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ARTICLE

Outcomes of blastocysts biopsied and vitrified once versus those cryopreserved twice for euploid blastocyst transfer

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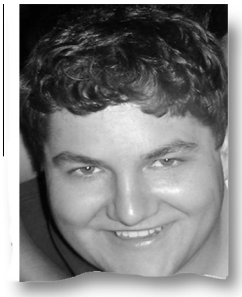

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Abstract Trophectoderm biopsy with comprehensive chromosome screening (CCS) has been shown to increase implantation and pregnancy rates. Some patients desire CCS on previously cryopreserved blastocysts, resulting in blastocysts that are thawed/warmed, biopsied, vitrified and then warmed again. The effect of two cryopreservation procedures and two thawing/warming procedures on outcomes has not been effectively studied. Cycles were divided into two groups: group 1 patients underwent a cryopreserved embryo transfer with euploid blastocysts that were vitrified and warmed once; group 2 patients had a cryopreserved embryo transfer of a euploid blastocyst that was cryopreserved, thawed/warmed, biopsied, vitrified and warmed. Groups 1 and 2 included 85 and 17 women aged 35.6 ± 3.9 and 35.3 ± 4.9 years, respectively (not significantly different). Blastocyst survival in group 1 (114/116, 98.3%) and survival of second warming in group 2 (21/24, 87.5%) was significantly different ($P = 0.0354$). There was no difference between biochemical (68.2% and 62.5%) and clinical (61.2% and 56.3%) pregnancy rates, implantation rate (58.4% and 52.4%) and live birth/ongoing pregnancy rate (54.0% and 47.6%) between groups 1 and 2, respectively. Although it is unconventional to thaw/warm, biopsy, revitrify and rewarm blastocysts for cryopreserved embryo transfer, the results indicate that outcomes are not compromised.

KEYWORDS: aneuploidy, blastocyst, biopsy, IVF, vitrification, warming

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Introduction

Typically, only good-quality blastocysts derived from a fresh cycle of IVF are utilized in trophectoderm biopsy with comprehensive chromosome screening (CCS). This approach has yielded pregnancy outcomes higher than standard morphological assessment alone (Scott et al., 2013a). Clinical pregnancy rates utilizing this technology are 60–75%, which is comparable to anonymous oocyte donation (Grifo et al., 2013). However promising, the utilization of this technology is typically limited to blastocysts derived from fresh IVF cycles.

In order to benefit from trophectoderm biopsy and CCS, a patient has to undergo a fresh IVF procedure or have zygote- or cleavage-stage embryos previously cryopreserved thawed and cultured to the blastocyst stage. There are a large number of patients who have had IVF previously and have good-quality, unbiopsied blastocysts cryopreserved (Zhu et al., 2013). These patients could simply want to utilize the current technology or they have previous outcomes that may warrant utilization of CCS with trophectoderm biopsy. For example, if a patient suffered a miscarriage or had failed attempts with fresh embryos, they may choose to utilize trophectoderm biopsy and CCS on previously cryopreserved blastocysts to allow for the transfer of a euploid embryo.

Blastocyst biopsy involves the removal of 3–10 cells from the trophectoderm of blastocysts on either day 5 or 6 of culture (Scott et al., 2013b). If a blastocyst is biopsied on day 5, it is possible to get results by day 6 for a fresh transfer. Most clinics do not conduct on-site CCS; therefore, a majority of the time, blastocysts are vitrified post biopsy. Current research indicates that the transfer of an embryo into an unstimulated uterus may yield higher pregnancy outcomes than a transfer during a fresh cycle (Shapiro et al., 2011).

After biopsy, the sample is sent to the genetics laboratory while the blastocysts remain cryopreserved awaiting results. Even in the hands of the most experienced embryologist and geneticists, readings are not possible 100% of the time (Harton et al., 2011). Therefore, it is possible that the CCS report would reveal a 'no result'. In this particular instance, the patients are left with a cryopreserved blastocyst that has no genetic result.

Although biopsy, obtaining CCS results and transfer without the need to vitrify can be achieved, particularly with methods such as 4-h quantitative real-time PCR, this approach cannot be utilized by every IVF clinic due to logistics (Treff and Scott, 2013). Because of this, blastocysts with a 'no result', as well as blastocysts that have been previously cryopreserved without undergoing trophectoderm biopsy during the fresh cycle, would need to be thawed/warmed for biopsy or rebiopsy and subsequently cryopreserved again while awaiting CCS results. Furthermore, if euploid, these blastocysts would undergo an additional warming procedure before being transferred into the uterus. Few studies have focused on patients that have previously cryopreserved blastocysts, which undergo thawing/warming, biopsy, vitrifying and a second warming prior to a cryopreserved embryo transfer. The purpose of this study was to test the hypothesis that blastocysts that were previously cryopreserved can be successfully utilized for subsequent trophectoderm biopsy and CCS and to determine the clinical efficiency of those blastocysts when used in a subsequent cryopreserved embryo transfer cycle.

Materials and methods

This retrospective chart review was deemed exempt by Sterling Institutional Review Board. Patients attending Reproductive Endocrinology Associates of Charlotte from 1 January 2009 to 31 April 2013 were included in this study. Cycles were subdivided into two groups (**Figure 1**). Group 1 ($n = 85$ cycles, 116 blastocysts) consisted of patients who underwent the traditional method of trophectoderm biopsy and CCS, by having their oocytes retrieved via IVF, embryos cultured to the blastocyst stage and all viable blastocysts biopsied and vitrified according to laboratory protocol. Group 2 consisted of cycles that had cryopreserved blastocysts and subsequently desired to have their blastocysts biopsied ($n = 19$ cycles, 70 blastocysts) or those who desired a rebiopsy due to a 'no result' ($n = 2$ cycles, three blastocysts). Outcomes consisted of biochemical pregnancy (positive β-human chorionic gonadotrophin test), clinical pregnancy (visualization of gestational sac on ultrasound), fetal cardiac activity and ongoing/live birth rate.

In-vitro fertilization and embryo culture

Oocytes were retrieved under ultrasound guidance and placed in HEPES-buffered solution (Cooper Surgical, Trumbull, CT, USA) and 10% serum protein substitute (Cooper Surgical) overlaid with oil (Irvine Scientific, Santa Ana, CA, USA). All oocytes were designated for intracytoplasmic sperm injection and trimmed and stripped of excess cumulus cells, as described by Taylor et al. (2006). Oocytes were separated based on maturity and placed back into the incubator. After 2 h, all mature oocytes underwent intracytoplasmic sperm injection (Nagy et al., 1995).

Because this study occurred over a long period of time, two different culture systems were utilized. From January 2009 to August 2012, sequential media (Cooper Surgical) and 10% serum protein substitute overlaid with oil was used. From September 2012 to April 2013, continuous sequential culture media (Irvine Scientific) and 10% serum substitute supplement (Irvine Scientific) overlaid with oil was utilized. Regardless of culture system, all oocytes and embryos were cultured in 95% N_2, 5% CO_2 and 98% humidity.

Trophectoderm biopsy and array comparative genomic hybridization

With the aid of a laser (Zilos-tk; Hamilton Thorne, Beverly, ME, USA), all embryos from group 1 underwent assisted hatching on day 3. Group 2 included blastocysts previously cryopreserved, these were thawed/warmed and assisted hatching was performed during the thawing/warming procedure while the blastocyst was compacted. Only blastocysts that presented with a good-quality inner cell mass and trophectoderm were biopsied. Blastocysts were placed in a drop of modified human tubal fluid (Irvine Scientific) and 10% serum substitute supplement. Gentle suction was