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# Blastocyst vitrification, cryostorage and warming does not affect live birth rate, infant birth weight or timing of delivery

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**KEY MESSAGE**

We found no evidence of an effect of blastocyst vitrification and cryostorage on the likelihood of implantation, clinical pregnancy, early pregnancy loss, live birth, low birth weight or preterm delivery.

A B S T R A C T

**Research question:** Does vitrification and warming affect live birth rate, infant birth weight and timing of delivery?

**Design:** Retrospective, cohort study comparing outcomes of donor oocyte recipient fresh ($n$ = 25) versus vitrified ($n$ = 86) euploid blastocyst transfers; donor oocyte recipient singleton live births from fresh ($n$ = 100) versus vitrified ($n$ = 102) single embryo transfers (SET); and autologous vitrified euploid SET ($n$ = 1760) (cryostored 21–1671 days).

**Results:** Group 1: fresh and vitrified–warmed blastocysts had similar live birth (OR 1.7; 95% CI 0.5 to 5.9), implantation (OR 0.9; 95% CI 0.2 to 3.9), clinical pregnancy (OR 3.4; 95% CI 0.9 to 13.0) and pregnancy loss (OR 1.2; 95% CI 0.98 to 1.4); group 2: low birth weight (OR 0.44; 95% CI 0.1 to 1.6) and preterm delivery (0.99; 95% CI 0.4 to 2.3) rates were similar in fresh and vitrified–warmed blastocyst transfers; group 3: cryostorage duration did not affect live birth (OR 1.0; 95% CI 1.0 to 1.0), implantation (OR 1.0; 95% CI 0.99 to 1.01), clinical pregnancy (OR 1.0; 95% CI 1.0 to 1.0]), pregnancy loss (OR 0.99; 95% CI 1.0 to 1.0), birth weight (β = −15.7) or gestational age at delivery (β = −0.996).

**Conclusions:** Vitrification and cryostorage (up to 4 years) are safe and effective practices that do not significantly affect clinical outcome after embryo transfer.



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## Introduction

Since the first live birth from a cryopreserved human embryo almost 40 years ago (Downing et al., 1985), embryo cryopreservation techniques have greatly improved in efficacy and efficiency. The past decade has seen a shift from slow-freezing to vitrification, which involves ultrarapid freezing and suspension of embryos in a glass-like state. Compared with slow-freezing, vitrification reduces the formation of ice crystals (Liebermann and Tucker, 2006; Son and Tan, 2009; Zegers-Hochschild et al., 2009) and results in increased embryo cryosurvival (Kolibianakis et al., 2009; Loutradi et al., 2008), clinical pregnancy (AbdelHafez et al., 2010; Stehlik et al., 2005; Wong and Wong, 2011), and live birth rates (Li et al., 2014). Embryo cryopreservation facilitates ovarian hyperstimulation syndrome prevention through freeze-all; elective fertility preservation for social and medical reasons; embryo transfer in a physiologic endometrial hormonal milieu; and preimplantation genetic testing (PGT) before transfer (Pandian et al., 2009; Wang et al., 2017). Compared with fresh embryo transfer, the more physiologic uterine environment of frozen embryo transfer (FET) might be more favourable for implantation and placentation (Amor et al., 2009; Healy et al., 2010; Kansal Kalra et al., 2011; Zeilmaker et al., 1984). The finding of improved pregnancy and live birth rates in FET cycles (Roque et al., 2013; Shapiro et al., 2011) was confirmed by a recent randomized controlled trial in which patients undergoing PGT were randomized to fresh embryo tranfser or freeze-only cycles followed by subsequent FET (Coates et al., 2017). Live births achieved using FET have been associated with a decreased incidence of low birth weight (Pelkonen et al., 2010; Schwarze et al., 2015) and preterm delivery (Wennerholm et al., 2013).

The rapid adoption of vitrification into IVF practice and the growing proportion of IVF live births arising from FET warrants the evaluation of the effect of this technology on peri-implantation, i.e. short-term, and perinatal, i.e. long-term, outcomes to ensure its safety and efficacy. During vitrification, embryos are exposed to cryoprotectants and, in open-vitrification systems, are directly in contact with liquid nitrogen (Bielanski et al., 2003; Gosden, 2011). One or more of these exposures might alter early embryo development and affect implantation and growth potential. Compared with naturally conceived pregnancies, FET has been reported to lead to an increased risk of large for gestational age or macrosomic (Pinborg et al., 2014; Sazonova et al., 2012) infants, suggesting that the cryopreservation process may influence placentation and fetal growth. Most studies of FET cycles to date have not been appropriately designed to isolate for the independent effects of embryo cryopreservation and warming on clinical outcome. Studies comparing pregnancies from FET and natural conception are confounded by laboratory handling and programmed hormonal preparation of endometria, whereas studies comparing pregnancies from FET and fresh embryo transfer are confounded by the effect of ovarian stimulation on endometrial receptivity in fresh transfers. Furthermore, prior studies have not accounted for embryo ploidy status, a key factor affecting implantation potential.

Donor oocyte IVF provides a unique model for evaluating whether vitrification and warming of embryos has an independent effect on embryo implantation and placentation. In donor cycles, the ovarian stimulation of the donors is separated from the endometrial environment of the recipients (Navot et al., 1991). Initial studies comparing fresh and frozen embryo transfers in donor oocyte recipients reported reduced pregnancy rates after FET (Check et al., 1995; Tatpati et al., 2010). These studies, however, involved the transfer of pronuclear and cleavage-stage embryos that underwent slow-freezing. Therefore, the results cannot be applied to the current treatment paradigm of blastocyst culture and vitrification. Recent studies using the donor oocyte IVF model have focused on perinatal outcome, reporting no effect of embryo vitrification on infant birth weight or gestational age at delivery (Galliano et al., 2015; Kalra et al., 2011). Interpretation of these results is limited as these studies pooled data from multiple IVF centres and included mixed cohorts of blastocyst and cleavage-stage embryo transfers.

Few studies have explored a possible effect of cryostorage duration on embryo viability, implantation potential and perinatal outcome. Although cryopreservation is thought to halt metabolism and ageing, it is reasonable to question the stability of vitrified embryos over time. Vitrification involves rapid solidification of fluid into a glassy, disorganized, unstable state. As the temperature decreases below the threshold for glass transition, the disordered mole cular pattern of a liquid is maintained despite the physical transition to a solid (Wowk, 2010). Within this state, cooling by only 10°C can induce an increase in viscosity by a factor of 1000 (Wowk, 2010). Therefore, the molecular structure of vitrified cells may be sensitive to storage temperature variations and affected by the duration of cryostorage in liquid nitrogen (Wirleitner et al., 2013). Most studies have failed to demonstrate a time-related effect of cryostorage on pregnancy and live birth rates (Aflatoonian et al., 2013; Riggs et al., 2010; Wirleitner et al., 2013); however, few have evaluated the effect of cryostorage duration on perinatal outcome.

Given the widespread clinical use of vitrification, a robust, continued evaluation of this technology is necessary to confirm whether blastocyst vitrification has independent effects on embryo–jendometrial interaction and implantation, and whether this translates to any downstream effects influencing perinatal outcome. This study provides a comprehensive assessment of whether blastocyst vitrification, storage and warming affect reproductive and perinatal outcome after vitrified–warmed embryo transfer. To assess the effect of vitrification on embryonic implantation potential, donor oocyte recipients that underwent transfer of single, euploid fresh and vitrified–warmed blastocyst were evaluated. To assess the effect of blastocyst vitrification on birth weight and gestational age at delivery, donor oocyte recipients that achieved a singleton live birth after fresh and vitrified–warmed SET were compared. Finally, to assess the effect of cryostorage duration on IVF and perinatal outcome, we evaluated a cohort of patients with euploid blastocysts derived from autologous oocytes, who underwent PGT, vitrification and warming before SET.

## Materials and methods

### *Study design and patient population*

A single-centre, retrospective, cohort analysis of three distinct patient groups was conducted, analysing blastocyst transfers carried out between 2011 and 2016. All embryo transfers involving blastocysts derived from previously cryopreserved oocytes were excluded. Patients were identified from an electronic medical records database.

#### *Group 1: donor oocyte recipients undergoing transfer of fresh versus vitrified–warmed, PGT-screened blastocysts*

To evaluate the effect of blastocyst vitrification on IVF and embryo transfer cycle outcome, donor oocyte recipients who underwent a fresh



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