RBMO

ARTICLE

Time-lapse imaging algorithms rank human preimplantation embryos according to the probability of live birth

BIOGRAPHY

Simon Fishel, Founder and President of CARE Fertility Group, Fellow of the Royal Society of Biology worked with IVF pioneer and Nobel Laureate Robert Edwards from 1975-1985 at Cambridge University and as Deputy Scientific Director of the first IVF clinic, Bourn Hall. In 1978 he received the Beit Memorial Fellowship and was elected Research Fellow of Churchill College, Cambridge. In 2009 was awarded Liverpool John Moores University Honorary Fellowship for "outstanding contributions to humanity and science in the field of fertility treatment including embryology and IVF"

Simon Fishel[1], Alison Campbell[1,*], Sue Montgomery[2], Rachel Smith[3], Lynne Nice[4], Samantha Duffy[2], Lucy Jenner[5], Kathryn Berrisford[5], Louise Kellam[5], Rob Smith[6], Fiona Foad[7], Ashley Beccles[1]

KEY MESSAGE

This retrospective study demonstrated for the first time that human blastocyst embryos can be objectively ranked according to their propensity to produce a live birth using an in-house derived morphokinetic-based algorithm from time-lapse imaging. This appears to have greater discriminating power than subjective, conventional morphology assessment.

ABSTRACT

Research question: Can blastocysts leading to live births be ranked according to morphokinetic-based algorithms?

Design: Retrospective analysis of 781 single blastocyst embryo transfers, including all patient clinical factors that might be potential confounders for the primary outcome measure of live birth, was weighed using separate multi-variable logistic regression models.

Results: There was strong evidence of effect of embryo rank on odds of live birth. Embryos were classified A, B, C or D according to calculated variables; time to start (tSB) and duration (dB{tB – tSB}) of blastulation. Embryos of rank D were less likely to result in live birth than embryos of rank A (odds ratio [OR] 0.3046; 95% confidence interval [CI] 0.129, 0.660; $P < 0.005$). Embryos ranked B were less likely to result in live birth than those ranked A (OR 0.7114; 95% CI 0.505, 1.001; $P < 0.01$), and embryos ranked C were less likely to result in live birth than those ranked A (OR 0.6501, 95% CI 0.373, 1.118; $P < 0.01$). Overall, the LRT (Likelihood Ratio Test) p-value for embryo rank shows that there is strong evidence that embryo rank is informative as a whole in discriminating between live birth and no live birth outcomes ($p = 0.0101$). The incidence of live birth was 52.5% from rank A, 39.2% from rank B, 31.4% from rank C and 13.2% from rank D.

Conclusions: Time-lapse imaging morphokinetic-based algorithms for blastocysts can provide objective hierarchical ranking of embryos for predicting live birth and may have greater discriminating power than conventional blastocyst morphology assessment.

[1] CARE Fertility Group, John Webster House, 6 Lawrence Drive, Nottingham Business Park, Nottingham NG8 6PZ, UK
[2] CARE Manchester, 108–112 Daisy Bank Road, Victoria Park, Manchester M14 5QH, UK
[3] CARE Sheffield, 24–26 Glen Road, Sheffield S7 1RA, UK
[4] CARE Northampton, 67 The Avenue, Cliftonville, Northampton NN1 5BT, UK
[5] CARE Nottingham, John Webster House, 6 Lawrence Drive, Nottingham Business Park, Nottingham NG8 6PZ, UK
[6] CARE London, Park Lorne, 111 Park Road, London NW8 7JL, UK
[7] CARE Dublin, Beacon CARE Fertility, Beacon Court, Sandyford, Dublin 18, Ireland

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*Corresponding author. E-mail address: alison.campbell@carefertility.com (A Campbell). https://doi.org/10.1016/j.rbmo.2018.05.016 1472-6483/© 2018 Published by Elsevier Ltd on behalf of Reproductive Healthcare Ltd.
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KEYWORDS

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INTRODUCTION

Assessment of the value of time-lapse imaging (TLI) following its recent introduction into clinical IVF practice (Cruz et al., 2011; Pribenszky et al., 2010; Wong et al., 2010) has largely centred on the incidence of pregnancy in comparison to conventional culture (Rubio et al., 2014; Wu et al. 2016). Some studies have tried to evaluate algorithms predictive of blastulation (Cruz et al., 2012; Dal Canto et al., 2012; Hashimoto et al., 2016; Herrero et al., 2013; Kirkegaard et al., 2014; Milewski et al., 2015; Motato et al., 2016), and others have searched for algorithms predictive of euploidy or aneuploidy (Campbell et al., 2013a,b; Del Carmen et al., 2017; Franasiak et al., 2014; Kramer et al., 2014; Lagalla et al., 2017; Minasi et al., 2016; Mumusoglu et al., 2017; Rienzi et al., 2015). Of late, there have been several reviews looking at the use of TLI in all these domains (Armstrong et al., 2015; Milewski and Ajduk, 2017; Polanski et al., 2014; Pribenzsky et al., 2017; Racowsky et al. 2015). A large retrospective analysis of live births comparing TLI to conventional culture was recently published, concluding that the former can improve the incidence of live births by 19% in this system (Fishel et al., 2017). There are conflicting views on the value of TLI for improving IVF outcome, which in part is due to what has been measured; for instance, assessing TLI outcome solely while treating the embryo as an independent factor (see Kirkegaard et al., 2016); or comparing TLI algorithms to using a time-lapse incubator as a closed incubation system only, without considering any algorithms (Rubio et al., 2014). Furthermore, different days of embryo transfer and different culture systems (Ciray et al., 2012), and different embryo phenotypes (Athayde Wirka et al., 2014) have been used. Few studies have focused on live birth outcome.

In this retrospective analysis, treatment outcome using single embryo transfer at the blastocyst stage was examined; all embryos were cultured in the same TLI device with an identical culture system. All potentially confounding clinical factors were evaluated, and an assessment was done of whether embryos could be objectively and successfully ranked for their potential to result in a live birth based on a simple TLI algorithm.
The relevance of conventional blastocyst morphology in comparison to using the TLI algorithm was also tested.

MATERIALS AND METHODS

This multicentre study included 843 transfers for 781 unique patients attending CARE fertility centres from January 2013 to December 2015, at CARE Nottingham, CARE Northampton, CARE Manchester, CARE Sheffield, Beacon CARE Fertility Dublin and CARE London. All embryos were cultured in the EmbryoScope (Vitrolife, Sweden). Only embryos at the blastocyst stage were assessed, both using conventional morphological criteria and the TLI algorithm. All protocols for patient treatments complied with UK regulation (Human Fertilization and Embryology Act, 1990, 2008) and all UK facilities are regularly inspected by the Human Fertilization and Embryology Authority (HFEA), which includes the use of TLI. The retrospective analysis of the use of TLI algorithms for embryo selection did not require ethical or Institutional Review Board (IRB) approval, as confirmed by the chair of the IRB on 13 January 2017, having been performed according to previously validated procedures, and practised under licence from the HFEA. All patients were fully counselled and gave their signed consent. TLI was undertaken using the EmbryoScope with strict adherence to annotation protocols. All embryos were selected for transfer based on their in-house-derived TLI algorithm rank for transfer; standard morphology of the selected embryos was also recorded in the conventional manner at embryo transfer. The primary end-point of this study was a live birth event, i.e. the number of patients achieving a delivery of a live birth for each embryo transfer. Only 'fresh' single embryo transfer cases were included, and all preimplantation genetic testing cases were excluded.

The following clinical variables were categorized for inclusion in the analysis: patient age, day of embryo transfer, number of embryos transferred, donor age (where applicable), body mass index (BMI), anti-Müllerian hormone (AMH), antral follicle count (AFC), gonadotrophin type, gonadotrophin dosing days and gonadotrophin total dose. The groupings applied are presented for each variable in TABLE 1. Patient age was considered as a binary variable in the modelling (<38 and 38+), corresponding to the common grouping used by HFEA, which is familiar to patients. The groupings for categorizing BMI, AMH and AFC were chosen to correspond with clinically meaningful categories, i.e. to reflect what might be considered to be above, below or within a normal/healthy range. The oocyte provider ages (<29, 29–32 and 33+), gonadotrophin dosing days and the total dose were categorized based on the quantiles of the observed distribution to ensure that sufficient information was present in each of the categories for a robust analysis.

The following definitions were used for the BMI, AMH and AFC categories, relating to the data presented:

- BMI: <18.5 (underweight), 18.5–<25 (healthy weight), 25–<30 (overweight), 30–<40 (obese), 40+ (extremely obese).
- AMH (pmol/l): <6 (low), 6–<24 (normal), 24–<70 (high), 70+ (very high).
- AFC: <4 (extremely low), 4–<10 (low), 10–<14 (somewhat low), 14–<22 (normal), 22–<35 (high), 35+ (very high).

OVARIAN STIMULATION PROTOCOLS

Pituitary suppression for ovarian stimulation was performed either with gonadotrophin-releasing hormone agonist (Suprecur; 0.5 ml subcutaneously daily; Sanofi Aventis, UK) or antagonist (Cetrotide; 0.25 mg daily; Merck Serono, UK), and ovarian stimulation was achieved using human menopausal gonadotrophin (Menopur; Ferring, UK) and/or recombinant FSH (Gonal-F; Merck Serono), as previously described (Campbell et al., 2013b; Fishel et al., 2016).

TABLE 1 VARIABLES OFFERED TO THE MODELS DURING THE STEPWISE SELECTION PROCEDURE. FOR EACH OUTCOME RESPECTIVELY, THOSE MARKED Y WERE INCLUDED IN THE FINAL MODEL

Variable	Included in live births model?	Included in implantation model?	Included in clinical miscarriage model?
Patient age (years)	Y	Y	Y
Embryo rank (A/B/C/D)	Y	Y	N
Total previous cycles	Y	Y	Y
Total previous miscarriages	N	N	Y
Aspirin (Y/N)	N	N	N
Intralipids (Y/N)	N	N	N
Clexane (Y/N)	N	N	N
Prednisolone (Y/N)	N	N	N
BMI	N	N	N
Day 2 FSH	N	N	N
AMH	N	N	N
AFC	N	N	N
Gonadotrophin type	Y	Y	N
Maximum endometrial thickness (mm)	N	Y	N
Ethnicity	N	N	N
Duration of infertility (years)	N	N	N
Gonadotrophin total dose	N	N	N
Gonadotrophin dosing days	N	N	N
MTHFR (Y/N)	N	N	Y
Catheter used	N	N	N
HCG name	N	N	N
Eggs collected	N	N	N
Mature eggs inseminated	N	Y	N
Mature eggs inseminated out of eggs collected ratio	N	N	N
Transfer grade/morphology	Y	Y	N
Patient type (oocyte recipient/standard)	Y	Y	Y
Donor age (years)	N	N	N
Gonadotrophin total dose (IU)	N	Y	N
Oocyte recipient/patient age interaction	N	N	N
Embryo rank/patient age interaction	N	N	N
Embryo rank/oocyte recipient interaction	N	N	N
Embryo rank/donor age interaction (where applicable)	N	N	N
Embryo rank/day of embryo transfer interaction	N	N	N
Embryo rank/patient age/oocyte recipient interaction	N	N	N
Embryo rank/transfer grade interaction	N	Y	N
Embryo rank/transfer stage interaction	N	N	N
Embryo rank/day of embryo transfer and transfer grade interaction	N	N	N
Embryo rank/day of embryo transfer and transfer stage interaction	N	N	N

AFC = antral follicle count; AMH = anti-Müllerian hormone; BMI = body mass index; HCG = human chorionic gonadotrophin.

OOCYTE RETRIEVAL, DENUDATION AND INTRA-CYTOPLASMIC SPERM INJECTION

Female sedation was achieved with a combination of propofol (Braun, Germany), fentanyl (Auden McKenzie, UK) and midazolam (Hamelyn, UK), and transvaginal ultrasound-guided oocyte retrieval took place approximately 36 h post human chorionic gonadotrophin injection (10,000 IU; Pregnyl; Organon, UK; or Ovitrelle; Merck Serono) or agonist trigger (Buserelin 0.5 ml subcutaneous; Suprefact, Sanofi SA, France), using an aspiration needle (Vitrolife, Sweden) connected to a vacuum pump (Rocket Medical, UK). Oocyte–cumulus complexes were recovered from follicular aspirates using a stereomicroscope in a class II hood with a heated stage, washed and cultured in Ferticult IVF medium (Fertipro, Belgium) at 5% CO_2 in air, 37.0°C, and maximum humidity, in standard small volume box or flatbed incubators (Galaxy 48R, New Brunswick, UK; Miri, ESCO, Japan).

Oocytes allocated for intracytoplasmic sperm injection (ICSI) were cultured for 2–4 h before cumulus cell denudation with 15–20 IU/ml cumulase (Origio, Denmark) in the same medium and complete removal of the coronae radiatae with a 140 µm pipette (EZ Squeeze; Research Instruments, UK). Oocytes at the metaphase II stage underwent insemination by ICSI within 2 h of denudation, following which they were placed in the EmbryoScope. Oocytes allocated for IVF were inseminated following sperm preparation using SupraSperm density gradient (Origio, Denmark) and washing in Ferticult IVF medium (Fertipro) at a concentration of 0.2 mmol/l, between 3 and 6 h post oocyte recovery. Culture was performed in standard incubators for 18 ± 1 h before fertilization was assessed. The sperm preparation method was the same for IVF and ICSI.

EMBRYO CULTURE AND INCUBATION

For TLI, following ICSI or IVF, oocytes or zygotes, respectively, were placed individually in microwells of EmbryoSlides (Vitrolife, Sweden) in 25 µl Global IVF medium (LifeGlobal) supplemented with 10% dextran serum supplement (Irvine Scientific); the wells were overlaid with 1.4 ml mineral oil (Fertipro, Belgium) and the slides were placed in the EmbryoScope at 37.0°C in 5.5% CO_2, 5% O_2 and 89.5% N_2 for up to 6 days. EmbryoSlides were prepared with medium and oil that had equilibrated overnight. The built-in microscope was used to acquire images of each fertilized oocyte every 10–20 min through seven focal planes.

Selection of the embryos was based on time-lapse algorithm ranking (A to D) and morphological assessment was recorded for all transferred blastocysts using a scoring system modified from the Gardner and Schoolcraft (1999) annotation of trophectoderm and inner cell mass morphology where scores AA/AB/BA, etc. were replaced by numbers: 1:1/1:2/2:1, etc., as per the Istanbul consensus recommendation (*The Istanbul Consensus, 2011*).

EVALUATION OF TIME-LAPSE IMAGES

Time-lapse images were collected for the duration of the culture period, until embryo transfer. The images were used for the assessment of fertilization following ICSI and *in vitro* embryo development. For ICSI, the time of insemination was programmed into the EmbryoScope as the time-point midway through the ICSI procedure. For IVF, the time of insemination was recorded as the time spermatozoa were added to the oocytes. Because of the difference in timing of sperm penetration in ICSI versus IVF, the designation of time post insemination (hpi) was based on pronuclei fading (PNf) after carefully controlled annotation. For ICSI, the mean time was 23.23 ± 3.7 h (n = 2547 zygotes); for IVF, the mean was 25.18 ± 3.6 h (n = 785). Hence ICSI zygote PNf occurred on average 1.95 h before IVF embryos. This difference in the modelling is accounted for as described below.

The EmbryoViewer image analysis software (Vitrolife) was used to log and display the precise timing of developmental events as they were annotated by the embryologists studying the time-lapse images. The morphokinetic variables of interest have been described in detail previously (Campbell et al., 2013a, 2013b; Fishel et al. 2017). All times were recorded in hpi. Following implementation of an in-house-derived time-lapse algorithm to rank embryos according to likelihood of live birth, the algorithm was used prospectively for selection of blastocysts for transfer. For this study, blastocyst ranking depended upon annotating for the relative time to the start of blastulation (rtSB) and the duration of blastulation (dB), with the following weighting: rank A = rtSB ≤ 93.1 h; B: rtSB > 93.1 h, dB ≤ 12.5 h; C: rtSB > 93.1 h, dB > 12.5 h. D was scored for those embryos in which the start of blastulation could not be annotated; this may occur due to obscuring fragmentation, presence of multiple fluid-filled vacuoles, or anomalous kinetics. As the algorithm's morphokinetic variable, tSB, is based on time from insemination, and as we observed a 1.95 h delay for IVF embryo development (unpublished data) compared with ICSI, the algorithm was adjusted for IVF embryos as follows in order to generate the ranking (A–D): RelSBIVF = tSB – 1.95 h.

EMBRYOLOGY ANNOTATION PROTOCOLS AND QUALITY CONTROL

Following training in annotation and competency assessment, CARE embryologists participate in regular quality assurance (QA) exercises and use a centralized annotation QA protocol whereby example embryos are annotated by each practitioner and their values are compared with those of their colleagues. Intra correlation coefficients (ICC) are calculated for each morphokinetic value. Annotation quality is considered assured where the ICC is greater than 0.9, demonstrating close correlation between practitioners, and competency in annotation.

EMBRYO SELECTION AND TRANSFER

Following TLI, embryos were objectively selected using user-defined time-lapse algorithms programmed into the 'Compare and Select' software as described above. All embryos were annotated before decision on transfer. Rank A was given the highest priority while rank D had the lowest. Morphology was scored at the time of transfer, but for decision on transfer, morphology was considered only secondary to the morphokinetic algorithm.

Embryo transfer was performed using a Wallace (UK) embryo transfer catheter under ultrasound guidance.

STATISTICS

The primary outcome measurement was live birth, i.e. the delivery of one or more babies; and the secondary outcome was the incidence of miscarriage (including HCG-determined implantation which did not result in a clinical pregnancy and clinical loss, defined as a loss of pregnancy following fetal heart detection on ultrasound). Separate multi-variable logistic regression models were fitted to the study data in order to assess the effects of embryo rank on each outcome of interest. A logistic regression analysis models the probability of the binary outcome as a function of the supplied explanatory variables. To explicitly control for differences in the patient populations between the embryo rank groups, potential confounding variables from the available clinical data were also considered as other explanatory variables in the models.

To choose which of the available variables to include as covariates in each model, a forward–backward stepwise variable selection procedure was performed. This procedure started with an initial model that included just an intercept term and new explanatory variables were added (and removed) one at a time to look for the model that has the best value of the Akaike information criterion (AIC) – a measure of model fit that has a penalty for the number of parameters in the model. TABLE 1 shows the variables made available to the variable selection procedure and those that were selected to be in the final model for each outcome.

A number of interactions ('effect modifications') were made available to the variable selection procedure. An interaction allows the level of one or more variables to change the effect of another variable. Any combination of variables may interact with each other, resulting in an enormous number of potential interactions. To make the variable selection and model fitting practicable, the interactions were limited to the following:

- Patient age with an indicator for whether the patient was an oocyte recipient, to allow for the possibility that the patient's age is less (or even more) important when they have received an egg donation.
- Embryo rank with each of patient age, oocyte recipient indicator, donor age (where applicable), day of embryo transfer, transfer grade and transfer stage (number of cells) and day of embryo transfer and morphology. These interactions were included to allow for the possibility that the sizes and directions of the embryo rank effects vary for different groups of patients.
- A three-way interaction between embryo rank, oocyte recipient status and patient age was also made available. This was included because the corresponding two-way interaction without embryo rank was being included. Importantly, these interactions were included to allow for the possibility that the size and direction of the embryo rank effects vary for different groups of patients.

Fitted final models were used to provide estimates of the directions and sizes of the effects of interest (presented as odds ratios [OR] which describe the relative difference in the odds of a live birth or clinical miscarriage between different treatment cycles) for each outcome (TABLE 2). The estimated effect sizes are accompanied by profile likelihood confidence intervals (CI), which quantify the uncertainty in the estimates arising from the sample data. Likelihood ratio tests (LRT) are provided to assess the overall significance of each variable, across the multiple groups of a categorical variable. A LRT tests the null hypothesis that including the variable in question does not improve the model fit (as measured by the likelihood).

All analyses were performed using the statistical software package R version 3.3.1 (R Core Team, 2016). The logist package was used to implement Firth's penalized maximum likelihood logistic regression method (Heinze and Ploner, 2016) when analysing clinical miscarriage.

RESULTS

A total of 843 single blastocyst transfers were examined for LB according to their TLI algorithm value of the transferred embryos. The mean ages of patients were 34.8 (n = 373), 35.4 (n = 297), 34.8 (n = 93) and 37.1 (n = 80) for embryos with algorithm ranks of A, B, C and D, respectively. An assessment of the value of a hierarchical ranking of embryos using all the available clinical data was undertaken, as shown in TABLE 1. A full data set was available for 781 transfers, included in a detailed analysis; summary statistics for each continuous or discrete variable were considered in the analysis versus embryo rank and are shown in TABLE 2. Of the 781 transfers, 354 (45.3%) had an embryo ranked A, 273 (35.0%) ranked B, 86 (11.0%) ranked C, and 68 (8.7%) ranked D. There were 329 (42.1%) live births; 186 (52.5%) from transfers with an embryo ranked A, 107 (39.2%) from rank B, 27 (31.4%) from rank C, and 9 (13.2%) from D-ranked embryos. There was strong evidence of an effect of embryo rank on the odds of live birth. Embryos of rank D were less likely to result in a live birth than embryos of rank A (OR 0.3046; 95% CI 0.129, 0.660; $P < 0.010$), and similarly embryos of rank D were less likely to result in a live birth than embryos of rank B (OR 0.428; 95% CI 0.190, 0.963; $P < 0.01$). Embryos ranked B were less likely to result in a live birth than those ranked A (OR 0.7114; 95% CI 0.505, 1.001; $P = 0.0101$), and embryos ranked C were less likely to result in a live birth than those ranked A (OR 0.6501, 95% CI 0.373, 1.118; $P = 0.0101$). Overall, the data provide strong evidence of an effect of embryo rank on the odds of live births (LRT p-value = 0.0101). There was no evidence of an independent effect of embryo rank on the odds of clinical miscarriage.

The role of morphology assessment was also examined; in the absence of TLI data, this would have been the primary factor in deciding which embryo to transfer. The highest incidence of live birth was achieved with blastocysts graded '2:2' when compared with other morphology grades (FIGURE 1). The comparison showed that selecting for an embryo grade 2:2 for transfer is associated with an increase in the odds of a live birth compared with grades 1:1/1:2/2:1 (OR 0.6795; 95% CI 0.485, 0.988), or with grades 2:3/3:2/3:3; OR 0.3181; 95% CI: 0.171, 0.573).

DISCUSSION

In summary, using the EmbryoScope for culture to the blastocyst stage, coupled with objective embryo selection criteria based on a morphokinetic algorithm, embryos can be successfully ranked based on their chance of achieving a live birth. An embryo rank of D is estimated be associated with a 69.5%

TABLE 2 SUMMARY STATISTICS FOR EACH CONTINUOUS OR DISCRETE VARIABLE CONSIDERED IN THE ANALYSIS VERSUS EMBRYO RANK. THE MEAN (AND SD = STANDARD DEVIATION) AND MEDIAN (AND RANGE), BY RANK

Variable	Summary	A (*n* = 354)	B (*n* = 273)	C (*n* = 86)	D (*n* = 68)	Total (*n* = 781)
Patient age (years)	Mean (SD)	35.4 (5.11)	36.0 (4.96)	36.2 (5.13)	36.5 (5.20)	35.8 (5.07)
Patient age (years)	Median (range)	35.0 (22–48)	35.0 (24–49)	36.0 (25–50)	36.5 (26–47)	35.0 (22–50)
Total previous cycles	Mean (SD)	3.2 (2.02)	3.1 (1.62)	3.6 (2.52)	3.9 (2.42)	3.3 (2.00)
Total previous cycles	Median (range)	2.0 (2–16)	2.0 (2–12)	3.0 (2–18)	3.0 (2–16)	2.0 (2–18)
Total previous miscarriages	Mean (SD)	0.3 (0.61)	0.3 (0.69)	0.4 (0.84)	0.4 (0.77)	0.3 (0.68)
Total previous miscarriages	Median (range)	0.0 (0–4)	0.0 (0–5)	0.0 (0–5)	0.0 (0–3)	0.0 (0–5)
Maximum endometrial thickness (mm)	Mean (SD)	10.9 (2.60)	10.6 (2.28)	10.9 (2.62)	10.5 (2.16)	10.8 (2.46)
Maximum endometrial thickness (mm)	Median (range)	10.6 (6–20)	10.2 (6–19)	10.2 (6–17.5)	10.5 (7–19.5)	10.4 (6–20)
Donor age (years)	Mean (SD)	29.3 (3.98)	29.6 (3.78)	30.8 (3.75)	30.3 (4.38)	29.6 (3.92)
Donor age (years)	Median (range)	30.0 (20–36)	30.0 (21–35)	31.0 (22–37)	29.0 (24–38)	30.0 (20–38)
Duration of infertility (years)	Mean (SD)	3.3 (2.47)	3.0 (2.27)	2.9 (2.38)	3.6 (3.10)	3.2 (2.46)
Duration of infertility (years)	Median (range)	3.0 (0–12)	3.0 (0–16)	2.0 (0–15)	3.0 (0–21)	3.0 (0–21)
Gonadotrophin total dose (IU)	Mean (SD)	2283.1 (981.18)	2294.3 (1112.53)	2439.2 (1075.45)	2862.5 (1199.18)	2356.2 (1069.58)
Gonadotrophin total dose (IU)	Median (range)	2250.0 (450–5400)	2250.0 (450–6000)	1800.0 (675–5400)	2700.0 (525–5400)	2250.0 (450–6000)
Gonadotrophin dosing days	Mean (SD)	10.2 (2.42)	9.8 (2.73)	10.3 (2.31)	10.6 (1.72)	10.1 (2.48)
Gonadotrophin dosing days	Median (range)	12.0 (3–12)	10.0 (3–12)	12.0 (3–12)	10.5 (6–12)	10.0 (3–12)
Eggs collected	Mean (SD)	12.2 (5.72)	11.2 (5.52)	9.8 (5.45)	7.2 (3.71)	11.1 (5.65)
Eggs collected	Median (range)	11.0 (1–34)	11.0 (1–30)	9.0 (2–26)	7.0 (1–21)	10.0 (1–34)
Mature eggs inseminated	Mean (SD)	9.8 (4.71)	9.2 (5.00)	8.1 (4.68)	5.3 (3.56)	9.0 (4.88)
Mature eggs inseminated	Median (range)	9.0 (0–28)	9.0 (0–28)	7.0 (1–25)	4.5 (1–18)	8.0 (0–28)
Mature eggs inseminated out of eggs collected ratio	Mean (SD)	0.8 (0.17)	0.8 (0.19)	0.8 (0.18)	0.7 (0.24)	0.8 (0.19)
Mature eggs inseminated out of eggs collected ratio	Median (range)	0.9 (0–1)	0.9 (0–1)	0.9 (0.25–1)	0.8 (0.125–1)	0.9 (0–1)
Oocyte recipient patient age (years)	Mean (SD)	41.4 (4.01)	41.4 (4.74)	41.6 (5.61)	40.0 (5.41)	41.3 (4.55)
Oocyte recipient patient age (years)	Median (range)	42.5 (24–48)	42.0 (27–49)	44.0 (29–50)	40.5 (26–47)	42.0 (24–50)
Non-oocyte recipient patient age (years)	Mean (SD)	33.6 (3.88)	34.4 (3.72)	34.6 (3.78)	35.6 (4.79)	34.2 (3.94)
Non-oocyte recipient patient age (years)	Median (range)	34.0 (22–43)	34.0 (24–46)	35.0 (25–41)	35.5 (26–45)	34.0 (22–46)

decrease in the odds of a live birth compared with an embryo of rank A. The 95% CI for this comparison ranges from an 87.1% decrease to a 34.0% decrease in the odds. The analysis also highlighted the weakness of dependence on conventional morphology alone as a selection tool. For example, a grade 2:2 blastocyst, which is the equivalent of the Gardner and Schoolcraft (1999) ranking BB, results in live birth at a significantly higher frequency than embryos graded 1:1/1:2 or 2:1, which contradicts previous publications (*Gardner and Schoolcraft, 1999; Gardner et al., 2000;* Istanbul Consensus, 2011). While TLI algorithms are objective and likely to achieve greater consistency in embryo selection, static morphology assessments remain subjective and prone to variability. Additionally, morphology grading is likely to reflect a limited number of single daily time-points when the grade is assigned, whereas TLI can provide photographs of the embryo at frequent intervals throughout culture; this in turn provides the opportunity to witness and evaluate changes in embryo morphology over time and therefore avoid the unreliability of a single-moment, subjective morphology grading. One of the limitations of most TLI studies is the comparison between TLI and conventional incubators for culturing embryos, irrespective of the use of algorithms; this type of comparison leaves open the question of whether uninterrupted incubation alone is an advantage, whether or not morphokinetics are considered for embryo selection. This study compares embryos cultured only in the EmbryoScope from Day 0 (ICSI) or Day 1 (IVF) up to Day 5 of development.

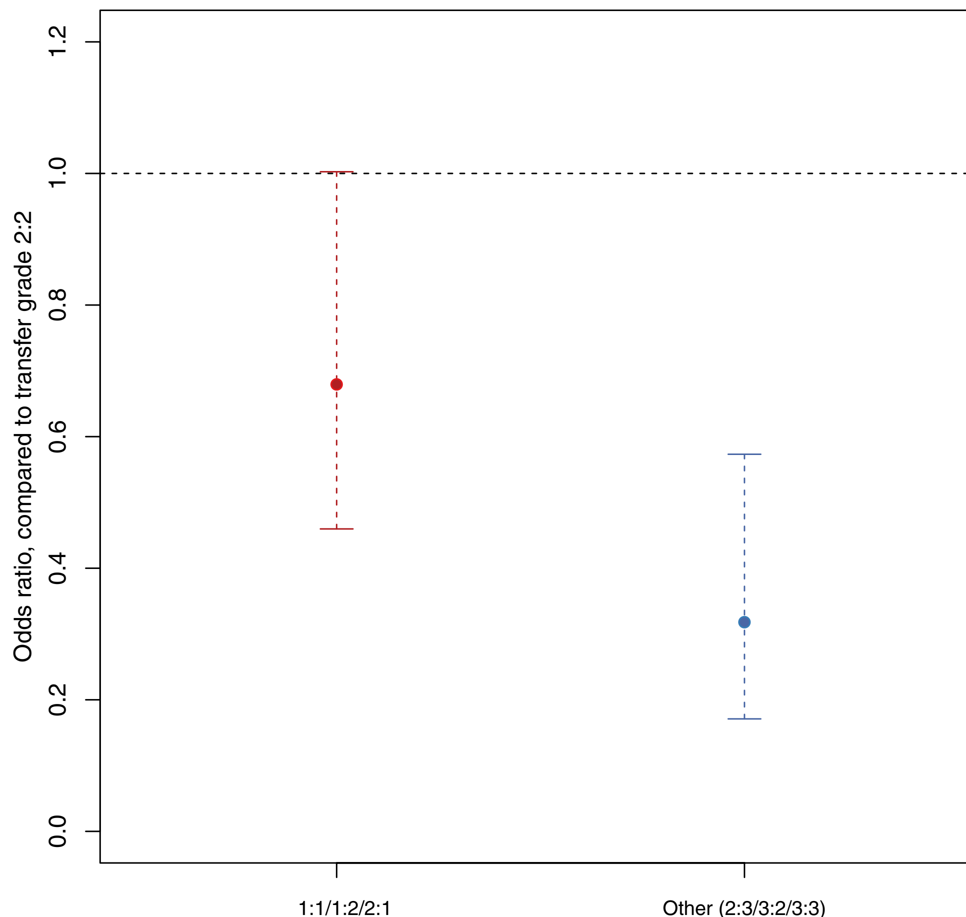

FIGURE 1 Model estimated odds ratios, comparing the occurrence of live birth between transfer grades. The bars indicate 95% confidence intervals.

It also includes single blastocyst transfers only, to avoid any ambiguity of the effects of more than one embryo transferred. The results demonstrate the value of an objective algorithm for embryo selection. The main limitations of this study are, first, that the analysis was undertaken to validate the effectiveness of an in-house-derived algorithm and this algorithm may not produce similar results in other settings (Freour et al., 2015; Liu et al., 2016). Secondly, the efficacy of this ranking system remains to be demonstrated prospectively. Further, it is not possible to account for any clinical or physiological differences not contained within the available dataset and therefore this investigation may not have eliminated all confounding effects in this non-randomized study.

TLI has been used in clinical practice for over 10 years (*Hlinka et al.*, 2012; Lemmen et al., 2008) with several recent reviews (Armstrong et al., 2015; *Chen et al.*, 2017; Kaser & Racowsky, 2014; Polanski et al., 2014; Racowsky et al., 2015). Systematic reviews have either considered studies comparing TLI incubation to conventional incubation, or the overall impact of TLI as compared with conventional methods, or the potential effect of using morphokinetics for embryo selection. Van Loendersloot *et al.* (2014) applied a retrospective multi-variable clinical pregnancy prediction model to rank embryos following Day 3 transfer, distinguishing those embryos with high, moderate or low implantation potential. Petersen *et al.* (2016) also used a morphokinetic algorithm for embryos transferred on Day 3 to predict blastulation based on data from 24 clinics over a 5-year period. However, apart from patient age, and whether IVF or ICSI was performed, embryos were treated as independent variables; there is concern regarding the validity of such studies because cohorts of embryos should not be considered independent. When associating embryological data with clinical outcome, all potential clinical confounders must be considered (*Fishel et al.*, 2017; Kirkegaard et al., 2016).

Several studies have, however, reported on the use of TLI as a potential prognosticator in clinical practice (Adamson et al., 2016; Chen et al., 2016; Kong et al., 2016; Liu et al., 2016; Milewski et al., 2015; Mizobe et al., 2016; Rubio et al., 2014; VerMilyea et al., 2014; Wu et al., 2016; Yang et al., 2014). Other studies have disagreed (Freour et al., 2015; Wu et al., 2016). Given the complex nature of the effects of culture conditions and the milieu on preimplantation development, in addition to their inherent genetic and chromosomal complements, it is difficult to pinpoint any single feature that may directly impact outcome, especially because development of the implanting embryo to a live baby depends also on the maternal environment. A few studies have purported to relate discriminating morphokinetics of euploid and aneuploid embryos (Campbell et al., 2013a, 2013b; Minasi et al., 2016; Vera-Rodriguez et al., 2015) although

this has been disputed (Rienzi et al., 2015). However, Bronet et al. (2015) even found distinctive morphokinetic differences between male and female embryos. More recently Mumusoglu et al. (2017), studying morphokinetics and the prediction of ploidy status when patient and ovarian stimulation–related factors were taken into account, concluded that aneuploid embryo development appears to be delayed at post-cleavage stages, but that the predictive ability was 'low to moderate'. Of previously reported cut-off points for various TLM parameters, they only noted tSB within 96.6 h of insemination as having significant predictive ability (Campbell et al., 2014). Kong et al. (2016) reported a relationship between early cell division behaviour and developmental potential with elongation or shortening of the cell cycle affecting cell number. This study concluded that by excluding such embryos, the incidence of implantation and live birth following Day 3 transfer of embryos increased when cell number was maximal. Recently Ottolini et al. (2017), in an important study using TLI and genome-wide SNP genotyping and meiomapping of both polar bodies, analysed tripolar and other abnormal mitoses demonstrating that failure to coordinate the cell cycle in early cleavage and regulation of centrosome duplication is a major cause of human preimplantation developmental arrest *in vitro*.

Morphological evaluation of the embryos at specific time-points has been the method of choice for embryo selection for decades (Cummins et al., 1986; Fishel et al., 1983, 1983), although its limitations have later been recognized (Fehilly et al., 1985; Guerif et al., 2007; Hartshorne et al., 1991; Racowsky, 2009). Blastocysts also undergo a normal cycle of collapse and re-expansion and are often difficult to grade reliably. Although they can be reassessed at other time-points (The Istanbul Consensus, 2011), TLI highlights the transient nature of certain morphologies and thus a source of weakness of morphology-based grading as a prognosticator. A number of studies have found weak correlation between blastocyst morphology and chromosomal abnormalities, including those incompatible with post-implantation development (Fragouli et al., 2010; Schoolcraft et al., 2010).

In the most recent meta-analysis on five randomized controlled trials using TLI algorithms, Pribenszky et al. (2017) reported a significantly higher incidence of ongoing clinical pregnancy and live birth, and a significantly lower incidence of early pregnancy loss following time-lapse incubation and algorithm-based embryo selection compared with conventional culture with embryo selection based on single time-point morphology. The studies included a heterogeneous patient population, days of transfer, the way the visual information from the time-lapse devices was used to support embryo evaluation and end-points, and as such the quality of the evidence was deemed moderate to low owing to inconsistencies across the studies. Indeed, the studies in general did not include a comprehensive clinical confounder analysis. Motato et al. (2016) used the timing of expanded blastocyst formation (tEB) as the primary variable at ≤122.9 h and the synchronicity of the third round of cleavage divisions (s3 = t8 – t5) as the secondary variable setting the optimal range of ≤5.67 h. Motato et al. (2016) used these data to divide embryos into four categories (A–D), with a decreasing implantation potential (from 72.2% for A to 39.7% for D). However, there are now many different algorithms of apparent efficacy, but in some laboratories advantages have not been proven, as comprehensively reviewed recently by Milewski and Ajduk (2017).

In conclusion, this study demonstrates the unique live birth capacity of individual blastocysts based on an algorithm that incorporates time to start and duration of blastulation. These parameters must be carefully annotated by embryologists, with tight quality control on annotation principles, and within a single culture system. This study further demonstrates the advantage of objective data, which can be revisited any time (as TLI images, for example) without embryo disruption, over the capricious and subjective morphological scoring at one or more discrete time-points. The study also highlights the need to ensure that analyses do not treat embryos as independent variables because this can lead to erroneous conclusions; it also argues in favour of inclusion of full clinical data in analyses of this type before drawing conclusions on clinical impact. The data importantly implicate a relatively simple algorithm to rank and therefore select embryos in clinical practice to improve the chance of a live birth. Although more high-quality evidence, such as large, well-controlled prospective randomized studies, are needed to definitively demonstrate the value of TLI, further research into other cellular processes such as chromosome segregation, cytoskeleton function and energy metabolism are required to reduce embryo wastage and unnecessary embryo transfers and cryopreservation and enable selection of only those embryos with the capacity to reach full term.

REFERENCES

Adamson, G.D., Abusief, M.E., Palao, L., Witmer, J., Palao, L.M., Gvakharia, M. **Improved implantation rates of day 3 embryo transfers with the use of an automated time-lapse-enabled test to aid in embryo selection.** Fertil. Steril. 2016; 105: 369–375.e6. doi:10.1016/j.fertnstert.2015.10.030

Armstrong, S., Arroll, N., Cree, L.M., Jordan, V., Farquhar, C. **Time-lapse systems for embryo incubation and assessment in assisted reproduction.** Cochrane Database Syst Rev 2015;CD011320. https://doi.org/10.1002/14651858.CD011320.pub2. PMID: 25721906

Athayde Wirka, K., Chen, A.A., Conaghan, J., Ivani, K., Gvakharia, M., Behr, B., Suraj, V., Tan, L., Shen, S. **Atypical embryo phenotypes identified by time-lapse microscopy: high prevalence and association with embryo development.** Fertility and Sterility 2014; 101: 1637–1648

Bronet, F., Nogales, M.-C., Martínez, E., Ariza, M., Rubio, C., García-Velasco, J.-A., Meseguer, M. **Is there a relationship between time-lapse parameters and embryo sex?** Fertil Steril 2015; 103: 396–401.e2. doi: 10.1016/j.fertnstert.2014.10.050

Campbell, A., Fishel, S., Bowman, N., Duffy, S., Sedler, M., Hickman, C.F.L. **Modelling a risk classification of aneuploidy in human embryos using non-invasive morphokinetics.** Reprod. Biomed. Online 2013; 26: 477–485 doi: 10.1016/j.rbmo.2013.02.006

Campbell, A., Fishel, S., Bowman, N., Duffy, S., Sedler, M., Thornton, S. **Retrospective analysis of outcomes after IVF using an aneuploidy risk model derived from time-lapse imaging without PGS.** Reprod. Biomed. Online 2013; 27: 140–146. doi: 10.1016/j.rbmo.2013.04.013

Campbell, A. **Non-invasive techniques: embryo selection by timelapse imaging.** In: Montag, M. (ed.), A Practical Guide to Selecting Gametes and Embryos, CRC Press, Boca Raton, FL, USA 2014: 177–189

Chen, F., De Neubourg, D., Debrock, S., Peeraer, K., D'Hooghe, T., Spiessens, C. **Selecting the embryo with the highest implantation potential using a data mining based prediction model.** Reprod. Biol. Endocrinol. RBE 2016; 14. doi: 10.1186/s12958-016-0145-1

Ciray, H.N., Aksoy, T., Goktas, C., Ozturk, B., Bahceci, M. **Time-lapse evaluation of human embryo development in single versus sequential culture media–a sibling oocyte study.** Journal of assisted reproduction and genetics 2012; 29: 891–900

Core Team, R. **R: A language and environment for statistical computing.** R Foundation for Statistical Computing, Vienna, Austria; 2016. Vienna, Austria http://www.R-project.org/

Cruz, M., Gadea, B., Garrido, N., Pedersen, K.S., Martínez, M., Pérez-Cano, I., Muñoz, M., Meseguer, M. **Embryo quality, blastocyst and ongoing pregnancy rates in oocyte donation patients whose embryos were monitored by time-lapse imaging.** J. Assist. Reprod. Genet. 2011; 28: 569–573

Cruz, M., Garrido, N., Herrero, J., Pe´rez-Cano, I., Munõz, M., Meseguer, M. **Timing of cell division in human cleavage-stage embryos is linked with blastocyst formation and quality.** Reproductive BioMedicine 2012; 25: 371–381

Dal Canto, M., Coticchio, G., Mignini Renzini, M., De Ponti, E., Novara, P.V., Brambillasca, F., Comi, R., Fadini, R. **Cleavage kinetics analysis of human embryos predicts development to blastocyst and implantation.** Reprod Biomed Online 2012; 25(5): 474–480

Del Carmen Nogales, M, Bronet, F, Basile, N, Martinez, EM, Linan, A, Rodrigo, L, Meseguer, M. **Type of chromosome abnormality affects embryo morphology dynamics.** Fertility and sterility 2017; 107: 229–235.e2

Fehilly, C.B., Cohen, J., Simons, R.F., Fishel, S.B., Edwards, R.G. **Cryopreservation of cleaving embryos and expanded blastocysts in the human: a comparative study.** Fertil Steril 1985 Nov; 44(5): 638–644

Fishel, S., Baker, D., Elson, J., Ragunath, M., Atkinson, G., Shaker, A., Omar, A., Kazem, R., Beccles, A., Greer, I.A. **Precision Medicine in Assisted Conception: A Multicentre Observational Treatment Cohort Study of the Annexin A5 M2 Haplotype as a Biomarker for Antithrombotic Treatment to Improve Pregnancy Outcome.**EBioMedicine 2016; 10: 298–304. doi: 10.1016/j.ebiom.2016.06.024

Fishel, S., Campbell, A., Montgomery, S., Smith, R., Nice, L., Duffy, S., Jenner, L., Berrisford, K., Kellam, L., Smith, R., D'Cruz, I., Beccles, A. **Live births after embryo selection using morphokinetics versus conventional morphology: a retrospective analysis.** Reproductive Biomedicine Online 2017; 35: 407–416

Fishel, SB, Edwards, RG, Purdy, J **Fertilization of the Human Egg *in vitro*, Biological Basis and Clinical Application.** In: Beier H.M., Lindner, H.R. (Ed.), Fertilization of the Human Egg *In vitro*, Biological Basis and Clinical Application. Springer-Verlag, Berlin. 1983: 251–270

Franasiak, JM, Forman, EJ, Hong, KH, Werner, MD, Upham, KM, Treff, NR, Scott, RTJr **The nature of aneuploidy with increasing age of the female partner: a review of 15,169 consecutive trophectoderm biopsies evaluated with comprehensive chromosomal screening.** Fertility and sterility. 2014; 101: 656–663

Fragouli, E., Katz-Jaffe, M., Alfarawati, S., Stevens, J., Colls, P., Goodall, N., Tormasi, S., Gutierrez-Mateo, C., Prates, R., Schoolcraft, W.B., Munne´, S., Wells, D. **Comprehensive chromosome screening of polar bodies and blastocysts from couples experiencing repeated implantation failure.** Fertil. Steril. 2010; 94: 875–887

Freour, T., Basile, N., Barriere, P., Meseguer, M. **Systematic review on clinical outcomes following selection of human preimplantation embryos with time-lapse monitoring.** Hum. Reprod. Update 2015; 21: 153–154. doi:10.1093/humupd/dmu054

Gardner, D.K., Lane, M., Stevens, J., Schlenker, T., Schoolcraft, W.B. **Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer.**Fertil. Steril. 2000; 73: 1155–1158

Gardner, D.K., Schoolcraft, W.B. **Culture and transfer of human blastocysts.** Curr. Opin. Obstet. Gynecol. 1999; 11: 307–311

Guerif, F., Le Gouge, A., Giraudeau, B., Poindron, J., Bidault, R., Gasnier, O., Royere, D. **Limited value of morphological assessment at days 1 and 2 to predict blastocyst development potential: a prospective study based on 4042 embryos.** Hum Reprod. 2007 Jul; 22(7): 1973–1981

Hartshorne, G.M., Elder, K., Crow, J., Dyson, H., Edwards, R.G. **The influence of in-vitro development upon post-thaw survival and implantation of cryopreserved human blastocysts.** Hum Reprod. 1991 Jan; 6(1): 136–141

Hashimoto, S., Nakano, T., Yamagata, K., Inoue, M., Morimoto, Y., Nakaoka, Y. **Multinucleation per se is not always sufficient as a marker of abnormality to decide against transferring human embryos.** Fertil. Steril. 2016; 106: 133–139. doi: 10.1016/j.fertnstert.2016.03.025

Heinze, G., Ploner, M. **logistf: Firth's bias reduced logistic regression.** R package version 2016; 1: 22. http://CRAN.R-project.org/package=logistf

Herrero, J., Meseguer, M. **Selection of high potential embryos using time-lapse imaging: the era of morphokinetics.** Fertil Steril 2013 Mar 15; 99(4): 1030–1034

Hlinka, D., Kaľatová, B., Uhrinová, I., Dolinská, S., Rutarová, J., Rezáčová, J., Lazarovská, S., Dudáš, M. **Time-lapse cleavage rating predicts human embryo viability.**Physiol. Res. Acad. Sci. Bohemoslov 2012; 61: 513–525

Kaser, D.J., Racowsky, C. **Clinical outcomes following selection of human preimplantation embryos with time-lapse monitoring: a systematic review.** Hum. Reprod. Update 2014; 20: 617–631. doi: 10.1093/humupd/dmu023

Kirkegaard, K., Campbell, A., Agerholm, I., Bentin-Ley, U., Gabrielsen, A., Kirk, J., Sayed, S., Ingerslev, H.J. **Limitations of a time-lapse blastocyst prediction model: a large multicentre outcome analysis.** Reprod. Biomed. Online 2014; 29: 156–158. doi: 10.1016/j.rbmo.2014.04.011

Kirkegaard, K., Sundvall, L., Erlandsen, M., Hindkjær, J.J., Knudsen, U.B., Ingerslev, H.J. **Timing of human preimplantation embryonic development is confounded by embryo origin.** Hum. Reprod. Oxf. Engl. 2016; 31: 324–331. doi: 10.1093/humrep/dev296

Kramer, YG, Kofinas, JD, Melzer, K, Noyes, N, McCaffrey, C, Buldo-Licciardi, J, McCulloh, DH, Grifo, JA.l **Assessing morphokinetic parameters via time lapse microscopy (TLM) to predict euploidy: are aneuploidy risk classification models universal?.** Journal of assisted reproduction and genetics 2014; 31: 1231–1242

Kong, X., Yang, S., Gong, F., Lu, C., Zhang, S., Lu, G., Lin, G. **The Relationship between Cell Number, Division Behaviour and Developmental Potential of Cleavage Stage Human Embryos: A Time-Lapse Study.** PloS One 2016; 11e0153697. doi: 10.1371/journal.pone.0153697

Lagalla, C, Tarozzi, N, Sciajno, R, Wells, D, Di Santo, M, Nadalini, M, Distratis, V, Borini, A. **Embryos with morphokinetic abnormalities may develop into euploid blastocysts.** Reproductive biomedicine online 2017; 34: 137–146

Lemmen, J.G., Agerholm, I., Ziebe, S. **Kinetic markers of human embryo quality using time-lapse recordings of IVF/ICSI-fertilized oocytes.** Reprod. Biomed. Online 2008; 17: 385–391

Liu, Y., Chapple, V., Feenan, K., Roberts, P., Matson, P. **Time-lapse deselection model for human day 3 *in vitro* fertilization embryos: the combination of qualitative and quantitative measures of embryo growth.**

Fertil. Steril 2016; 105: 656–662. doi: 10.1016/j.fertnstert.2015.11.003

Milewski, R., Ajduk, A. **Time-lapse imaging of cleavage divisions in embryo quality assessment.** Reproduction 2017 Aug; 154(2): R37–R53

Milewski, R., Kuć, P., Kuczyńska, A., Stankiewicz, B., Łukaszuk, K., Kuczyński, W. **A predictive model for blastocyst formation based on morphokinetic parameters in time-lapse monitoring of embryo development.** J. Assist. Reprod. Genet. 2015; 32: 571–579. doi: 10.1007/s10815-015-0440-3

Minasi, M.G., Colasante, A., Riccio, T., Ruberti, A., Casciani, V., Scarselli, F., Spinella, F., Fiorentino, F., Varricchio, M.T., Greco, E. **Correlation between aneuploidy, standard morphology evaluation and morphokinetic development in 1730 biopsied blastocysts: a consecutive case series study.** Hum. Reprod. Oxf. Engl. 2016; 31: 2245–2254. doi: 10.1093/humrep/dew183

Mizobe, Y., Oya, N., Iwakiri, R., Yoshida, N., Sato, Y., Miyoshi, K., Tokunaga, M., Ezono, Y. **Effects of early cleavage patterns of human embryos on subsequent *in vitro* development and implantation.** Fertil. Steril 2016; 106: 348–353. doi: 10.1016/j.fertnstert.2016.04.020

Motato, Y, de los Santos, MJ, Escriba, MJ, Ruiz, BA, Remohi, J, Meseguer, M **Morphokinetic analysis and embryonic prediction for blastocyst formation through an integrated time-lapse system.** Fertility and Sterility 2016; 105: 376–384. doi: 10.1016/j.fertnstert.2015.11.001

Mumusoglu, S, Yarali, I, Bozdag, G, Ozdemir, P, Polat, M, Sokmensuer, LK, Yarali, H. **Time-lapse morphokinetic assessment has low to moderate ability to predict euploidy when patient- and ovarian stimulation-related factors are taken into account with the use of clustered data analysis.** Fertility and sterility 2017; 107: 413–421.e4

Ottolini, Christian S., Kitchen, John, Xanthopoulou, Leoni, Gordon, Tony, Summers, Michael C., Handyside, Alan H. **Tripolar mitosis and partitioning of the genome arrests human preimplantation development *in vitro*.** Sci Rep. 2017 Aug 29; 7(1): 9744

Polanski, LT, Coelho Neto, MA, Nastri, CO, Navarro, PA, Ferriani, RA, Raine-Fenning, N, Martins, WP. **Time-lapse embryo imaging for improving reproductive outcomes: systematic review and meta-analysis.** Ultrasound Obstet Gynecol. 2014; 44: 394–401 https://doi.org/10.1002/uog.13428

Pribenszky, C., Mátyás, S., Kovács, P., Losonczi, E., Zádori, J., Vajta, G. **Pregnancy achieved by transfer of a single blastocyst selected by time-lapse monitoring.** Reprod. Biomed. Online 2010; 21: 533–536

Pribenszky, C., Nilselid, A-M., Montag, M. **Time-lapse culture with morphokinetic embryo selection improves pregnancy and live birth chances and reduces early pregnancy loss: a meta-analysis.** Reprod. Biomed. Online 2017, in press, http://creativecommons.org/licenses/by-nc-nd/4.0/)

Rienzi, L., Capalbo, A., Stoppa, M., Romano, S., Maggiulli, R., Albricci, L., Scarica, C., Farcomeni, A., Vajta, G., Ubaldi, F.M. **No evidence of association between blastocyst aneuploidy and morphokinetic assessment in a selected population of poor-prognosis patients: a longitudinal cohort study.** Reprod. Biomed. Online 2015; 30: 57–66. doi: 10.1016/j.rbmo.2014.09.012

Racowsky, C, Kovacs, P, Martins, WP. **A critical appraisal of time-lapse imaging for embryo selection: where are we and where do we need to go?** J Assist Reprod Genet 2015; 32: 1025–1030

Racowsky, C., Ohno-Machado, L., Kim, J., Biggers, J.D. **Is there an advantage in scoring early embryos on more than one day?** Hum Reprod. 2009 Sep; 24(9): 2104–2113

Rubio, I., Galán, A., Larreategui, Z., Ayerdi, F., Bellver, J., Herrero, J., Meseguer, M. **Clinical validation of embryo culture and selection by morphokinetic analysis: a randomized, controlled trial of the EmbryoScope.** Fertil. Steril. 2014; 102: 1287–1294. doi: 10.1016/j.fertnstert.2014.07.738

Schoolcraft, W.B., Fragouli, E., Stevens, J., Munne´, S., Katz-Jaffe, M.G., Wells, D. **Clinical application of comprehensive chromosomal screening at the blastocyst stage.** Fertil. Steril. 2010; 94: 1700–1706

The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Hum. Repro., 26, 2011; 26: 1270–1283

Vera-Rodriguez, M., Chavez, S.L., Rubio, C., Reijo Pera, R.A., Simon, C. **Prediction model for aneuploidy in early human embryo development revealed by single-cell analysis.** Nat. Commun. 2015; 6: 7601.doi: 10.1038/ncomms8601

VerMilyea, M.D., Tan, L., Anthony, J.T., Conaghan, J., Ivani, K., Gvakharia, M., Boostanfar, R., Baker, V.L., Suraj, V., Chen, A.A., Mainigi, M., Coutifaris, C., Shen, S. **Computer-automated time-lapse analysis results correlate with embryo implantation and clinical pregnancy: a blinded, multi-centre study.** Reprod. Biomed. Online 2014; 29: 729–736. doi: 10.1016/j.rbmo.2014.09.005

Wong, C.C., Loewke, K.E., Bossert, N.L., Behr, B., De Jonge, C.J., Baer, T.M., Reijo Pera, R.A. **Non-invasive imaging of human embryos before embryonic genome activation predicts development to the blastocyst stage.** Nat. Biotechnol. 2010; 28: 1115–1121

Wu, L., Han, W., Zhang, X., Wang, J., Liu, W., Xiong, S., Huang, G. **A retrospective analysis of morphokinetic parameters according to the implantation outcome of IVF treatment.** Eur. J. Obstet. Gynecol. Reprod. Biol. 2016; 197: 186–190. doi: 10.1016/j.ejogrb.2015.12.002

Yang, Z., Zhang, J., Salem, S.A., Liu, X., Kuang, Y., Salem, R.D., Liu, J. **Selection of competent blastocysts for transfer by combining time-lapse monitoring and array CGH testing for patients undergoing preimplantation genetic screening: a prospective study with sibling oocytes.** BMC Med. Genomics 2014; 7: 38. doi: 10.1186/1755-8794-7-38

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