Assessment of the implantation of day-2 human embryos by morphometric nonsubjective parameters

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Objective: To demonstrate the usefulness of image analysis in designing objective embryonic morphometric variables.
Design: Retrospective study of 214 top-quality day-2 embryo photographs from 50 double-embryo transfers resulting in no pregnancy (group 0) and 57 resulting in twin pregnancy (group 1).
Setting: Human reproduction unit.
Patient(s): Study of 107 in vitro fertilization–intracytoplasmic sperm injection (IVF–ICSI) cycles in women age <36 years with double-embryo transfer of top-quality embryos. Only the first cycle of IVF–ICSI was included.
Intervention(s): Standard IVF–ICSI protocols.
Main Outcome Measure(s): The embryo photographs were analyzed using the ImageJ program. The effects of the embryo variables and the clinical variables on embryo implantation were evaluated using a stepwise dichotomous logistic regression.
Result(s): Significant differences were observed, owing to the women's ages, internal perimeter, roundness factor, and zona pellucida thickness. Embryos with smaller internal perimeter, circular shape, and thinner zona pellucida were more likely to implant.
Conclusion(s): Morphometric variables lower the subjectivity of the current embryo grading systems. These variables are nonsubjective factors to consider when predicting implantation. Embryo image analysis is an accurate tool that can improve IVF–ICSI outcomes and reduce the number of twin pregnancies. (Fertil Steril® 2014;102:1022–8. ©2014 by American Society for Reproductive Medicine.)
Key Words: Embryo selection, embryo score, morphological and morphometric embryo variables, images analysis, embryo implantation, embryo grading systems

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One of the major issues in assisted reproductive technology (ART) is multiple pregnancies, which carry a significant risk to maternal–fetal health (1–3). The woman's age and the number and quality of transferred embryos are correlated with high multiple pregnancy rates (2, 4). These risks can be reduced by lowering the number of transferred embryos (4, 5). The ideal approach to studying the morphologic determinants of a single embryo's implantation would be to analyze exclusively single-embryo transfers (ETs). However, in most single-ET programs, only 'top'-quality embryos are transferred, so an optimal span of variables for statistical evaluation cannot be reached. The progressive implementation of a top-quality single ET produces an important decrease in multiple pregnancies without a significant reduction in pregnancy rates (6–10). Nevertheless, the linear implementation of single ET produces an unacceptably low pregnancy rate, particularly in older patients and those with poor embryo quality (10). Thus, it is important to increase knowledge of the implantation potential of each individual embryo to select top-quality embryos for transfer.

Until now, embryo selection has been routinely based on embryo development and morphologic characteristics by using various classification

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and scoring systems to evaluate embryo quality (6–8). Embryo quality assessment based on morphologic criteria of transferred embryos is highly subjective with interobserver variability in the evaluation of morphologic parameters (9–11). The absence of a clearly defined standard method to measure specific characteristics determines a loss of essential information.

The evaluation in real time of all the morphologic characteristics is time consuming. The embryo evaluation time has to be as short as possible to prevent embryo exposure to suboptimal culture conditions. Fluctuations in pH and temperature have deleterious effects on embryo development, quality, and implantation (12).

Time-lapse imaging has been proposed as a method for embryo selection by adding new dynamic predictors of viability to the assessment. The time-lapse embryo assessment allows the evaluation of embryonic morphokinetic parameters (13–15). However, the high costs of these technologies do not allow their implementation in many laboratories.

There is increasing international recognition of the value of performing elective single- or double-ET to prevent higher-order multiple pregnancies, demonstrating the importance of selecting embryos that result in live birth. Therefore, it is essential to develop new objective tools for selecting embryos.

Work has been performed linking morphometric embryo variables to embryo quality parameters such as embryo fragmentation and multinuclearity, as well as embryonic segmentation and 3-dimensional reconstruction (13–17). However, few studies compare the embryo morphometric parameters with embryo implantation. Recently, Partenot et al. (11, 15) demonstrated a better prediction of implantation rate based on blastomere number and size. In addition, they have shown correlations between total embryo volume and clinical pregnancy in day-3 embryos.

In our previous study (18), morphometric embryo variables were demonstrated to be more objective and powerful in predicting embryo implantation. However, the low number of successfully implanted embryos (27 embryos) did not allow conclusive results. The aim of the current study is to demonstrate the usefulness of the ImageJ program (National Institutes of Health) for image analysis in the design of objective embryonic morphometric variables. These were obtained from a selected population of women age <36 years with double ET of morphologically selected top-quality embryos successfully implanted.

MATERIALS AND METHODS

This study was approved by the Institutional Review Board of the hospital. All procedures in the Methods section were compliant with ethical guidelines, i.e., approved by the Ethical Committee.

Patients and Embryos

A retrospective study was performed, from January 2008 to December 2010, of 214 transferred embryos coming from 100 intracytoplasmic sperm injection (ICSI) cycles and 7 in vitro fertilization (IVF)–ICSI cycles in a selected population of women age <36 years, with double ET of top-quality day-2 embryos of the same morphologic characteristics. An IVF–ICSI cycle was only performed when more than 12 oocytes were retrieved. Half of the oocytes were micro-injected, and the best-quality embryos were transferred regardless of which insemination technique was used (IVF or ICSI). Only 2 embryos corresponding to 2 different IVF–ICSI cycles were derived from an IVF insemination technique that resulted in no pregnancy. All the embryos had 4 cells, with equal, symmetrical, and mononucleated unfragmented blastomeres. The stimulation protocol used in this study has been previously published (19).

Experimental Design

The embryos were distributed in the following groups: group 0 (0% implantation), 50 double ETs without pregnancy; and group 1 (100% implantation), 57 double ETs with a twin pregnancy (2 gestational sacs). Only the first cycle of IVF–ICSI was included. Cycles with endometriosis, low response, uterine malformations, recurrent abortions, or donor gametes were excluded.

All the embryos were morphologically top-quality day-2 embryos, and all the women had a good reproductive prognosis. Therefore, the differences between implanted and no implanted embryos should have been due to the morphometrically evaluated embryo variables, which could not have been evaluated using only simple observation through an inverted microscope.

ART Procedure

After oocyte retrieval, the oocytes were placed separately in 200-microliter drops of culture medium (IVF medium, Medicult) under mineral oil (Medicult). Semen samples for the IVF–ICSI cycles were prepared using standard swim-up procedures. They were diluted and centrifuged twice at 300 g for 10 minutes. Standard IVF–ICSI procedures were performed between 2 and 6 hours after oocyte retrieval. In the IVF procedure, oocytes were inseminated with 100,000–300,000 progressively motile sperm per oocyte.

In the ICSI cycles, injected oocytes were incubated together in 20-microliter drops of culture medium (IVF medium, Medicult) under mineral oil (Medicult). On day 1 (16–20 hours after insemination/injection), fertilization was evaluated. Only normally fertilized oocytes were cultured individually in a 20-microliter droplet of culture medium (IVF medium, Medicult) covered with mineral oil.

On day 2 (41–44 hours after insemination/injection), the embryo evaluation was based on the assessment of cell number, size, and degree of fragmentation. Replacements of only 2 top-quality embryos were considered. All the embryos were photographed immediately before transfer. Photographs were taken using “Cronus 3” software (Research Instruments LTD) implemented in a phase contrast inverted microscope (Nikon Eclipse) with a 20x optic magnification and Hoffman modulation contrast. An ongoing twin pregnancy was defined as the presence of 2 intrauterine gestational sacs after 6–8 weeks of pregnancy.