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ARTICLE

No evidence of association between blastocyst aneuploidy and morphokinetic assessment in a selected population of poor-prognosis patients: a longitudinal cohort study

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Abstract Recent studies involving a limited number of patients have indicated a correlation between aneuploidy and various morphokinetic parameters during preimplantation development. The results among different groups, however, have been inconsistent in identifying the parameters that are able to predict chromosomal abnormalities. The aim of this study was to investigate whether aneuploidy of human blastocysts was detectable by specific morphokinetic parameters in patients at increased risk of aneuploidy because of advanced maternal age, history of unsuccessful IVF treatments, or both. A longitudinal cohort study was conducted using 455 blastocysts from 138 patients. Morphokinetic features of preimplantation development were detected in a timelapse incubator. Blastocysts were subjected to trophectodermal biopsy and comprehensive chromosomal screening. Analyses were conducted by means of logistic mixed-effects models, with a subject-specific intercept. No statistical correlation between 16 commonly detected morphokinetic characteristics of in-vitro embryo development and aneuploidy was found. Results suggest that morphokinetic characteristics cannot be used to select euploid blastocysts in poor-prognosis patients regarded as candidates for pre-implantation genetic screening.

KEYWORDS: biopsy, blastocyst, PGS, qPCR, time-lapse

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Introduction

Improvement of culture conditions, extended culture and elective single blastocyst transfer were major achievements in human assisted reproduction during the past decade, resulting in a considerable reduction of twin pregnancies while increasing implantation and maintaining pregnancy rates. Results, however, are still far from optimal. It has been revealed that the aneupolidy rate of in-vitro produced embryos can exceed 60% (Fragouli et al., 2011), resulting in implantation failures or spontaneous abortions. Advanced maternal age, a common problem in human assisted reproduction, further increases the risk of aneuploidy. Consequently, a safe method of selecting euploid blastocysts with high in-vivo developmental competence would be crucial to improve overall efficiency further.

The most commonly used approach for blastocyst selection is the application of semiquantitative grading system based on the static inverted microscopic view before transfer (Gardner and Sakkas, 2003; Gardner and Schoolcraft, 1999). Although routinely applied worldwide, the limitations of this method to detect aneuploidy have been revealed and new approaches sought. Definitive diagnosis with high accuracy can be obtained with blastocyst biopsy (Capalbo et al., 2013; de Boer et al., 2004; Jansen et al., 2008; McArthur et al., 2005) and comprehensive chromosomal screening (CCS) for aneuploidies. Although blastocyst biopsy was found to be harmless, and the intervention increased implantation and delivery rates considerably (Scott et al., 2013a), the invasiveness of the procedure and the complexity of the task still discourage many specialists, and search for alternative, non-invasive solutions has been intensified (Cohen et al., 2013).

Morphokinetic analysis of embryo development *in vitro* has become one of the most attractive advances of our decade in human embryology. Sophisticated timelapse equipment and new culture conditions allow uninterrupted embryo development, continuous control over the cohort and detailed analysis of various events (e.g. syngamy, cleavages, compaction and blastulation). Studies have increasingly focused on the possible value of these parameters to detect aneuploidies and to indicate implantation potential. Various correlations have been found (Campbell et al., 2013a, 2013b; Chamayou et al., 2013; Chavez et al., 2012; Meseguer et al., 2011; Wong et al., 2010), but conclusions are controversial (Kaser and Racowsky, 2014).

The aim of this study was to investigate the following: the correlation between individual morphokinetic time-lapse parameters of in-vitro embryo development; and molecular karyotype investigated by trophectoderm biopsy and 24 chromosomes screening based on quantitative polymerase chain reaction; in a selected group of patients prone to aneuploidies. Moreover, the reproducibility of previous published algorithms based on different morphokinetic parameters to predict embryo aneuploidy was also analysed.

Materials and methods

Study design, target population and outcome measures

In this longitudinal cohort study, 455 blastocyts obtained from 138 consecutive patients undergoing an intracytoplasmic sperm injection (ICSI) cycle with preimplantation genetic screening (PGS) at the GENERA Centre, Rome, recruited between December 2012 and December 2013 were included. Infertile patients of advanced maternal age (>36 years) ($n = 102$), with a history of unsuccessful IVF treatments (more than two failed IVF cycles) ($n = 16$), previous spontaneous abortion (more than two spontaneous abortions) ($n = 20$), or all three, were offered PGS.

All embryos were individually cultured in a timelapse incubator (EmbryoScope, Unisense, Denmark) from the insemination procedure up to blastocyst development. All biopsies and subsequent cryopreservations were carried out at blastocyt stage. Full morphokinetic information (from pronuclear formation up to blastocyst expansion) and chromosomal status (as assessed by comprehensive chromosomal screening) were obtained for each included blastocyst. At warming, single euploid blastocyst transfers were carried out. Implantation rate was defined as number of fetuses with heart activity beyond 12 weeks of gestation per transferred embryo.

Blastocysts were classified as euploid (normal chromosomal complement) or aneuploid (presence of monosomy or trisomy). Aneuploid embryos were further separated as single or complex aneuploid ones (with two or more chromosomal errors in the trophectoderm cell samples for the latter). The relationship between morphokinetic data and euploidy was then assessed.

The study and the informed consent were approved by the Institutional Review Board of the Clinic on 14 August 2014.

Sample size

In this equivalence study, the power of the statistical tests were asessed on the basis of the planned sample size. A minimal clinically significant effect for each predictor, according to Cohen (1988) convention, was given by an odds ratio of 1.49 for the increase of one standard deviation. At a significance level of 5%, our samples gave a power of 95% to detect an odds ratio of 1.41 or more for each standard deviation. The resulting minimal odds ratio per unit increase of each predictor was often very close to 1 (**Appendix: Supplementary Table S1**). Consequently, a lack of significance at the 5% level could be interpreted as equivalence (i.e. a true odds ratio above the minimally clinically significant level only with 5% or lower probability). Even at the Bonferroni corrected level of $5/16 = 0.31\%$, our sample gave a satisfactory power of 80%, and similarly minimally clinically significant odds ratios only slightly larger.

Ovarian stimulation, oocyte collection, denudation, insemination and embryo evaluation

Two protocols were used for ovarian stimulation: gonadotrophin-releasing hormone (GnRH) agonist long protocol and GnRH-antagonist protocol, as described previously (Ubaldi et al., 2010).

Details of laboratory procedures have been described previously (Rienzi et al., 2010). Briefly, oocytes were collected at 35 h after HCG administration. After 24-h incubation, cumulus-oocyte complexes were exposed to 40 IU/ml

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