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ARTICLE

A minimally invasive methodology based on morphometric parameters for day 2 embryo quality assessment

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Abstract The risk of multiple pregnancy to maternal–fetal health can be minimized by reducing the number of embryos transferred. New tools for selecting embryos with the highest implantation potential should be developed. The aim of this study was to evaluate the ability of morphological and morphometric variables to predict implantation by analysing images of embryos. This was a retrospective study of 135 embryo photographs from 112 IVF–ICSI cycles carried out between January and March 2011. The embryos were photographed immediately before transfer using Cronus 3 software. Their images were analysed using the public program *ImageJ*. Significant effects ($P < 0.05$), and higher discriminant power to predict implantation were observed for the morphometric embryo variables compared with morphological ones. The features for successfully implanted embryos were as follows: four cells on day 2 of development; all blastomeres with circular shape (roundness factor greater than 0.9), an average zona pellucida thickness of 13 μm and an average of 17695.1 μm^2 for the embryo area. Embryo size, which is described by its area and the average roundness factor for each cell, provides two objective variables to consider when predicting implantation. This approach should be further investigated for its potential ability to improve embryo scoring.

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Introduction

The number of transferred embryos in IVF–ICSI cycles needs to be reduced because of the increased obstetric and perinatal risks involved in multiple pregnancies. Both the number and quality of transferred embryos are correlated with high multiple pregnancy rates, which is why the selection of one top quality embryo for transfer has been proposed by Hu et al. (1998), Strandell et al. (2000) and Wright et al. (2006). Current embryo quality assessment, based on the morphological criteria of a transferred embryo, is highly subjective. Therefore, a scoring system for ranking implantation is essential when aiming for singleton pregnancies without a significant decrease in pregnancy rates (Bergh, 2005; Catt et al., 2003; De Neubourg and Gerris, 2006; De Neubourg et al., 2004; Van Montfoort et al., 2005). Single-embryo transfer (SET) produces an unacceptably low pregnancy rate, particularly in older women and in those with a poor embryo quality (De Neubourg and Gerris, 2006).

The woman's age and embryo quality are the variables that influence the implantation rate the most (Giorgetti et al., 1995; Hardarson et al., 2001; Holte et al., 2007; Hunault et al., 2002; Shulman et al., 1993; Steer et al., 1992; Terriou et al., 2001; Van Royen et al., 1999). The first variable is unchangeable, but when a sufficient number of embryos are available, embryos with the greatest implantation potential can be selected for transfer according to morphological criteria (Ebner et al., 2001, 2003; Holte et al., 2007; Rienzi et al., 2005; Scott, 2002, 2003; Scott et al., 2007; Van Royen et al., 1999, 2001, 2003). It is, therefore, important to increase knowledge about the characteristics of embryos with a high implantation potential as well as of non-top embryos (Debón et al., 2013).

Evaluation of the implantation potential of transferred embryos has generally been based on the construction of accumulated embryonic scores. Assumptions need to be made about the overall quality of transferred embryos and their subsequent implantation owing to ignorance about the exact quality of the embryo, which finally implants (Copperman et al., 1995; Cummins et al., 1986; Giorgetti et al., 1995; Laasch and Puscheck, 2004; Steer et al., 1992; Terriou et al., 2001; Visser and Fourie, 1993). In more recent studies, logistic regression models have been used to predict the implantation rate of embryos (Debón et al., 2013; Holte, 2006; Holte et al., 2004, 2007).

Traditionally, embryo quality assessment is based primarily on the morphological criteria of transferred embryos, which is highly subjective and therefore variable (Paternot et al., 2009, 2011a). As a result, embryonic classification systems based on the use of objective parameters of embryo morphology should be developed. That is, measurements should be taken directly from the embryo and used in place of the observer's opinion, thus totally avoiding the subjectivity of the measurements.

The use of morphometry in the standardization of elements and processes has been used for a long time in metallurgy, molecular biology, and electron microscopy (Pertusa, 2010). Studies have been carried out linking morphometric embryo variables to embryo quality parameters, such as embryo fragmentation and multinuclearity, as well as embryonic segmentation and three-dimensional reconstruction (Agerholm et al., 2008; Beuchat et al., 2008; Giusti et al., 2010; Hnida et al., 2004; Santos et al., 2010). A few studies, however, have evaluated the predictive implantation ability of embryo morphometric parameters. Recently, Paternot et al. (2011b; 2013) showed better prediction of implantation rates based on the number and size of blastomeres on day 3 and correlations between total embryo volume and clinical pregnancy.

The aim of this study was to evaluate the predictive implantation reliability of the morphometric variables using image analysis of embryos that have already been transferred and whose fate is known (implanted or not implanted). The incorporation of these variables into the current embryo classification may lead to the development a new embryo classification system, based on a combination of morphological and morphometric variables, which could be investigated for its potential ability to improve embryo scoring.

Materials and methods

This study was approved by the Institutional Review Board of the hospital, La Fe in Valencia, Spain (approved 5 September 2011, IRB reference number 2011/0329). All procedures in the Methods section were compliant with ethical guidelines and approved by the Ethical Committee.

Assisted reproduction techniques

After oocyte retrieval, the oocytes were placed separately in 200 μl drops of culture medium (IVF medium; Medicult, Denmark) under mineral oil (Mineral oil; Medicult). Spermatozoa for the IVF procedure were prepared using standard swim-up procedures. Sperm samples for ICSI were diluted and centrifuged twice at 300 g for 10 min. Standard IVF/ICSI procedures were carried out 2–6 h after oocyte retrieval. In the IVF procedure, oocytes were inseminated with 300,000 progressively motile spermatozoa per oocyte.

In the case of an ICSI cycle, injected oocytes were incubated together in 20 μl drops of culture medium (IVF medium; Medicult, Denmark) under mineral oil (Mineral oil; Medicult). On Day 1 (16–20 h after insemination/injection), fertilization was evaluated. Only normally fertilized oocytes (2PN) were cultured individually in a 20 μl droplet of culture medium (IVF medium; Medicult) covered with mineral oil.

On day 2 (44–47 h after insemination/injection) embryo evaluation was carried out based on the assessment of cell number, size and degree of fragmentation. The best available embryos were chosen for transfer based on the standard embryo scoring system (Van Royen et al., 1999).

All the embryos included in this study were morphologically re-evaluated and photographed immediately before