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ARTICLE

Conventional morphology performs better than morphokinetics for prediction of live birth after day 2 transfer

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Aisling Ahlström completed her Bachelor of Science and Honours Degree at the University of Adelaide, Australia in 1997. Thereafter, she worked as a research assistant at the Department of Obstetrics and Gynaecology focusing on reproductive immunology. From the start of the year 2000 until 2013 she worked at a private IVF clinic in Gothenburg and during this time obtained her PhD in Medical Science at the University of Gothenburg, Sweden. Currently she is working at the Unit of Reproductive Medicine, Sahlgrenska University Hospital. Her main interests are basic and clinical embryology, including methods for prediction of embryo viability, blastocyst culture and cryopreservation.

Abstract Numerous studies have reported on the potential value of time-lapse variables for prediction of embryo viability. However, these variables have not been evaluated in combination with conventional morphological grading and patient characteristics. The aim of this study was to assess the ability of patient characteristics and embryo morphology together with morphokinetic variables to predict live birth after day 2 transfer. This retrospective analysis included 207 transferred embryos from 199 couples cultured in a time-lapse system up to day 2 of development. Good prediction of live birth or ranking of embryos with respect to live birth potential was achieved with early cleavage combined with fragmentation grade at 43–45 h. These variables were selected as the strongest predictors of live birth, as assessed by stepwise logistic regression, and additional inclusion of morphokinetic variables did not improve the model significantly. Also, neither logistic regression models nor classification tree models with morphokinetic variables were able to achieve equally good prediction of live birth, as measured by AUC on an external data set not used for model development. In conclusion, for fresh day 2 transfers early cleavage in combination with fragmentation grade at 43–45 h should be considered when selecting between good quality embryos.

KEYWORDS: early cleavage, embryo selection, IVF, live birth, time-lapse

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Introduction

A large number of studies have been published regarding the potential value of time lapse. It was first introduced on a larger scale into IVF laboratories starting around 2010, and has become increasingly popular since then. The technique is used in different ways; as an embryo selection/deselection tool and/or as a logistic tool for the laboratory in order to be able to plan the work in a more efficient way. Divergent results concerning the potential value of time lapse to increase blastocyst, implantation, and/or pregnancy rates have been published and reviewed (Kirkegaard et al., 2012a, 2015). In one randomized controlled trial (RCT) (Rubio et al., 2014) it was found that, using the time-lapse system, ongoing pregnancy rates were significantly improved. Another RCT also showed a significant difference in ongoing pregnancy rate when one or two euploid embryos from preimplantation genetic screening (PGS) patients were selected using the morphokinetic criteria by Meseguer (Meseguer et al., 2011) compared with conventional morphological scoring and a standard incubator system (Yang et al., 2014). However, other published RCT (Goodman et al., 2016; Kirkegaard et al., 2012b; Park et al., 2015) have failed to show any benefit in implantation or pregnancy/live birth by using time lapse, and a recent Cochrane review concluded that there was insufficient evidence of differences in live birth, miscarriage, stillbirth or clinical pregnancy between the time-lapse system and conventional incubation (Armstrong et al., 2015). Furthermore, the efficacy of time-lapse variables assessed in combination with other known predictors of live birth, such as to conventional grading and patient characteristics, has not been studied.

The aim of this study was to determine in a retrospective data set whether morphokinetic variables when analysed in combination with conventional morphology and other patient variables could be used to predict live birth.

Materials and methods

Patients

This is a retrospective analysis of patients allocated to embryo culture in a time-lapse system as part of an RCT conducted at Reproductive Medicine, Sahlgrenska University Hospital, in Gothenburg between May 2010 and February 2014 (Park et al., 2015). In total, 364 patients (365 cycles) were randomized between May 2010 and February 2014. Their oocytes were cultured in either the TLI incubator (241 cycles, 2280 oocytes) or a standard incubator (124 cycles, 1180 oocytes). Patients were eligible if they were ≤40 years of age, undergoing their first fresh IVF cycle, with own gametes using intracytoplasmic sperm injection (ICSI) and where at least one oocyte was retrieved. In this retrospective analysis only patients where the number of live births matched the number of transferred embryos or where no live birth occurred ($n = 199$ patients, 207 transferred embryos) were included. Excluded from this analysis were patients who gave birth to a singleton child after double embryo transfer ($n = 1$), not receiving embryo transfer ($n = 11$ total embryo cryopreservation, $n = 1$ no mature oocytes, $n = 6$ failed fertilization, $n = 4$ no optimal embryo development) and when no time-lapse images were available due to technical failure ($n = 19$).

The study was approved by the Ethical committee of the University of Gothenburg on 9 December 2009 (reference number 666–09).

Ovarian stimulation, IVF and embryo culture

Stimulation protocols were performed as previously described (Park et al., 2015). Briefly, patients were down-regulated with gonadotrophin-releasing hormone (GnRH) agonists (Suprecur, Sanofi, Paris, France) in a long protocol and ovarian stimulation was achieved with either recombinant FSH (Gonal-F, Merck Serono, Darmstadt, Germany or Puregon, MSD, USA) or urinary-derived gonadotrophins (Menopur, Ferring, Copenhagen, Denmark). In a few cases ($n = 16$), patients were down-regulated in a short protocol using a GnRH antagonist (Orgalutran, MSD, NJ, USA). Human chorionic gonadotrophin (HCG; Pregnyl 5000 or 10,000 IU, MSD or Ovitrelle 6500 IU, Merck Serono) was administered when two or more follicles reached ≥18 mm diameter. Follicles were aspirated using vaginal ultrasonography 36 ± 2 h after HCG injection. Retrieved cumulus–oocyte–complexes were rinsed in MOPS (Vitrolife, Gothenburg, Sweden) and placed in G-IVF medium (Vitrolife). Mature oocytes were fertilized using conventional ICSI procedures. Directly after injection oocytes were placed in pre-equilibrated EmbryoSlides® (Vitrolife) containing 25 μl G1 media under 1.2 ml Ovoil (Vitrolife) and cultured in the Embryoscope® (Vitrolife) at 37°C, 6% CO_2 and atmospheric O_2 concentration until embryo transfer on day 2.

Embryo assessment and transfer

Conventional morphological embryo assessments were performed during embryo culture by at least two embryologists without removing the embryos from the EmbryoScope and in accordance with the Istanbul consensus (ALPHA Scientists in Reproductive Medicine and ESHRE Special Interest Group Embryology, 2011). Fertilized oocytes were confirmed by the presence of two pronuclei at 16–18 h and early cleavage was assessed at 25–27 h post-ICSI. On day 2 (43–45 h) embryos were graded according to blastomere number and size, degree of fragmentation and presence of multinucleation. Good quality embryos, defined as an embryo with four to six blastomeres, less than 20% fragmentation and no observed multinucleation, were primarily selected for transfer. Early cleavage was also considered when selecting between embryos of equal quality. Selections of embryos to transfer were based solely on morphological criteria. Time-lapse recordings and morphokinetic parameters were not annotated during the RCT study period or used for embryo selection. Validations of embryo scoring are performed annually to assess variations between individuals at our clinic and between eight different IVF clinics in Sweden. A high level of agreement for all morphological parameters graded was maintained, both within the group of embryologists at our clinic and in relation to other clinics.