2 in diameter). Type B ICM was the same size as type A but did not show compaction between cells. A type A trophoectoderm was well defined, uniform, and formed of many cells, and a type B trophoectoderm had an irregular appearance and was made up of fewer cells. Type A blastocysts had both type A ICM and type A trophoectoderm. Type B blastocysts had type A ICM with type B trophoectoderm or type B ICM with type B trophoectoderm (ASEBIR criteria). Type A and B blastocysts were selected for vitrification. Artificial collapsing of blastocysts was occasionally performed in hatching embryos.

The number of embryos to be replaced varied in each case, but typically two day-3 embryos were transferred and elective single-embryo transfer (SET) was considered for blastocyst-stage ET.

Endometrial Preparation for ET

For natural cycles (n = 70 and 831 for groups 1 and 2, respectively), follicular growing was monitored. When the leading follicle reached a diameter of 18 mm, 6,500 UI hCG (Ovitrelle; Merck Serono) was administered to trigger ovulation. Micronized progesterone (P) was initiated 3 days later (400 mg/d, vaginally). Day-3 ETs were conducted 5 days after hCG administration. Day-5 and day-6 blastocyst ETs were scheduled 7 and 8 days after hCG, respectively. Transfers were routinely performed under transabdominal ultrasound guidance. The protocol for hormonal replacement therapy (HRT) was that described previously (29), with slight modifications. Women with functioning ovaries were first down-regulated in the luteal phase with a single dose of GnRH agonist depot (3.75 mg Decapeptyl [Ipsen Pharm] or 3.75 mg Gonapeptyl [Ferring]) and received 6 mg oral estradiol valerate (EV; Progynovaw; Schering Spain) after menses. Approximately 10–15 days after initiation of EV, serum E_2 levels and endometrial thickness were determined. If E_2 levels were >150 pg/mL and a triple layer endometrial pattern was confirmed, administration of micronized P (800 mg/d, vaginally; Progeffik; Effik Laboratories) was initiated. Day-3, day-5, and day-6 ETs were scheduled 2, 4, and 5 days after P administration, respectively. The GnRH antagonist protocol (Cetrotide; Merck Serono) was almost identical except for the administration of 0.25 mg per day for 7 days from day 1 of the cycle. EV and P were administered as described above. The numbers of patients undergoing a warming cycle under HRT were 401 in group 1 and 1,798 in group 2.

Embryo Vitrification Protocol

The Cryotop method for embryo vitrification was that described by Kuwayama et al. (30) with slight modifications. Early-cleavage and blastocyst-stage embryos were equilibrated in a single step for 10–12 minutes at room temperature in 7.5% (v/v) ethylene glycol (EG) + 7.5% dimethylsulfoxide (DMSO) in TCM199 medium + 20%