Reproductive BioMedicine Online (2014) **28**, 485–491

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ARTICLE

Blastocyst culture selects for euploid embryos: comparison of blastomere and trophectoderm biopsies

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Abstract Preimplantation genetic diagnosis and screening improves the chances of achieving a viable pregnancy, not only free of undesired single-gene defects but also aneuploidy. In addition, improvements in vitrification provide an efficient means of preserving embryos (blastocysts). By combining trophectoderm biopsy with recent improvements in vitrification methods, only those embryos that have proved themselves viable and potentially more competent are tested. Using array comparative genomic hybridization (aCGH) to assess all 24 chromosomes, aneuploidy rates were compared between day-3 blastomere biopsy and day-5 trophectoderm biopsy. Of those 1603 embryos, 31% were euploid, 62% were aneuploid and 7% not analysable. A significantly larger proportion of embryos were euploid on day-5 biopsy (42%) compared with day-3 biopsy (24%, $P < 0.0001$). The number of euploid embryos per patient was not significantly different. Combining extended culture, trophectoderm biopsy and aneuploidy assessment by aCGH and subsequent vitrification can provide a more efficient means of achieving euploid pregnancies in IVF.
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KEYWORDS: aneuploidy, array comparative genomic hybridization, blastomere biopsy, preimplantation genetic screening, trophectoderm biopsy, whole-genome amplification

Introduction

A limiting issue in IVF is the age-dependent high incidence of aneuploidy with an abnormal number of chromosomes in the resulting embryos/fetuses. There are aneuploidies that are paternal in origin or are post meiotic, occurring after fertilization but the majority has been shown originating from meiotic nondisjunction in the oocyte (Handyside, 2012).

http://dx.doi.org/10.1016/j.rbmo.2013.11.018
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This has been shown to increase with advancing maternal age (Munne, 2003; Munne et al., 1994). These untested, abnormal embryos, whether transferred or conceived via intercourse, do not implant, lead to miscarriage (Hodes-Wertz et al., 2012) or result in a fetus with an abnormal chromosome compliment often leading to termination.

In order to screen embryos for euploid status, traditionally day-3 cleavage-stage blastomere biopsy is performed with chromosome analysis of the most common aneuploidies diagnosed by fluorescence in-situ hybridization (FISH). The cells' DNA can also be amplified using PCR to test for known single-gene disorders and can provide results in time for a day-5 embryo transfer (Gutierrez-Mateo et al., 2009). Higher pregnancy rates are obtained in some patient groups using day-3 blastomere biopsy with fresh transfer on day 4 or 5 (Munne et al., 2003).

With the advancement in the detection of the full chromosome complement available with array comparative genomic hybridization (aCGH), embryos can be more fully screened to avoid transferring embryos that do not contain a normal karyotype (Hellani et al., 2008). In addition, with improvement in media as well as culture conditions, blastocyst formation can reach an average of 40–60% (Gardner and Lane, 2003). Blastocyst culture has been shown to improve pregnancy and implantation rates by allowing selection to occur in the Petri dish. The extended culture gives an embryo time to better express its potential and has become an invaluable tool for embryo selection. Observing development in a cohort of embryos and basing selection of embryos for transfer on blastocyst morphology alone gives better implantation and birth rates (Grifo et al., 2007). However, there are some embryos that arrest at the cleavage stage: they simply stop growing. These embryos prove that they have no further potential, and most have degraded DNA and/or are aneuploid (Munne, 2006).

This work hoped to improve success by combining two selection tools: selecting euploid embryos (genetic selection) that had achieved the blastocyst stage (developmental competence selection). This was accomplished by performing trophectoderm biopsy and 24-chromosome preimplantation genetic screening using aCGH onlyon embryos reaching the blastocyst stage (Forman et al., 2012; Schoolcraft et al., 2010). We compared the euploidy rates of day-3 blastomere biopsy to trophectoderm biopsy at the blastocyst stage to determine if extended embryo culture also selects for euploid status and then compared pregnancy and implantation rates between the two different days of biopsy.

Materials and methods

Patients

Patients presented to New York University Fertility Center with interest in preimplantation genetic screening since it had been performed here for years prior to this study. The use of aCGH as the method for genetic screening began in 2010 as the centre was convinced that screening all chromosomes was an improvement over screening a limited number of chromosomes using FISH probes. Recruiting patients for a new unproven technique (trophectoderm biopsy) was difficult. Laboratory personnel and physicians counselled patients regarding the possible benefits of performing biopsies only on blastocysts. Recruiting patients to participate in this study with cryopreservation and no fresh transfer as described below was a challenge. Each case was evaluated and options discussed with patients. Some patients were interested in the variation in treatment options, while others were more reticent. Others became aware of the new procedure and requested trophectoderm biopsy. Patients were enlisted for the study between 1 January 2010 and 1 September 2012. All patients desiring to undergo blastomere biopsy with preimplantation genetic screening for aneuploidy determination with or without gender selection were considered for the study. Patients with preimplantation genetic diagnosis for single-gene disorders or using donor oocytes were excluded from this study. Patients who presented prior to onset of trophectoderm biopsy were provided only the day-3 biopsy option.

Several patients undergoing trophectoderm biopsy had suffered repeated miscarriages and were diagnosed with Asherman's syndrome or other uterine issues and subsequently successfully used gestational carriers to carry their euploid embryos to term. These patients were excluded from the study.

Ovarian stimulation

All underwent ovarian stimulation involving: (i) luteal-phase down-regulation with a gonadtrophin-releasing hormone (GnRH) analogue; (ii) follicular-phase flare using GnRH analogue; and (iii) antagonist-controlled stimulations in which GnRH antagonist was administered when lead follicles achieved diameters of roughly 12 mm. All cycles included daily injections of gonadotrophins, either recombinant FSH (Gonal F, EMD Serono, Rockland, MA, USA; or Follistim, Merck, Whitehouse Station, NJ, USA) or a combination of recombinant FSH and human menopausal gonadotrophin (Menopur, Merck). Injection of human chorionic gonadotrophin (HCG; 10,000 IU urinary HCG or two vials of recombinant HCG (Ovidrel; EMD Serono) or leuprolide acetate (Lupron, Tap Pharmaceuticals, Lake Forrest, IL, USA) plus 1000 IU urinary HCG was used to trigger the final maturation, when at least two follicles had reached 18 mm, although individualized criteria are often used when a patient has had prior cycles. The oocyte retrieval was scheduled 36 h after the trigger.

Day-3 biopsy and genetic screening

Cleavage-stage blastomere biopsy was performed on day-3 embryos that had at least 4 cells. A hole was created in the zona pellucida using acidified Tyrode's solution (Sigma, St Louis, MO, USA). Calcium/magnesium-free medium (Global, Guilford, CT, USA) was used to loosen the cell–cell adhesion allowing for less traumatic suction removal of the cell from the embryo. The biopsied cell was then loaded into a PCR tube (Eppendorf) labelled with the corresponding embryo number and sent to the reference laboratory (Reprogenetics, Livingston, NJ, USA) on dry ice for analysis using whole-genome amplification. Embryos were then placed back in culture until aCGH results were obtained in time for a day-5 embryo transfer. The best-quality euploid

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