ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Examining the efficacy of six published time-lapse imaging embryo selection algorithms to predict implantation to demonstrate the need for the development of specific, in-house morphokinetic selection algorithms

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Objective: To study the efficacy of six embryo-selection algorithms (ESAs) when applied to a large, exclusive set of known implantation embryos.
Design: Retrospective, observational analysis.
Setting: Fertility treatment center.
Patient(s): Women undergoing a total of 884 in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) treatment cycles (977 embryos) between September 2014 and September 2015 with embryos cultured using G-TL (Vitrolife) at 5% O_2, 89% N_2, 6% CO_2, at 37°C in EmbryoScope instruments.
Intervention(s): None.
Main Outcome Measure(s): Efficacy of each ESA to predict implantation defined using specificity, sensitivity, positive predictive value (PPV), negative predictive value (NPV), area under the receiver operating characteristic curve (AUC), and likelihood ratio (LR), with differences in implantation rates (IR) in the categories outlined by each ESA statistically analyzed (Fisher's exact and Kruskal-Wallis tests).
Result(s): When applied to an exclusive cohort of known implantation embryos, the PPVs of each ESA were 42.57%, 41.52%, 44.28%, 38.91%, 38.29%, and 40.45%. The NPVs were 62.12%, 68.26%, 71.35%, 76.19%, 61.10%, and 64.14%. The sensitivity was 16.70%, 75.33%, 72.94%, 98.67%, 51.19%, and 62.33% and the specificity was 85.83%, 33.33%, 42.33%, 2.67%, 48.17%, and 42.33%, The AUC were 0.584, 0.558, 0.573, 0.612, 0.543, and 0.629. Two of the ESAs resulted in statistically significant differences in the embryo classifications in terms of IR.
Conclusion(s): These results highlight the need for the development of in-house ESAs that are specific to the patient, treatment, and environment. These data suggest that currently available ESAs may not be clinically applicable and lose their diagnostic value when externally applied. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: Embryo development, embryo selection algorithm, morphokinetics

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Received July 18, 2016; revised November 4, 2016; accepted November 7, 2016.
A.B. has nothing to disclose. R.H. has nothing to disclose. G.M. has nothing to disclose. J.B. has nothing to disclose. C.K. has nothing to disclose. S.T. has nothing to disclose.
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Fertility and Sterility® Vol. ■, No. ■, ■ 2016 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2016.11.014

Traditional methods for embryo selection have been used for over 20 years. Numerous morphologic parameters are thought to be useful for correct embryo selection: pronuclear morphology (Z scoring) (1, 2), polar body alignment and appearance (3, 4), appearance of cytoplasm and zona pellucida (5), early cleavage (6, 7), multinucleation (8–10), and blastomere morphology (11–13). Basic embryo grading, including the number of blastomeres, evenness in the size of the blastomeres, and the level of fragmentation, remains the gold standard for embryo selection. However, using this method in a traditional sense (with a standard bench-top incubator) has two limitations: a restricted overview of an embryo's development and the exposure of the embryo to suboptimal temperatures and gas concentrations. With the introduction of time-lapse imaging, where an image of each embryo is taken every 10 to 20 minutes, more intricate embryo parameters can be viewed while leaving the embryos in an undisturbed environment. As the availability of time-lapse technologies increased, attention was first focused on assessing their clinical safety. Once the safety had been established and the available technologies were validated for clinical use (14–18), research then turned to determining how the time-lapse imaging systems could be used to increase pregnancy rates through in-depth embryo analysis and an undisturbed culture system.

Through the research performed previously and subsequently, many morphokinetic parameters were identified that correlated with the embryo's ability to create a pregnancy both in humans and animals: the appearance and disappearance of pronuclei and nuclei at each cell stage (3, 19–21), the length of time between early cytokinesis (22–29) and initiation of blastulation (30). Further embryologic phenomena have been observed using time-lapse imaging, including the reabsorption of fragments (31), direct cleavage of cells within embryos from one to three cells (32), and reverse cleavage (33). These phenomena have been shown to affect an embryo's implantation potential to varying degrees, but their discovery could lead to more effective embryo selection within a laboratory using time-lapse technology.

Single-embryo parameters such as those mentioned here have been linked to embryo viability (18), and now these parameters have been used to develop embryo-selection algorithms (ESAs). These ESAs seek to combine a number of morphokinetic parameters that have been linked to an embryo's viability expressed as the formation of a blastocyst, implantation, or a live birth. Here, the efficacy of six published ESAs for predicting an embryo's viability was examined, expressed as implantation rate (IR), in a clinically applicable setting (21, 27, 30, 34, 35) to demonstrate the need to develop specific, in-house ESAs. The ESAs examined were selected based on their clinical applicability to the test site, assessed superficially before analysis.

MATERIALS AND METHODS

This investigation was a single site, retrospective observational design approved by the North West Research Ethics Committee (ref: 14/NW/1043) and the institutional review board where necessary. All procedures and protocols complied with United Kingdom regulations (Human Fertilisation and Embryology Act, 1990, 2008). The data were obtained from 884 treatment cycles between September 2014 and December 2015. Clinical pregnancy was confirmed by the presence of a fetal heartbeat at ultrasound scan at 6 weeks' gestation. All treatments included in this analysis were from known implantation embryos; a single-embryo transfer or a double-embryo transfer where the transfer of two embryos resulted in either a negative test or two fetal heartbeats.

Ovarian Stimulation

Pituitary down-regulation was achieved using either a gonadotropin-releasing hormone agonist (buserelin, Suprecur; Sanofi Aventis) or antagonist (cetrorelix acetate, Cetrotide; Merck Serono). Ovarian stimulation was performed using urine-derived or recombinant follicle-stimulating hormone (Progynova [Bayer Germany]; Fostimon and Merional [IBSA]; Menopur [Ferring Fertility]; or Gonal f [Merck Serono]). Doses were adjusted based on the patient's demographic and response. Patients were given 5,000 IU of subcutaneous human chorionic gonadotropin (Gonasi HP; IBSA Pharmaceuticals) 36 hours before oocyte collection. Luteal support was provided via 400 mg of progesterone pessaries, twice daily (Cyclogest; Actavis), until the pregnancy test was taken.

Oocyte Retrieval and Embryology

Ultrasound-guided oocyte collection was performed transvaginally under sedation (Diprivan; Fresenius Kabi). Collected oocyte–cumulus complexes were cultured in four-well dishes (Nunc; Thermo Scientific) with each well containing 0.65 mL of G-IVF (Vitrolife) covered with 0.35 mL of OVOIL (Vitrolife) in a standard incubator (Sanyo Multigas MCO 18M). Sperm preparation was performed using a standard gradient separation at 0.3 relative centrifugal force (rcf) for 10 minutes (ISolate; Irvine Scientific) followed by two washes at 0.6 rcf for 10 minutes using G-IVF. The oocytes destined for intracytoplasmic sperm injection (ICSI) were prepared using enzymatic (Hyase 10X; Vitrolife) and mechanical digestion. Intracytoplasmic sperm injection was performed approximately 4 hours after collection, following which all injected oocytes were placed in individual culture drops of G-TL (Vitrolife) and cultured in the EmbryoScope (Vitrolife). The oocytes destined for standard insemination had this performed approximately 4 hours after collection and replaced into a standard incubator until the fertilization check the next day. Oocytes were then checked for fertilization approximately 16 to 18 hours postinsemination (hpi), and all fertilized oocytes along with all unfertilized metaphase II oocytes were placed in individual culture drops of G-TL and cultured in the EmbryoScope.

Embryo selection was performed using the national grading scheme (36) along with an internally derived ESA. This ESA was used as an additive to morphology at the test site and only used when two or three (where double-embryo transfer was to be performed) embryos of similar morphology were available for transfer. Morphology remained the gold