ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Impact of multiple blastocyst biopsy and vitrification-warming procedures on pregnancy outcomes

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Objective: To assess the impact of multiple blastocyst biopsy and vitrification-warming procedures on clinical outcomes.
Design: Retrospective study.
Setting: Private fertility clinic.
Patient(s): Preimplantation genetic diagnosis (PGD) patients undergoing comprehensive chromosome screening, including monogenic disorder and chromosome rearrangement cases.
Intervention(s): Warming and transfer of euploid blastocysts biopsied and vitrified-warmed once (group 1 [G1, control]; n = 2,130), biopsied once but vitrified-warmed twice (group 2 [G2]; n = 34), or biopsied and vitrified-warmed twice (group 3 [G3]; n = 29).
Main Outcome Measure(s): Thaw (for transfer) survival rate and clinical pregnancy rate (CPR).
Result(s): The thaw survival rates were 98.4% for G1, 97.3% for G2, and 93.3% for G3, with once biopsied and vitrified-warmed embryos being significantly higher than twice biopsied and vitrified-warmed embryos (G1 vs. G3; P=.032). There was a slight reduction in CPR with an additional vitrification-warming (G1 54.3% vs. G2 47.1%) and larger reduction with an additional embryo biopsy (G2 47.1% vs. G3 31.0%), but neither difference was statistically significant. However, the combined effect of both additional biopsy and vitrification-warming resulted in a significantly reduced CPR (G1 54.3% vs. G3 31.0%; P=.013).
Conclusion(s): This study indicates that blastocysts biopsied and vitrified-warmed twice have reduced clinical outcomes compared with blastocysts biopsied and vitrified-warmed once. PGD patients should be advised that performing a second biopsy and vitrification-warming in cases of failure to obtain a result from initial biopsy will reduce the chance of pregnancy. Patients with inherited disorders may elect to proceed with the second biopsy and vitrification to avoid transfer of embryos with the genetic condition. (Fertil Steril® 2017;■:■–■. ©2017 by American Society for Reproductive Medicine.)
Key Words: Blastocyst, preimplantation genetic screening, trophectoderm biopsy, vitrification

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The successful cryopreservation of excess embryos is an important component of assisted conception programs, with vitrification widely recognized as the criterion standard method (1, 2). There are many benefits of embryo cryopreservation, including the adoption of a single-embryo transfer policy to reduce the risk of multiple pregnancies and maximize the cumulative pregnancy rate (3, 4). Evidence also suggests that vitrified-warmed embryo transfers have equivalent or higher pregnancy rates and improved neonatal outcomes compared with fresh embryo transfers (5–8), which is hypothesized to be due to avoidance of deleterious effects from hormone stimulation on endometrial preparation and receptivity. In addition, embryo cryopreservation is critical to preimplantation genetic diagnosis (PGD) programs, allowing time for genetic testing of embryo biopsies for chromosomal content and inherited genetic disorders.

Embryo vitrification is performed with the use of high concentrations of cryoprotectants and ultrarapid cooling to avoid detrimental ice crystal formation (9). Although many studies have indicated vitrification to be a safe and efficient practice (5–8), it is unclear if multiple vitrification-warmings are detrimental to assisted conception outcomes. One reason this question has arisen is due to the request for chromosome screening on already cryopreserved embryos by patients hoping to improve their chance of pregnancy or reduce the risk of miscarriage from a given embryo transfer. Taylor et al. (10) attempted to address this question in their twice cryopreserved-warmed preimplantation genetic screening (PGS) population consisting of blastocysts both

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initially slow-frozen (n = 7) and vitrified (n = 12). They reported a lower embryo survival rate after warming compared with control samples, 87.5% vs. 98.3%, with the three twice cryopreserved-warmed embryos that failed to survive all being initially slow-frozen. However, pregnancy outcomes from their 14 euploid twice cryopreserved-warmed blastocyst transfers were similar to those of the control group.

Another reason for twice vitrifying-warming embryos is failure to achieve a result from the initial biopsy specimen, thus also requiring a second trophectoderm biopsy. This is particularly critical for inherited single-gene disorder and chromosomal rearrangement cases, and is increasingly being considered by PGS patients hesitant to transfer an embryo with unknown chromosomal status. However, there is concern that removal of too many trophectoderm cells could be detrimental to an embryo's pregnancy potential (11), and very little information is available on outcomes of twice-biopsied blastocysts. Zhang et al. (12) reported ten single euploid embryo transfers with twice biopsied and vitrified-warmed blastocysts, which resulted in five live births, although no control groups were presented. Likewise, Minasi and Greco (13) noted as unpublished data that they performed eight single-embryo transfers with the use of blastocysts biopsied twice but cryopreserved once, resulting in four live births. Further studies are required to determine if an additional blastocyst trophectoderm biopsy, as well as an additional vitrification-warming, is detrimental to pregnancy and neonatal outcomes.

In this retrospective analysis we analyzed the impact of multiple blastocyst biopsy and vitrification-warming procedures on clinical outcomes.

MATERIALS AND METHODS

Study Design

This retrospective study analyzed the impact of multiple blastocyst biopsy and vitrification-warming procedures on clinical outcomes at the Australian private fertility clinic Genea (previously Sydney IVF; Sydney, Liverpool, Northwest, and Canberra clinics). The first part of this study examined biopsy outcomes of vitrified embryos warmed with the intention of biopsy for PGD from January 28, 2013, to September 12, 2016. Data were separated into blastocysts that were vitrified without biopsy and blastocysts that were biopsied and vitrified but failed to produce a PGD result. All PGD cases were included regardless of reason for PGD or intended screening method. The second part of the study examined the outcomes of single or double trophectoderm biopsy and once or twice vitrified euploid blastocysts that were warmed with the intention of uterine transfer from May 29, 2014 (first transfer), to May 15, 2017. Data included PGD cycles undergoing PGS with the use of next-generation sequencing (NGS) or comparative genomic hybridization (CGH), with or without testing for single-gene disorders or chromosome rearrangements. The data were separated into three groups: blastocysts biopsied and vitrified-warmed once (group 1), blastocysts biopsied once but vitrified-warmed twice (group 2), and blastocysts both biopsied and vitrified-warmed twice (group 3). In both parts of the study, embryos were first vitrified as blastocysts (including very early blastocysts) on day 5 or 6, with or without trophectoderm biopsy, with a minority of the nonbiopsied blastocysts being imported from external clinics. Ethical approval for retrospective cohort studies with the use of deidentified patient clinical data was granted by Genea's Human Research Ethics Committee in December 2012.

General Assisted Reproduction Procedures

Ovarian stimulation, oocyte collection, and fertilization with sperm were performed as described previously (6, 14). Embryo culture to the blastocyst stage was performed in groups of up to five embryos as described previously (6) with either in-house manufactured sequential media identical in formulation to Sydney IVF embryo culture medium suite (Cook Medical) or Gems sequential embryo culture media (Genea Biomedx). A minority of embryos were cultured in the Geri time-lapse incubator with the use of Gems one-step media (Genea Biomedx) as described by the manufacturer. Note that these details may not be applicable to blastocysts imported from other clinics. Blastocysts were scored on the morning of day 5 of development onward and just before vitrification according to a simplified Gardner blastocyst grading system, whereby grade 1, grade 2, and grade 3 were considered to be an excellent, good, and poor-quality embryo, respectively (15). Blastocysts confirmed as euploid were warmed and transferred as described previously (6).

Embryo Hatching and Blastocyst Biopsy

Assisted hatching was performed on PGD-designated day-3 cleavage-stage embryos with the use of a Zilos TK Laser (Hamilton Thorne Biosciences) to create a 10-μm opening in the zona pellucida, with the exception of embryos cultured in the Geri time-lapse incubator which had assisted hatching on day 4. On day 5, blastocysts were assessed for the presence of trophectoderm herniating from the zona breach location. Blastocysts with suitable trophectoderm herniation underwent biopsy as described previously (16), whereas embryos not yet suitable for biopsy were reassessed after an additional 6–24 hours of culture. Embryo biopsies were immediately stored at −20°C for preservation of DNA for genetic analyses. After trophectoderm biopsy blastocysts were allowed to recover in culture for a minimum of 1 hour and then cryopreserved by means of vitrification.

For cryopreserved nonbiopsied blastocysts designated for PGD, embryos were mostly warmed in the late afternoon and hatched (if not already done previously), then cultured overnight and assessed for biopsy early the next morning. The exception to this was embryos warmed, biopsied, and revitrified on the same day. Similarly, cryopreserved biopsied blastocysts requiring a second biopsy because of unsuccessful genetic analysis were mostly warmed in the late afternoon, cultured overnight, and assessed for biopsy early the next morning. The exception to this was fully hatched blastocysts, which were warmed in the morning just before assessment for rebiopsy. Note that there was one warmed day-6 blastocyst that required culture for 2 nights before being suitable for biopsy.