ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Automatic time-lapse instrument is superior to single-point morphology observation for selecting viable embryos: retrospective study in oocyte donation

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Objective: To correlate the different categories provided by a commercial diagnostic test with blastocyst formation, quality, implantation potential, and ongoing pregnancy (OPR) for the purpose of validating the automatic annotations and the classification algorithm.

Design: Observational, retrospective, multicenter cohort study.

Setting: University-affiliated private IVF center.

Patient(s): A total of 3,002 embryos, including 521 transferred embryos with known implantation, from 626 IVF cycles that were incubated in a conventional incubator and monitored with an automatic time-lapse test.

Interventions(s): None.

Main Outcome Measure(s): Embryo selection was based on morphology and the classification provided by a commercial diagnostic test. Implantation was the primary end point, and OPR, blastocyst formation (BR), and embryo morphology were secondary end points.

Result(s): BR and number of optimal blastocysts were related to the classification test. This correlation was also observed when analyzing implantation rates (day 3 transfer: high 38.2%, medium 31.7% and low 26.1%; day 5 transfer: high 66.7%, medium 50%, low 31%). Patients where no high embryos were transferred ($n = 75$) had an OPR of 46.70%, and those patients where at least one high embryo was transferred ($n = 109$) significantly increased OPR to 67%. A logistic regression analysis studying other confounding factors (day of transfer, number of oocytes obtained, and embryo morphology classification) was included. In that model, if at least one of the embryos was labeled as high, OPR was 2.567 times higher than a cycle where no high embryos were transferred.

Conclusion(s): Our study presents, to our knowledge, the largest set of transferred embryos after time-lapse analysis with the use of an automatic time-lapse test. The provided classification was related to reproductive outcome. Our results suggest that the automated embryo diagnostic test provided extra information to the embryologist to select the best embryos, independently from clinical features of the patient or day of transfer. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)

Key Words: Embryo, implantation, pregnancy, time lapse, automatic, morphokinetics

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From the very beginning of assisted reproduction and up to today, embryo selection was based on only morphologic assessment. Different theories have been proposed and some morphologic evaluation parameters accepted and universally used by many embryologist without much scientific evidence (1, 2). The reason for this is not that embryologists a couple of decades ago were less smart than we are at the moment, but in the past decade the evolution of new technologies has provided us extra information and has let us learn more about embryo evolution.

Morphology evaluations are subjective and done at discrete time points owing to the negative effects that

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manipulation has on embryo development, limiting the information for selecting the best embryos. Very recently, time-lapse systems have been introduced into laboratory procedures because of their precise and continuous recordings of embryonic development (3).

This technology may improve effectiveness of IVF cycles, increasing the ability to identify embryos with higher implantation potential, not only to increase implantation and pregnancy rates, but also to perform elective single-embryo transfer, reducing multiple pregnancy rates, which is at the moment one of the golden goals in assisted reproduction cycles. It provides improved culture conditions based on embryo evaluation without removal from the incubator, which minimizes manipulation. In addition, more objective and detailed information is obtained with the ability to evaluate embryos from a dynamic point of view, determining some phenomena that can be observed only with a continuous evaluation and studying exact timings of some important parameters of embryo development (4, 5).

There is growing interest in analyzing the abundant image data that have been gathered from time-lapse imaging systems. Even though time-lapse represents an advance on embryo evaluation and embryo development knowledge, the truth is that analysis is laborious and requires extensive training and practice for each time-lapse user. Moreover, the time needed for even highly trained users to perform analysis of large stacks of images in the limited time available before embryo transfer is sometimes too much in the work flows common to IVF clinics. Finally, potential interobserver and intra-observer variability may affect time-lapse marker interpretation, similarly to what has been found with manual embryo morphology grading (6).

Taking these arguments into account, the first computer-automated platform for time-lapse image analysis and blastocyst prediction has been developed: EEVA (Early Embryo Viability Assessment). This novel technology overcomes many of these problems with the introduction of automation in embryo evaluation.

Numerous studies have recently focused on early cleavage markers to select embryos with a higher implantation potential. Wong et al. (7) found that development of human embryos to the blastocyst stage was associated with key timings in earlier development, and they proposed that the extra information acquired with the use of time-lapse systems would in future negate the need for prolonged culture. Using the algorithm developed by Meseguer et al. (8), Cruz et al. (9) developed the largest time-lapse analysis of human blastocysts to date to demonstrate associations between various cleavage-stage kinetic parameters and the ability of the embryos to reach the blastocyst stage. That study compared the blastocyst rate and morphologic features for cleavage-stage embryos, which were graded according to their morphokinetic development. From their results, Cruz et al. concluded that time-lapse–based evaluation of the exact timing of early events in embryo development is a tool for the prediction of blastocyst formation and quality. Dal Canto et al. (10) studied time-lapse images and found human embryo cleavage rates to be suggestive of their ability to develop to the blastocyst stage and to implant.

The EEVA software includes not only automation but a software based on these early parameters to select the embryos with a higher probability of reaching the blastocyst stage, with the advantages involved.

Conaghan et al. (11) completed a multicenter prospective clinical trial in the United States. Results obtained in that study suggested that adjunctive use of morphology plus EEVA improved embryo selection by enabling embryologists to better discriminate which embryos would be unlikely to develop to blastocyst and was particularly beneficial for improving selection among good-morphology embryos. Embryologists using EEVA were able to improve their ability to identify nonviable embryos compared with traditional methods alone. Additionally, EEVA was able to increase the consistency of embryo assessment across embryologists. This was an important first step in the validation of the EEVA test. The results of this study were also confirmed by VerMilyea et al. (12), who studied the results from six different clinics, observing that embryos with high and medium scores have significantly higher implantation rates than those with low scores. They also pointed out that pregnancy rates in patients which had at least one embryo classified as high were higher than those with only embryos classified as low.

Diamond et al. (13) moved in the same direction and developed a study comparing blastocyst formation prediction of five different embryologists based only on morphology and then supplemented with EEVA test information. Results showed that, when EEVA prediction was used adjunctively with morphology, there was an evident improvement in the average specificity and positive predictive values. Because EEVA helps distinguish false positives, sensitivity also declined and overall odds ratio (OR) was higher than with morphology alone, determining the importance of this software. Moreover, a further analysis was developed focusing on good/fair-morphology embryos, with notable differences in the quantitative indicators mentioned above, confirming that the EEVA test can help to distinguish among similar-looking embryos that are evaluated first by morphologic criteria. Adamson et al. (14) studied cycles in which the EEVA test was combined with morphology, resulting in higher implantation and pregnancy rates than in cycles where only morphology was used for embryo selection.

All of these studies suggest that a method to predict not only blastocyst formation, but especially embryos with high implantation potential at day 3 would be very useful and that obviously time-lapse would definitely have an important role providing further information at early stages.

A key limitation of the EEVA system is the image provided by dark field. Morphology evaluation in some cases is quite difficult owing to the image quality. Moreover, parameters, such as multinucleation, which are usually taken into account in morphologic evaluation can not be evaluated with the use of these images. Another limitation of this system is that the images are taken in only one focal plane, which explains why fertilization can not be evaluated under these conditions and is also detrimental for precise embryo evaluation and cell count.