Single-embryo transfer of vitrified-warmed blastocysts yields equivalent live-birth rates and improved neonatal outcomes compared with fresh transfers

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Objective: To compare pregnancy and neonatal outcomes after fresh and vitrified-warmed single-blastocyst transfers.
Design: Retrospective study.
Setting: Private in vitro fertilization (IVF) clinic.
Patient(s): 1,209 infertile patients who underwent a total of 1,157 fresh and 645 vitrified-warmed embryo transfers.
Intervention(s): Day-5 single-blastocyst transfers using fresh or vitrified-warmed (Cryotop method) grade I and grade II embryos.
Main Outcome Measure(s): Fetal heart pregnancy rate, live-birth rate, gestational age, and live-birth weight.
Result(s): The overall blastocyst thaw survival rate was 94.4% and was not significantly different between blastocyst grades or developmental stages. Similar clinical outcomes were achieved for fresh and vitrified-warmed blastocyst transfers; for example, grade I blastocysts had a live-birth rate of 52.8% versus 55.3%, respectively, and grade II blastocysts had a rate of 34.9% versus 30.4%, respectively. Significantly improved neonatal outcomes were evident for vitrified-warmed blastocyst transfers for gestational age, being on average 0.3 weeks longer, and for live-birth weight with babies born on average 145 g heavier (3,296 g versus 3,441 g for fresh and vitrified-warmed groups, respectively), as compared with fresh transfers.
Conclusion(s): Embryo transfer of vitrified-warmed blastocysts yields equivalent live-birth rates and improved neonatal outcomes compared with fresh transfers. (Fertil Steril® 2014;101:1294–301. ©2014 by American Society for Reproductive Medicine.)
Key Words: Assisted reproduction, blastocyst, human, single-embryo transfer, vitrification

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In human assisted reproductive technology (ART), the use of exogenous gonadotropins for in vitro fertilization (IVF) commonly results in the production of numerous embryos. Although the transfer of two or more of these embryos may increase the chance of pregnancy for a patient, it may also result in multiple pregnancies, with increased maternal and neonatal complications. Therefore, many ART clinics are now opting for a single-embryo transfer protocol that aims to limit the complications associated with multiple pregnancies (1, 2). However, to implement a successful single-embryo transfer protocol, an ART clinic must have in place an effective cryopreservation process to allow for the storage of supernumery embryos and thus maximize cumulative pregnancy rates. Additionally, cryopreservation programs are essential for patients at risk of ovarian hyperstimulation syndrome as well as to allow time for the testing of embryos for genetic anomalies before transfer.

It is imperative that the cryopreservation protocol employed by the ART clinic has minimal impact on the embryo being preserved. The last decade has seen a shift from conventional controlled-rate (slow) freezing toward

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vitrification, which typically uses a high concentration of cryoprotectants and rapid cooling rates to minimize ice crystal formation (3). This has resulted in improvements in clinical outcomes from frozen transfers, with some clinics reporting a modest increase (4) and others a doubling of pregnancy rates from vitrified embryos compared with slow-frozen embryos (5, 6). Furthermore, vitrification has improved blastocyst survival rates upon warming from approximately 60% with slow-freezing to above 90% with vitrification, thus significantly improving the cumulative pregnancy rate (7–9). There are several vitrification systems and protocols available on the market today (10–12) including the Cryotop (4), a room temperature vitrification method that has been used extensively and offers the highest cooling and warming rates, allowing for excellent embryo survival and recovery after cryopreservation.

To date, there have been a handful of studies comparing pregnancy outcomes from vitrified-warmed blastocysts with fresh transfers, the majority of which were comparably small and all of which performed multiple embryo transfers (13–16). The largest of these was by Takahashi et al. (14), who found pregnancy rates from 413 vitrified-warmed blastocysts were equivalent to those of fresh transfers. This included pregnancy and implantation rates of 44.1% and 29.0%, respectively, for vitrified-warmed embryos as compared with 44.4% and 23.4%, respectively, for fresh transfers. Furthermore, neonatal outcomes from vitrified-warmed births appear promising, with a recent Japanese study of 4,092 vitrified-warmed singleton births finding a modest but significant increase in birth weight: an average of 2,943 g for fresh transfers compared with 3,028 g for vitrified-warmed transfers (17). Further studies are now required to confirm whether transfer of vitrified-warmed embryos could in fact be beneficial for ART outcomes.

In this study, we report our findings on clinical pregnancy and neonatal outcomes of 1,157 fresh and 645 vitrified-warmed day-5 single-embryo blastocyst transfers. This includes analysis of the data separated by embryo grades as well as further subdivision to evaluate the effect of maternal age and blastocyst developmental stage.

MATERIALS AND METHODS

Study Design

A retrospective cohort analysis comparing clinical outcomes from transfer of fresh embryos versus embryos cryopreserved by vitrification was performed on ART procedures at Genea (formerly Sydney IVF; Sydney, Australia) between March 2010 and November 2011. Data for the fresh group were restricted to patients with three or fewer stimulation cycles who had a single-blastocyst (blastocyst, expanding blastocyst, or hatching blastocyst) transfer on day 5 and at least one excess blastocyst for cryopreservation. Data for the vitrified-warmed group were restricted to patients who had three or fewer stimulation cycles and a single-embryo transfer of a day-5 vitrified-warmed blastocyst (blastocyst, expanding blastocyst, or hatching blastocyst). The exception to this was the calculation of total thaw survival rates, which included all blastocysts warmed (no exclusion criteria). Preimplantation genetic diagnosis patients and donor-recipient and surrogacy cases were excluded from all analyses. Retrospective cohort studies using deidentified patient clinical data were approved by Genea's ethics committee, an independent institutional human research ethics committee registered with Australia's National Health and Medical Research Council.

Ovarian Stimulation and Oocyte Retrieval

Stimulation and oocyte retrievals were conducted as reported previously elsewhere (1, 18). Patients were stimulated with recombinant follicle-stimulating hormone (FSH) with either an agonist or antagonist protocol. The agonist treatments usually involved midluteal long down-regulation with leuprorelin acetate (Lucrin; Abbott Australia) or nafarelin acetate (Synarel; Pfizer), with ovarian stimulation from menstruation with follitropin-α (Gonal-F; Merck) or follitropin-β (Puregon; MSD). Antagonist cycles usually involved commencing recombinant FSH on day 2 or day 3 of the menstrual cycle, with either the gonadotropin-releasing hormone (GnRH) antagonist Cetrorelix (Cetrotide; Merck) or Ganirelix (Orgalutran; MSD) commenced on day 5 of stimulation. Final follicular maturation was induced with hCG (Pregnyl, 5,000 U, MSD; or Ovidryl, 250 mg, Merck) when the leading follicles exceeded 17 mm on transvaginal ultrasound. Oocyte retrieval was performed 36 to 37 hours later using a 17-gauge transvaginal follicle aspiration needle (Cook Medical).

In Vitro Fertilization and Intracytoplasmic Sperm Injection

Oocytes selected for IVF were coincubated for 2 hours with 150×10^3 density gradient separated spermatozoa approximately 39–41 hours after the hCG injection. The oocytes were then washed and placed into fresh fertilization medium (Cook Medical) for overnight culture. Successful fertilization was assessed the next morning, and zygotes were transferred to cleavage medium (Cook Medical).

Oocytes selected for intracytoplasmic sperm injection (ICSI) were exposed to Hyalase (80 IU; Sanofi-Aventis, Australia) in fertilization medium (Cook Medical) and then stripped of their cumulus cells using flame-pulled Pasteur pipettes. A single immobilized spermatozoon was injected into each mature oocyte, which was then cultured overnight in cleavage medium (Cook Medical) and assessed for fertilization.

Embryo Culture and Assessment

After fertilization, day-1 embryos were transferred into fresh cleavage media until day 3. The majority of embryos were further cultured to the blastocyst stage by transferring into blastocyst medium (Cook Medical) on the morning of day 3. All embryo cultures were performed under low-oxygen conditions (5% oxygen, 6% carbon dioxide, 89% nitrogen) provided in solid-state mini-incubators (MINCs; Cook Medical). Embryos were cultured using Nunclon four-well dishes (Nunc), each with up to five embryos in 10-μL drops under pre-equilibrated embryo-tested mineral oil (Cook Medical).