Reproductive BioMedicine Online (2016) 33, 398–403

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ARTICLE

Does slow embryo development predict a high aneuploidy rate on trophectoderm biopsy?

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Abstract The aneuploidy rates in expanded blastocysts biopsied on days 5 and 6 development were assessed in women undergoing IVF followed by array comparative genomic hybridization. This study included 1171 expanded blastocysts from 465 patients. Among the 465 patients, 215 and 141 underwent embryo biopsy on day 5 and day 6 (46.2% and 30.3%, respectively), and 109 underwent biopsy on both days 5 and 6 (23.4%). The cycles of 206 women were cancelled because only aneuploidy embryos were present (44.3%). The aneuploid embryos were classified according to the type as single, double or complex aneuploidy. No differences were observed in the distributions of these three categories according to the day of the biopsy. The aneuploidy rate was also evaluated according to maternal age, and was found to be higher in older patients; however, no differences in this rate were detected between embryos biopsied on days 5 and 6 according to maternal age. Biopsy was carried out when blastocysts reached the expanded stage. The embryos biopsied on day 6 had a higher rate of aneuploidy (69.9%) than those biopsied on day 5 (61.4%); however, the euploid embryos transferred had similar chances for successful and healthy gestation.

KEYWORDS: array-CGH, embryo aneuploidy, embryo culture, trophectoderm biopsy

http://dx.doi.org/10.1016/j.rbmo.2016.06.005
1472-6483/

Introduction

Preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS) increase the chance of achieving a viable pregnancy without a single-gene defect or aneuploidy (Adler et al., 2014). Ten per cent of human pregnancies are affected by monosomy or trisomy; however, this rate can exceed 50% in women approaching the end of their reproductive lives (Nagaoka et al., 2012). Although embryos are typically selected according to morphology, PGS is the best method for selecting chromosomally healthy embryos for transfer because aneuploidy occurs at a higher rate in oocytes and spontaneous abortion (Sugiura-Ogasawara et al., 2012) or implantation failure (Margalioth et al., 2006).

Currently, extended culturing to the blastocyst stage enables biopsy samples to be obtained for PGS, which results in high diagnostic accuracy. The trophectoderm can therefore be more accurately identified, allowing biopsied cells to be obtained without causing damage to the inner cell mass. These improvements have resulted in significant increases in the implantation and pregnancy rates (Forman et al., 2013; Scott et al., 2013b, 2013c). Fewer embryos, however, reach the blastocyst stage in extended cultures, as demonstrated by a genetic analysis of 15,169 trophectoderm biopsies showing that 50% of patients had three or fewer blastocysts available for biopsy and that 20% had only a single blastocyst (Scott et al., 2013a). Therefore, an efficient laboratory with an extended culture system is required for blastocyst biopsy, and appropriate cryopreservation techniques are needed to allow for sufficient time to conduct genetic analyses.

Moreover, some embryos exhibit a slow rate of development during cleavage but may reach the early blastocyst stage on day 5, thus preventing safe biopsy at the appropriate stage. Those embryos are commonly kept in culture until day 6 to reach the appropriate developmental stage for biopsy. It is not clear, however, whether the slow rate of embryo development itself and the consequent extension of embryo culturing for an additional day of development (day 6) are independently related to any type of genetic disease. We hypothesized that a slow rate of development might be associated with a higher risk of embryo aneuploidy. Therefore, this study evaluated the aneuploidy rates in biopsied expanded blastocysts on days 5 and 6 of development in patients undergoing IVF with PGS by aCGH.

Materials and methods

Experimental design, patients and inclusion criteria

In this retrospective cohort study, 1171 expanded blastocysts were evalutated from 465 women who underwent fertility treatment with IVF and array comparative genomic hybridization (aCGH) between February 2014 and May 2015 at Huntington Reproductive Medicine, Sao Paulo, Brazil. Written informed consent was obtained from all patients before treatment, and the patients consented to the use of their retrospective data in scientific publications. According to the ethical guidelines, institutional review board approval was not required for this study because of its retrospective nature and because the data were anonymized.

Patients and ovarian stimulation

All couples included in this study had embryos that were biopsied for PGS by aCGH. All patients with an abnormal karyotype or family history of genetic disease were excluded. The patients received ovarian stimulation according to the routine protocols of the clinic. Briefly, pituitary blockade was achieved with a GnRH antagonist (0.25 mg Orgalutran, MSD, Kenilworth, NJ, USA) or agonist (Lupron, Abbott, North Chicago, IL, USA) according to standard protocols (Al-Inany et al., 2016; Pacchiarotti et al., 2016; Siristatidis et al., 2015). Ovarian stimulation was conducted using recombinant FSH (Gonal, Merck Serono, Germany or Puregon, MSD, USA), combined (or not) with human menopausal gonadotrophin (Menopur, Ferring, Switzerland), initiated on day 2 or 3 of the menstrual cycle. The initial dose was determined according to a previous antral follicle count, and the dose was adjusted according to the ovarian response. Follicle development was monitored every 2 days by ultrasonographic assessment of follicle growth. When at least two follicles measuring 18 mm or more in diameter were present, final oocyte maturation was triggered with 250 μg recombinant HCG (rhCG, Ovidrel®, Merck Serono, Switzerland). Oocyte aspiration was carried out under sedation at 35–36 h after recombinent HCG triggering.

Intracytoplasmic sperm injection and embryo culture

Follicular fluid was examined, and oocytes were identified and cultured for 3 h after retrieval to achieve final maturation, following denudation and assessment for the presence of the first polar body, indicating mature metaphase II. Then, metaphase II oocytes were fertilized by intracytoplasmic sperm injection (Palermo et al., 1992). Normally fertilized oocytes, defined by the presence of two pronuclei and two polar bodies, were cultured in groups between days 1 and 3 in 1 ml of cell culture medium (G-1 Plus, Vitrolife, Sweden) supplemented with 10% synthetic serum substitute (SSS, Irvine Scientific) under a layer of paraffin oil (OVOIL, Vitrolife). From day 3 until the blastocyst stage (D5 or D6), the embryos were cultured in 1 ml medium containing 10% human albumin (CSCM, Irvine Scientific, USA) under a layer of paraffin oil. The embryos were incubated in triple gas incubators (90% N_2, 5% O_2 and 5% CO_2).

Trophectoderm biopsy, genetic screening and embryo transfer

The blastocysts were morphologically classified according to Gardner et al. (2000), and all expanded blastocysts with a grade of 3 or higher were biopsied on day 5 ($n = 730$) or day 6 ($n = 441$) for genetic analysis. The time of biopsy was selected according to embryonic growth and blastocyst expansion, and only expanded blastocysts were biopsied. During the early blastocyst stage, the embryo has not expanded enough for herniation to occur, which prevents the removal of satisfactory amount of trophectoderm cells for analysis. At this stage, it is difficult to identify the inner cell mass, and cell removal can interfere with embryo quality and development (McArthur et al., 2005). The blastocysts were ob-