

# The path of embryo selection for improving IVF success rates

## Opinion

Reproductive endocrinologists and clinical embryologists have the difficult and important task of selecting which of the available embryos created after in vitro fertilization (IVF) to transfer-those they think that are most likely to provide the best outcome for their patients. If they fail to identify and predict the embryos which are more likely to implant the result will be multiple-gestation pregnancies or negative IVF results.<sup>1,2</sup> The main goal of IVF is to precisely identify the most appropriate embryo(s) for transfer.<sup>3,4</sup> Although embryo grading has been proposed as the most appropriate system for selecting the right embryo(s), the fact that it is based on the assessment of morphological characteristics in an easy and non-invasive way make it subjective and with a limited predictive value which often results in different interpretations of embryo quality.<sup>2,5,6</sup>

The efficacy of blastocyst culture revealed a new fast and secure method to identify competent embryos. A self-selection system in which embryo viability to blastocyst stage can maximize the chance of implantation.<sup>7,8</sup> Although the philosophy of blastocyst culture is to improve both uterine and embryonic synchronicity and result in higher implantation rates,<sup>9,10</sup> it involves prolonged culture, which currently results in blastocyst development in approximately half of all good-quality day 3 (D3) embryos.<sup>11,12</sup> Therefore only if the patient has many embryos will benefit from embryo transfer at the blastocyst stage than with cleavage-stage embryo transfer.<sup>13</sup> Furthermore, some evidence (even though conflicting) suggests that prolonged embryo culture may also increase the risk of preterm delivery, low birth weight, epigenetic disorders, monozygotic twinning and other long-term health issues.<sup>11,12,14,15</sup> Hence, reliable prediction of blastocyst formation as early as on day 3 may be useful, especially for IVF patients with repeated failures after D3 embryo-transfers. Despite the duration of embryo culture, morphology assessment is still used to select the best-quality embryos when several embryos with good similar morphological characteristics are available for transfer or cryopreservation.

The identification of embryos that are most likely to result in a pregnancy can be improved by two different paths: by predicting the embryos that are more likely to develop correctly and finally implant or by non-selecting the embryos that have less possibilities to develop further and therefore may have lower implantation potential.<sup>16</sup> Therefore a further improvement in the design of innovative approaches can improve embryo selection at the time of transfer, thereby maximizing the chances of implantation and the delivery of a healthy baby.

Because of its noninvasiveness to the embryo and its rapid adoption in the IVF units, time-lapse microscopy is particularly well suited not only to identify new selection criteria but also provide information about the dynamic changes during the pre implantation period.<sup>17,18</sup> Time-lapse imaging provides a non invasive technique of predictive parameters based on developmental kinetics by detecting the dynamics of early embryo development. The main challenge of applying time-lapse microscopy in everyday IVF practice is ensuring

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embryo culture and light exposure safety, defining and validating predictive parameters using evidence-based data and processing abundant imaging data in real-time. “A picture tells 1000 words”, freeing the IVF lab from time restrictions, time-lapse microscopy allows the identification of parameters that may noninvasively predict the developmental potential of a cleavage-stage embryo.<sup>17,19</sup>

Published data suggest that morphokinetic observations can yield valuable information to aid the selection of embryos for transfer.<sup>15,20</sup> In many studies, cell cycle timing parameters that were retrospectively chosen from a variety of images of time-lapse videos have constantly shown strong parallel of human embryo development and implantation potential.<sup>19-21</sup> Although, even today, there is none prospective, multicenter clinical study, comparing the morphokinetic parameters with traditional embryo selection criteria based on embryo morphological features. Moreover, the embryologist cannot yet take advantage of the time-lapse parameters to ensure, as soon as possible, which embryos have the greatest potential to develop into blastocysts in order to be transferred or cryopreserved. A summary of the papers published in time-lapse research showing that standardization in embryo selection method is necessary.

Lately, it was reported a time-lapse microscopy-enabled embryo assessment test that was developed based on seminal scientific findings about the timings of early embryo development and state-of-the-art software that automatically measures cell division timings and provides quantitative information regarding embryo development potential.<sup>15,21</sup> The combination of abnormal developmental timings already described and atypical morphokinetic features of embryos<sup>21,22</sup> could improve further the process of embryo selection by permitting to the embryologist to distinguish the embryos that have good morphology but lower development potential.<sup>16</sup>

Clearly, the attributes of “optimal embryos” based exclusively on embryo morphological criteria given the constant introduction on new technical advances in ART are not enough. As a result, the limitations of morphological and morphokinetic approaches justified the development of new non-invasive “omics” approaches. The development of the “omics” technologies (epigenomics, genomics,

transcriptomics, proteomics and metabolomics) have as a main aim to identify new non-invasive biomarkers, by providing with a huge amount of information regarding the biological processes involved in reproductive success. Therefore, the “omics” technologies are suitable diagnostic tools to explore differences among embryos with similar morphological features.<sup>23</sup> Since a single embryo transfer is getting momentum across the IVF clinics, such platforms which diagnose the embryo viability based on the expression of biomarkers will be inevitable to select the embryos for transfer.<sup>24</sup>

Voluminous data suggest that soluble ligands and their receptors mediate human pre implantation embryo development and could therefore be suitable as noninvasive biomarkers of embryo implantation.<sup>25</sup> Cortezzi et al.,<sup>26</sup> used a smaller variant of high-performance liquid chromatography combined with electron spray ionization mass spectroscopy to analyze the secretome of day 3 embryo spent media, revealing 25 novel secretory proteins.<sup>26</sup> In another study by Dominguez et al.,<sup>27</sup> protein arrays, based on antigen-antibody reactions and multiplex technology, have also been used, identifying CXC chemokine ligand (CXCL) 13, stem cell factor (SCF), and tumor necrosis factor receptor (TNFR) 1 in implanted vs. non-implanted embryos.<sup>27</sup> Butler et al.,<sup>28</sup> reported that, the dominance of hCG (human chorionic gonadotropin) and hCGh (hyperglycosylated hCG) expression seen after blastocyst hatching may be indicative of potential implantation success<sup>28</sup> and Mains et al.,<sup>29</sup> proved that Apolipoprotein A1 is produced by human preimplantation embryos, and correlated the increased levels in spent culture media with higher morphological grade blastocysts.<sup>29</sup> Recently, Dominguez et al.,<sup>30</sup> analyzed seven proteins in the embryo spent media (SCF, TNFR1, PIGF-1, IFN- $\alpha$ 2, IL-6, CXCL13, and GMCSF) with the use of a bead-based multiplexing technology and combined this data with the exact timing (in hours) of cell cycle duration, blastomere synchrony, and 5-blastomere cleavage with the use of an incubator equipped with time-lapse videography.<sup>30</sup> Finally, only the presence or absence of IL-6 appeared to be useful. However, no single biomarker has yet been used in standard clinical practice, mainly due to the differences in culture conditions the complication of the laboratory techniques and the effect of biological variations.<sup>31</sup>

Animal models, so far, have been particularly useful to help scientists analyze whole proteome or single protein markers in follicular fluid and gametes. Several studies have generated large amounts of data, nonetheless, the perfect profile to predict the best oocytes and embryos suitable for implantation are still to be uncovered.<sup>32</sup> An overview of metabolomics and its complementary role with transcriptomics and proteomics, it highlights how non-invasive footprint analysis in the embryo spent culture media has many applications for the assessment of the biochemical status of the embryo.<sup>33</sup>

In conclusion, microfluidic devices for embryo culture and analysis may yet prove to be a practical means for the integration and application of these new technologies. Additionally, we are very close to understanding what relationships exist between embryo morphokinetics, gene expression and physiology. In the future, following time-lapse monitoring and analysis of spent culture media for biomarkers will help to formulate comprehensive and robust algorithms for generating a superior embryo selection diagnostic tool. Even if perfection is our dream, improvement is still a reasonable hope and such algorithms are a realistic proposal for improvement in IVF.

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## Conflicts of interest

The author declares there is no conflict of interest.

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