Patients of advanced maternal age should only transfer a single euploid blastocyst

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Abstract

Background: The IVF industry has been trying to reduce high order multiple pregnancies by promoting single embryo transfer for nearly two decades. Although improvements in embryo culture practices concurrently occurred, poor prognosis patients and those of advanced maternal age (≥38 years old) proved to be challenging cases when determining the number of embryos to transfer and yet still optimize pregnancy success. It was not until preimplantation genetic screening (PGS) of blastocysts was coupled with conservative embryo transfer decisions that worldwide progress occurred. The objective of this study was to determine the efficacy of single embryo transfer (SET) compared to dual embryo transfer (DET) in older patients (age ≥38) performing vitrified-warmed, euploid ET cycles.

Methods and findings: Retrospective cohort analysis was performed on 140 vitrified-warmed euploid blastocyst transfers of patients ≥38 years old performing either a SET (n=122) or DET (n=18). All full to hatched blastocysts were initially biopsied on Days 5, 6 or 7, and the trophectoderm samples were analyzed using NGS or aCGH. All transfers represented the patients first transfer attempt following PGS between January 2013 to June 2015. Implantation and live birth results per ET treatment were evaluated and compared using Chi-squared analysis (p<0.05). The average patient age was 39.7 years old, achieving a clinical pregnancy rate of 83% (116/140) and a live birth rate of 80% (112/140). SET achieved a live birth rate of 79.5% (97/122) similar to DET (15/18, 83.3%). Although pregnancy outcome comparisons were not different between age groups or treatments, a trend (p<0.10) toward higher implantation for SET was observed. Most significantly, the twinning rate was appreciably higher (p<0.001) with DET at 73% (11/15) compared to 1% for SET (1/97).

Conclusions: Independent of age, when using euploid blastocysts, we believe that SET should be adopted as the standard of care for clinics utilizing PGS. This is especially true for the first ET attempt by patients of advanced maternal age to optimize implantation rates and reduce the potential wastage of precious euploid embryos.

Introduction

Today’s human assisted reproductive technology (ART) industry strives to increase live birth rates, while decreasing the occurrence of multiple gestations. In turn, the current clinical focus is on how to best offer couples the option of transferring only one embryo to decrease the chance of a multiple pregnancy. High order multiple pregnancies, including twins, are associated with increased pregnancy complications and numerous perinatal risks [1,2], especially in women of advanced maternal age (≥38 years old). Numerous studies have advocated for elective single embryo transfer (eSET) given a 90-95% reduction in multiple gestations [3-5]. While some programs experienced a 20 to 40% reduction in live birth rates with eSET [3], other studies using improved embryo culturing practices showed no effect to a conservative ET approach [6,7]. As such, much of the literature argues in favor of SET only in optimal population groups [8], such as those patients age <35 years old and with >2 good quality blastocysts for ET [9]. Fujimoto and coworkers [7] recently reported that eSET actually improved live birth rates in patients age ≤37 years old, but not in older patients. It has also been reported that vitrification-all ET cycles can improve pregnancy outcomes by transferring embryos into a more progesterone synchronized, receptive uterine environment [10].

Considering the current move toward SET, importance has been placed on embryo selection. Subjective morphology grading has been the commonly accepted standard to judge embryo quality [11], but our ability to select embryos with high success of implantation has been limited by solely judging morphology [12]. Other recent developments using time-lapsed imaging (TLI) have shown promise for improving the selection process [13]. Yet the latter methods, morphologic grading and TLI, are not highly predictive of genetic normalcy once a blastocyst is produced. The application of preimplantation genetic screening (PGS), incorporating genomic array technologies, to confirm embryo euploidy status prior to transfer has proven to be a highly effective approach for embryo selection [14,15]. The clinical application of blastocyst biopsy/PGS practices has led to decreases in spontaneous abortion and multiple gestations, and overall improvements in live birth rates [5,14-16]. In fact, it has now been shown that the quality grading of blastocysts (>fair to good quality; 3BB or better) does not correlate to euploidy predictability [17].

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Materials and methods

Embryo culture, grading, biopsy, and PGS

Using MCO-5M mini Sanyo/Panasonic tri-gas incubators (5 % 02/5.3–6.0 % CO2) under humidified air conditions (37°C), we group cultured up to five embryos per 25 µL droplet of Global™ medium (LG; Life Global, Guilford, CT) supplemented with 7.5 % synthetic protein supplement under Ovoil™ (Vitrolife, Englewood, CO) until blastocyst biopsy [17,18]. All oocytes retrieved were evaluated for maturity and had ICSI performed 2–6 h post-egg retrieval. Embryos were initially evaluated on Day 3; laser zona dissection was performed using a 1480-nm diode laser (Zilos-tk™; Hamilton Thorne, Beverly, MA), and embryo incubation continued until Days 5, 6 and possibly Day 7 for evaluation and biopsy determination [5,18]. The zona opening created on Day 3 allowed trophectoderm (TE) to prematurely rupture through a 10–12-μm furrow in the zona. Blastocysts were graded at biopsy using a modified Gardner scale [11]. The modification was necessary to account for premature hatching: grade 3 = <10 % TE extrusion (full blastocyst), grade 4 = 10 %–50 % TE extrusion (expanded blastocyst), and grade 5 = 50 % TE extrusion (hatching blastocyst)[5,18]. Inner cell mass (ICM) and the TE were independently graded from top quality “A” to fair quality “B” and poor quality “C” with the first letter in the grade assigned to the ICM and the second to the TE. A grade of 3BB or better was required to initiate biopsy. All residual developing embryos were allowed to continue in vitro culture to Day 7 for possible late biopsy consideration. The diode laser was again used on Day 5/6/7 for biopsying, combining laser pulse ablation and mechanical aspiration to separate 3–10 TE cells [5]. All TE samples were aseptically tubed, frozen and shipped to Genesis Genetics (Plymouth, MI) or Ovation Fertility Genetics (Henderson, NV) for array CGH or NGS analysis.

Vitrification and embryo transfer

Fair to excellent quality blastocysts (≥3BB grade) were vitrified on Days 5, 6 or 7 using microSecure-VTF in glycerol based, non-DMSO vitrification (VTF) solutions (Innovative Cryo Enterprises, Linden, NJ; 19). Aseptic microSecure VTF was performed using a 3-step dilution (5 min/5 min/1 min); individual blastocysts were loaded into 300 µm ID flexipettes (Cook Medical, Spencer, IL; 3 µl volume); flexipettes were then dried and inserted tip first into prelabeled 0.3 ml CMS™ embryo straws; the straws were blue sealed and plunged directly into LN2 [19,20]. Rapid warming was achieved by direct placement of the vitrified flexipettes into a warm (37°C) 0.5 M sucrose bath [21]. Within 10 seconds, each blastocyst was pipette directly from the flexipette into an open 200 µL droplet of 1.0 M sucrose solution and then transferred into 100 µL droplets under oil for 3 min intervals. Embryos were serially diluted in declining sucrose solutions (T1–T4), before isotonic equilibration in Hepes-LG medium. Warmed blastocysts were then cultured in LG medium + protein for 1–3 h prior to vitrified ET (VFET).

All VFET’s involved hormone replacement cycles using oral estradiol, estradiol patches, or intramuscular (IM) estradiol valerate followed by IM progesterone in oil. Progesterone in oil was started when endometrial thickness was >8 mm after documentation of serum progesterone level of <1.0 ng/ml. VFET was performed after 5.5 days of IM progesterone administration. Transvaginal ultrasound guidance ET procedures were performed. Pregnancies were initially tested 10 days post-ET and implantation subsequently assessed by transvaginal ultrasound beginning 4 weeks later. Live births were confirmed by written or oral communication with patients.
Fundamentally, when embryos are screened genetically normal, their implantation potential outperforms the need to transfer more than one embryo. This data validates our decision to adopt SET as standard clinical practice for all first ET attempts using euploid blastocysts. PGS-ET cycles have been previously shown to increase implantation and live birth rates (50.9% and 45.5%, respectively) in women 40-43 years old [22], granted at a lower level than observed in our laboratory. Interestingly, a trend was noted favoring increased implantation rates in the SET group compared to DET. This may hint toward a possible adverse competition between embryos during implantation, however we have previously documented in vitro that blastocyst amalgamation can occur [18]. The latter event suggests that trophectodermal cells are highly compatible with other blastocysts and are indeed programmed toward cellular invasion by Day 6, even with one another on occasion (0.015%). Overall, our retrospective analysis was limited by a small DET sample size, but with a larger patient population we believe that these implantation rates differences would convey significance.

It is our clinical practice to promote blastocyst biopsy/PGS cycles for patients of advanced maternal age, knowing that the rate of embryo aneuploidy is progressively higher in this population [23]. Our experience is similar to other recent reports with euploidy rates ranging from 25.2-35.3% at 38-40 years old, 15.9-20.5% at 41-42 years old and 17-23.1% at >43 years old (Ovation Fertility, unpublished data 2017). Consequently, 24%, 42% and 81% of these patients, respectfully, will fail to produce euploid blastocysts for a subsequent VFET cycle. Yet, our approach provides optimism that we are offering patients an informative, direct, more emotionally balanced path to pregnancy success. Undoubtedly, PGS does add cost to the cycle, but one must not discount the enormous cost of emotional distress and trauma endured by women experiencing repeated failures and fetal loss transferring morphologically good quality, aneuploid embryos. Furthermore, fetal losses can potentially complicate the patients’ future fertility, as well as delay their ability to become pregnant again. Time is a precious commodity in the older age population evaluated in this study. We feel the ability to diagnose potential cycle failures and drastically reduce the rate of pregnancy losses in women ≥38 years old (i.e., SAB; 25-75% in untested ET cycles), strongly justifies blastocyst biopsy/PGS intervention. Factoring in the limited pregnancy loss and increased healthy singleton live births associated with euploid SET, we are now able to offer a more enjoyable, safer and positive IVF experience for the majority of our patients.

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