Reproductive BioMedicine Online (2013) **26**, 210–221

www.sciencedirect.com
www.rbmonline.com

SYMPOSIUM: EVIDENCE-BASED REPRODUCTIVE MEDICINE

Morphological systems of human embryo assessment and clinical evidence

Ronit Machtinger, Catherine Racowsky *

Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, United States
* Corresponding author. *E-mail address:* cracowsky@partners.org (C Racowsky).

Ronit Machtinger received her MD from Tel-Aviv University, Israel in 1999, followed by a postgraduate diploma in obstetrics and gynaecology in 2003. She completed her residency at the Sheba Medical Center at 2006 and has been part of the physician team of the IVF unit at the centre since then. She has been a research fellow at the REI division of Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA. She was awarded a 2-year part-time fellowship by the MRgFUS Foundation and the Environmental Health Fund (EHF). Her fields of interest include effects of the environment on the ovary, oocyte maturation *in vitro*, embryo morphology and MRI-guided focused ultrasound for uterine fibroids.

Abstract Success rates with IVF have improved remarkably since the procedure was first established for clinical use with the first successful birth in 1978. The main goals today are to perform single-embryo transfer in order to prevent multiple pregnancies and achieve higher overall pregnancy rates. However, the ability to identify the most viable embryo in a cohort remains a challenge despite the numerous scoring systems currently in use. Clinicians still depend on developmental rate and morphological assessment using light microscopy as the first-line approach for embryo selection. Active research in the field involves developing non-invasive methods for scoring embryos and ranking them according to their ability to implant and give rise to a healthy birth. Current attention is particularly being focused on time-lapse evaluation. Available data from preliminary studies indicate that these systems are safe; prospective data now need to be collected to determine whether these methods do improve implantation rates. This review gives brief consideration to the use of morphological evaluations in assisted reproduction treatment, discusses the types of embryo scoring, digital imaging and biometric approaches currently in use and comments on future developments for embryo evaluation.

KEYWORDS: clinical IVF, embryo morphology, embryo rank, embryo scoring systems, IVF, time-lapse imaging

Introduction

Over the past 35 years, success rates with IVF have improved remarkably. However, the ability to identify the most viable embryo in a cohort remains a challenge. Despite active research in the field, clinicians still depend on developmental rate and morphological assessment using light microscopy as the first-line approach for embryo selection (reviewed by Montag et al., 2011). A variety of non-invasive technologies for the assessment of human embryos have been developed but, to date, none have been proven superior to standard morphological evaluation. This review gives brief consideration to the use of morphological evaluations in assisted reproduction treatment, discusses the types of embryo scoring, digital imaging and biometric approaches

1472-6483/$ - see front matter
http://dx.doi.org/10.1016/j.rbmo.2012.10.021

currently in use and comments on future developments for embryo evaluation.

Types of studies used and preferred for embryo evaluation

Selection of the best embryos to transfer is largely based on clinical tradition and less derived from evidence-based medicine (Holte et al., 2007). The preferred studies for evidence-based medicine are prospective randomized trials. However, such trials are very challenging to perform when considering human embryos (Giorgetti et al., 1995; Ziebe et al., 1997; Van Royen et al., 1999; Holte et al., 2007) due to the following reasons: (i) in cases for which the number of embryos transferred exceeds the number of gestational sacs or viable fetuses present, the ability to record the outcome of each individual embryo is limited; (ii) as single-embryo transfer is not widely used in most IVF units, the majority of studies with this type of data are based on relatively small numbers of embryos; (iii) the variability in patient selection and embryo grading systems within different clinics make inter-clinic comparison very difficult; and (iv) every embryo and every patient is unique, making it difficult to compare different evaluation systems and outcomes. As a result, most of the studies available in this field are retrospective or include small sample sizes.

Original use of morphological studies in assisted reproduction treatment

Morphological scoring of gametes and embryos has been used since the inception of IVF, initially to define embryo development (Edwards et al., 1981) (**Figure 1**) and later as a tool for selecting the best embryos for transfer, i.e. those with the highest implantation potential (Hill et al., 1989; Desai et al., 2000; Racowsky et al., 2010; Montag et al., 2011; Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011). Since human embryonic development follows a specifically timed, co-ordinated sequence of events, developmental rate (assessed by certain milestones being reached at particular points in time) and morphological characteristics (defined at specified intervals after the day of insemination) have provided the two main morphological measures of embryonic development. Various approaches have been undertaken including those that assess embryos either sequentially at several stages during early development, or only once, immediately before transfer. These collective approaches have led to analyses and debate as to whether sequential, or so-called 'cumulative', scoring provides superior sensitivity and specificity for selection, compared with a single-step scoring approach (reviewed by Racowsky et al., 2009).

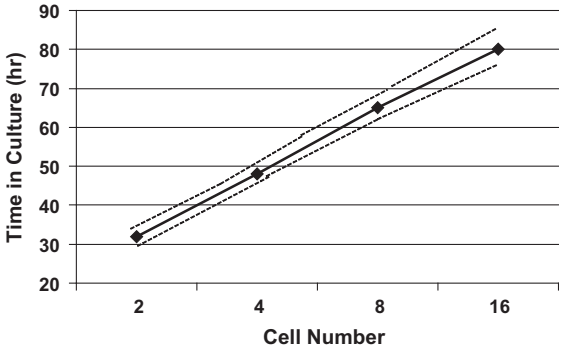

Figure 1 The estimated 'growth' curve of development of human embryos in culture. The solid line represents the average developmental timeline; the dashed lines indicate that 95% confidence limits. Reprinted from: Edwards et al., 1981. The growth of human preimplantation of embryos. Am. J. Obstet. Gynecol. 141, 408–416 (**Figure 3**). Copyright 1981, with permission from Elsevier.

Zygote pronuclear scoring systems

Over a decade ago, a number of systems were developed to score zygotes at the pronuclear stage (Scott and Smith, 1998; Tesarik and Greco, 1999; Scott et al., 2000). The most commonly used systems assess the number and relative position of the nucleolar precursor bodies (NPB) in each pronucleus (PN), with/without evaluation of the PN size and alignment, and appearance of the cytoplasm.

The predictive value of PN scoring is controversial and most of the studies are retrospective. Many investigators have found a relationship between PN scoring and improved embryo development/blastocyst formation (Balaban et al., 2001; Rienzi et al., 2002; Zollner et al., 2002; Nagy et al., 2003) and/or increased pregnancy and implantation potential (Wittemer et al., 2000; Montag and van der Ven, 2001; reviewed by Zollner et al., 2003). These studies have shown that the most viable zygotes have PN of similar size and that are centrally located, with each containing NPB of equal size and number aligned at the pronuclear interphase in preparation for syngamy (Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011). However, numerous other studies have failed to show that the addition of PN scoring to a cumulative scoring system does, in fact, improve selection of viable embryos (reviewed by Skiadas and Racowsky, 2007).

Cleavage-stage scoring systems

The features of cell number, degree of fragmentation, equality of size and shape of blastomeres and multinucleation have been frequently evaluated at this stage to determine viability. Both the Society for Assisted Reproductive Technology (SART) (Racowsky et al., 2010) and the Alpha Scientists in Reproductive Medicine and the ESHRE Special Interest Group of Embryology (Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011) have published cleavage-stage scoring systems.

Cell number

Edwards et al. (1981) showed that normal human embryos progress along a predictable timeline from the 1-cell stage to the 16-cell stage (**Table 1**). From their data, an 'average growth curve' was generated for normal development of the human embryo up to the 16-cell stage (**Figure 1**). Consistent with these observations, other studies have reported the presence of optimal cleavage rates and showed that

Table 1 Estimates of time from insemination to attainment of specified stages of development. Reprinted from: Edwards et al., 1981. The growth of human preimplantation of embryos. Am. J. Obstet. Gynecol. 141, 408–416 (**Table 4**). Copyright 1981, with permission from Elsevier.

Cell stage	*Estimate of midpoint of cleavage stages (mean hours ± SE)*	*Upper 95% point of distribution (h)*
2-cell	33.2 ± 1.3	47
4-cell	49.0 ± 1.3	63
8-cell	64.8 ± 1.8	84
16-cell	80.7 ± 2.4	106
Morula	96.8 ± 1.9	115
Early blastocyst	112.7 ± 2.9	130

Estimates for the 2-, 4-, 8- and 16-cell stages were obtained from the exponential growth curves; those for morula and early blastocyst, by direct computation. Upper 95% points of distribution give the estimated time from insemination by which 95% of the embryos will have attained the specified stage.

embryos cleaving either too quickly or too slowly were associated with compromised development (Giorgetti et al., 1995; Ziebe et al., 1997; Alikani et al., 2000). Of particular significance is the robust evidence confirming the early observation of Shoukir et al. (1997) that the time to first cell division is a marker of developmental competence: those zygotes cleaving to the 2-cell stage between 24–27 h (the so-called 'early cleavers') have a greater likelihood to form a viable fetus (reviewed by Skiadas and Racowsky, 2007).

Numerous studies have shown a direct correlation between the number of cells in day-3 embryos (up to 8) and implantation rates following day-3 transfer (Puissant et al., 1987; Steer et al., 1992; Carrillo et al., 1998; Racowsky et al., 2003). Some authors have also associated cell number on day 3 with blastocyst formation rate (Jones et al., 1998). Of all morphological characteristics typically assessed in cleavage-stage embryos, cell number is still believed to be the single most important indicator for embryo viability.

Fragmentation

A fragment is defined as an anuclear, membrane-bound extracellular cytoplasmic structure (Alikani et al., 1999). The presence of such fragments has been found to be linked to abnormalities in cell metabolism or cell division that may reflect apoptosis (Jurisicova et al., 1996; Perez et al., 1999) or anomalies in chromosomal segregation (Pellestor et al., 1994; Munné et al., 1994).

Recent data suggests that fragmentation may result from abnormalities in the oocyte membrane (Fujimoto et al., 2011). There are several scoring systems for fragmentation. The simplest one describes the percentage of the volume of the embryo occupied by fragments (e.g. score 0 = 0%; score 1 = <10%; score 2 = 10–25%; score 3 = >25%) which, overall, is negatively correlated with embryo developmental potential and implantation rate (Ziebe et al., 1997, 2007; Alikani et al., 1999; Racowsky et al., 2003). However, other scoring systems include the location of fragments relative to the size and position of nucleated cells and have shown that the distribution of the fragments is, in and of itself, a predictor of implantation potential (Alikani et al., 1999).

Symmetry

Symmetry defines the size and shape of the blastomeres within the cleavage-stage embryo. Asynchrony can result from abnormality in cell division or uneven distribution of various organelles between two sister cells (Rienzi, 2005). Compared with the number of cells or extent of fragmentation, fewer studies have assessed the relationship between asymmetry and implantation potential. However, embryos with significant asymmetry have been shown to have lower implantation rates (Giorgetti et al., 1995; Hardarson et al., 2001).

Multinucleation

The presence of more than one nucleus within a blastomere (multinucleation) is considered abnormal. Studies have found an association between multinucleation and decreased implantation/pregnancy rates (Jackson et al., 1998; Van Royen et al., 2003; Saldeen and Sundstrom, 2005), and this association has been related to an increased incidence of chromosomal aberrations (Kligman et al., 1996; Hardarson et al., 2001; Munné, 2006; Magli et al., 2007; Ambroggio et al., 2011).

Additional features

Other features such as the granularity and shape of blastomeres have also been suggested as useful criteria for grading early cleavage embryos, but there are no hard data regarding their relevance to developmental potential (Rienzi et al., 2003).

Blastocyst scoring systems

Morphological evaluation of blastocysts includes the stage (early, expanding, expanded, hatching or hatched) as well as the quality of the inner cell mass and trophectoderm. Dokras et al. (1991, 1993) were the first to publish a blastocyst grading system. In this system, the blastocysts were classified into three grades depending on their developmental pattern and morphology. While there was some relationship between grade and human chorionic gonadotrophin secretion *in vitro*, clinical utility was not assessed in these early studies. However, a correlation between blastocyst grading and implantation rate has since been shown using this grading system (Balaban et al., 2000, 2006).

The most used scoring system for evaluating blastocysts is the one proposed by Gardner and Schoolcraft (1999a,b), Gardner et al. (2000). This system, which has been shown to provide improved selection and higher implantation rates than that of Dokras et al. (Balaban et al., 2006), takes into consideration the extent to which the volume of the embryo is occupied by the blastocoele, as well as the number and organization of cells in each of the inner cell mass and trophectoderm (**Table 2**).

In addition to these characteristics, assessment of size and shape of the inner cell mass may provide further insight into predicting implantation potential (Richter et al., 2001). While not commonly included in blastocyst scoring, these grading refinements highlight the potential importance of

Table 2 Gardner's system for grading human blastocysts. Adapted from: Gardner and Schoolcraft, 1999a. *In vitro* culture of human blastocysts. In: Jansen R, Mortimer D (eds). *Toward Reproductive Certainty: Fertility and Genetics Beyond 1999*. London: Parthenon Publishing 1999; 378–388.

Blastocyst stage	*Grade*	*Characteristics*
Early blastocyst	1	The blastocoele is less than half the volume of the embryo
Blastocyst	2	The blastocoele is greater than or equal to half of the volume of the embryo
Full blastocyst	3	The blastocoele completely fills the embryo
Expanded blastocyst	4	The blastocoele volume is larger than that of the early embryo and the zona pellucida is thinning
Hatching blastocyst	5	The trophectoderm has started to herniate through zona pellucida
Hatched blastocyst	6	The blastocyst has completely escaped from the zona pellucida
Inner cell mass	A	Tightly packed, many cells
	B	Loosely grouped, several cells
	C	Very few cells
Trophectoderm	A	Many cells forming a tightly knit epithelium
	B	Few cells
	C	Very few cells forming a loose epithelium.

The inner cell mass and trophectoderm are only graded for blastocysts grade 3 to 6.

more subtle morphological characteristics for predicting viable embryos.

Standardized scoring systems

Systems from professional organizations

Standardized criteria for grading embryos have recently been proposed by two organizations. The first, from SART, is a simple grading system that assigns one grade to account for the overall appearance of the embryo but which on day 3 also assesses cell number, fragmentation, symmetry and stage, and on day 5 assesses quality of the inner cell mass and trophectoderm. Together, these characteristics are now collected into the SART national registry (SART CORS) (Racowsky et al., 2010; **Table 3**), and validity of the day-3 collections have been documented (Vernon et al., 2011; Racowsky et al., 2011).

The second standardized grading system from a professional group is from the Alpha Executive and ESHRE special Interest Group of Embryology (2011) and includes additional standards, as follows, resulting in a more detailed grading scheme for assessing human embryo development.

Timing of observations should be standardized relative to the time of insemination (**Tables 4** and **5**).

For checking fertilization, pronuclear size and location should be assessed. Atypical pronuclei were defined of different size, widely separated or having micronuclei. The committee concluded that pronuclear scoring is of value and can provide additional information to the fertilization check.

For cleavage-stage embryos, several stages of development according to specific time points post insemination were proposed. According to the suggested timeline, embryos are supposed to have 4 cells on day 2 and 8 cells on day 3. Fragmentation was stratified as: mild (<10%), moderate (10–25%) and severe (>25%). The location of the fragments was not part of the evaluation. It was agreed that in 2-, 4- and 8-cell embryos, blastomeres should have a

Table 3 Society for Assisted Reproductive Technology embryo grading system. Reprinted from: Racowsky et al., 2010. Standardization of grading embryo morphology. Fertil Steril 94, 1152–1153 (**Table 1**). Copyright 2010, with permission from Elsevier.

Growth phase	*Overall grade*	*Stage*
Cleavage	Good, fair, poor	Cell number: from 1 to >8 Fragmentation: 0, <10%, 11–25%, >25% Symmetry: perfect, moderately asymmetric, severely asymmetric
Morula	Good, fair, poor	Compaction: complete, incomplete Fragmentation: 0, <10%, 11–25%, >25%
Blastocyst	Good, fair, poor	Expansion: early, expanding, expanded, hatched Inner cell mass: good, fair, poor Trophectoderm: good, fair, poor

Table 4 Timing of observation of fertilized oocytes and embryos, and expected stage of development at each time point. Reprinted from: Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Reprod. Biomed. Online 22, 632–646 (**Table 4**). Copyright 2011, with permission from Elsevier.

Time of observation	*Timing post insemination*	*Expected stage of development*
Fertilization check	17 ± 1	Pronuclear stage
Syngamy check	23 ± 1	Expect 50% to be in syngamy (up to 20% may be at the 2-cell stage)
Early cleavage check	26 ± 1 ICSI 28 ± 1 post IVF	2-cell stage
Day-2 embryo assessment	44 ± 1	4-cell stage
Day-3 embryo assessment	68 ± 1	8-cell stage
Day-4 embryo assessment	92 ± 2	Morula
Day-5 embryo assessment	116 ± 2	Blastocyst

Values are mean hours ± SD.

Table 5 Comparison of estimates of time (in hours) from insemination and reaching of specified stages of development.

Expected stage of development	Edwards et al. (1981)	*Istanbul Consensus Workshop (2011)*[a]
2-cell stage	33.2 ± 1.3	26 ± 1 ICSI 28 ± 1 post IVF
4-cell stage	49.0 ± 1.3	44 ± 1
8-cell stage	64.8 ± 1.8	68 ± 1
Morula	96.8 ± 1.9	92 ± 2
Blastocyst	112.7 ± 2.9	116 ± 2

Values are mean hours ± SD.
ICSI = intracytoplasmic sperm injection.
[a]Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology (2011).

similar size. Multinucleation assessment should be performed on day 2 and observation of multinucleation in 1 cell is sufficient for the embryo to be considered as multinucleated. There was a consensus regarding a scoring system for cleavage-stage embryos (**Table 6**) that is very similar to that of the SART group.

For blastocysts, it was agreed that optimal blastocysts are fully expanded/hatching with a prominent inner cell mass. A consensus for blastocyst scoring system was achieved (**Table 7**).

It is important to mention that these grading characteristics represent only minimum standards and do not restrict laboratories from performing additional evaluations. Currently, there are no reports regarding validity of these data collections among many IVF centres.

Systems from individual countries

The Spanish society for professionals working in the IVF laboratory (ASEBIR) agreed that a dynamic system of embryo scoring was required, including all stages from gamete to blastocyst with specific agreement being made regarding characteristics for cleavage-stage embryo scoring and assessment of embryos on day 4 for evidence of compaction. Furthermore, a consensus was reached that a zygote should be discarded if it had either one polar body and two pronuclei, or two polar bodies and one pronucleus (Torello et al., 2005).

Table 6 Consensus scoring system for cleavage-stage embryos (in addition to cell number). Reprinted from: Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Reprod. Biomed. Online 22, 632–646 (**Table 6**). Copyright 2011, with permission from Elsevier.

Grade	*Rating*	*Description*
1	Good	10% fragmentation Stage-specific cell size No multinucleation
2	Fair	10–25% fragmentation Stage-specific cell size for majority of cells No evidence of multinucleation
3	Poor	Severe fragmentation (>25%) Cell size not stage specific Evidence of multinucleation

In the UK, the Association of Clinical Embryologists (ACE) and the British Fertility Society published practice guidelines for the assessment of embryo morphology (Cutting et al., 2008). For cleavage-stage embryos, scoring consists of the number of blastomeres, symmetry and fragmentation (after Hardarson et al., 2001 and van Royen et al., 2003). There were also guidelines for blastocyst evaluation based on the original system published by Gardner and Schoolcraft (1999a,b) with modifications by Stephenson et al. (2007).

Intra- and inter-observer difference in morphological assessment of early cleavage embryos

There are various electronic online systems available for grading embryos to help laboratories measure inter- and

Table 7 Consensus scoring system for blastocysts. Reprinted from: Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Reprod. Biomed. Online 22, 632–646 (**Table 8**). Copyright 2011, with permission from Elsvier.

	Grade	*Rating*	*Description*
Stage of development	1		Early
	2		Blastocyst
	3		Expanded
	4		Hatched/hatching
ICM	1	Good	Prominent, easily discernible, with many cells that are compacted and tightly adhered together
	2	Fair	Easily discernible, with many cells that are loosely grouped together
	3	Poor	Difficult to discern, with few cells
TE	1	Good	Many cells forming a cohesive epithelium
	2	Fair	Few cells forming a loose epithelium
	3	Poor	Very few cells

The scoring system for blastocysts is a combination of the stage of development and the grade of the ICM and the TE (e.g. an expanded blastocyst with a good ICM and a fair TE would be scored as 312). It is a numerical interpretation of the Gardner scale (Gardner and Schoolcraft, 1999a,b).
ICM = inner cell mass; TE = trophectoderm.

Table 8 Comparison of pregnancy rate and graduated embryo scoring of the highest-scoring transferred embryo. Reprinted from: Fisch et al. 2001. The graduated embryo scoring system (GES) predicts blastocyst formation and pregnancy rates from cleavage-stage embryos. Hum. Reprod. 16, 1970–1975 (**Table 5**). Copyright 2001, with permission from Oxford University Press.

Score	*Patients (n)*	*Pregnancies (n, %)*
70–100	74	44 (59)[a]
90–100	40	24 (60)
70–85	34	20 (59)[b]
0–65	35	12 (34)
30–65	32	11 (34)
0–25	3	1 (33)
Total	109	56 (51)

Score = graduated embryo scoring of highest-scoring transferred embryo.
[a] $P < 0.0015$ compared with score 0–65.
[b] $P < 0.05$ compared with score 30–65.

intra-observer differences among teams, as well as inter-lab variances. Examples include FertAid Australia (www.fertaid.com/FertAid/FertAid_RankIndex.asp) and GameteExpert, UK NEQAS (www.cmft.nhs.uk/saint-marys/our-services/ukneqasrepsci.aspx).

Several studies have analysed intra- and inter-observer variability. Baxter Bendus et al. (2006) evaluated 26 experienced embryologists who were asked to grade 35 video clips of day-3 embryos. The authors found considerable inter-observer variability and moderate intra-observer variability among participants.

Paternot et al. (2009) also investigated the intra- and inter-observer variability in the morphological evaluation of embryos. Multilevel images of embryos were performed on days 1, 2 and 3. Excellent intra- and inter-observer agreements were observed for the number of blastomeres on days 2 and 3. For day-1 assessment, there was a moderate intra-observer variability regarding the size of the pronuclei and the presence of cytoplasmic halo and poor inter-observer agreement for these two parameters.

Following these results, a multicentre study was conducted (Paternot et al., 2011a). Five embryologists from four IVF units participated and evaluated multilevel images of embryos (days 1, 2 and 3) on a website. For day-1 assessment, both intra- and inter-observer agreement were good to excellent for the position of the pronuclei but poor for pronuclear size and presence of cytoplasmic halo. There was good to excellent intra- and inter-observer agreement for the number of blastomeres on days 2 and 3 as well as the clinical decision for embryo fate (i.e. whether the embryos would be transferred, cryopreserved or discarded). However, the rates of inter- and intra-observer variation regarding the degree of fragmentation and the size of blastomeres on days 2 and 3 varied.

Taken together, the above results indicate how challenging it is to maintain consistency in scoring day-3 embryos and how important it is to perform quality control proficiencies to retain standardization in evaluations both within and among embryologists.

Complex types of embryo scoring

Numerous morphological systems, using various combinations of characteristics and days for evaluation, have been developed to grade and rank embryos (reviewed by Boiso et al., 2002). The fact that a large proportion of human embryos do not follow the expected developmental

timeline, has provided the rationale for more complex systems that either use a formula to predict pregnancy likelihood based on the appearance and development of an embryo at specific time points (Cummins et al., 1986) or that apply multiple day scoring instead of a single evaluation before retrieval (reviewed by Ceyhan et al., 2009). A large number of studies have been published in the last decade that propose various combinations of multiple day scoring (Scott et al., 2000 and Nagy et al., 2003; reviewed by Skiadas and Racowsky, 2007). However, there is no consensus on the optimum days of evaluation (reviewed by Ceyhan et al., 2009). This lack of consensus is likely due to patient heterogeneity and the impact of patient age (Racowsky et al., 2003), intracytoplasmic sperm injection (Hesters et al., 2008), differences in media (Van Soom et al., 2003) and, perhaps more critically, differences in the timing of evaluation (Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011).

Formulated embryo scoring

Cummins et al. (1986) developed a formula for scoring the growth rate of human embryos. The authors were interested in a rapid evaluation that would reflect the rate of embryo growth, independent of the time of observation and observer bias. Embryo quality was estimated both according to morphology and embryo developmental rating. Embryo developmental rating was based on the ratio between the time at which the embryos were observed at a particular stage and the time at which they would be expected to reach that stage according to an ideal growth rate. Analysis of 232 clinical pregnancies from these transfers showed that pregnancies were most likely to occur in cases in which embryos were scored as having good quality according to morphology and embryo developmental rating. Morphology and embryo developmental rating were significantly associated with each other.

Unfortunately, since this very early study, no more recent investigations have been undertaken to assess the efficacy of morphology and embryo developmental rating with more modern culture systems. This may be worth pursuing as this system obviates the need to score the embryos at specific time points, thereby facilitating integration into a busy clinical laboratory without the need for sophisticated imaging systems (see section below on imaging systems). However, a possible limitation of this method is missing an important specific developmental milestone which might be masked by an apparently normal growth rate.

Ranked embryo scoring

The need to compare embryo quality between cycles in the same patient and to standardize embryo grading between embryologists, led to the establishment of evidence-based embryo quality criteria. Holte et al. (2007) conducted a prospective study creating a scoring scale for embryo grading based on the number of blastomeres, fragmentation, blastomere size variation, symmetry of the cleavage-stage embryos and mononuclearity in the blastomeres. Using a regression analysis, blastomere number, blastomere size variation and mononuclearity in the blastomeres were highly correlated with embryo implantation potential, thereby enabling development of a ranked scoring system. More recently, Racowsky et al. (2009) used a similar approach to identify which characteristics on which days should be collected for day-3 transfers. Using multiple logistic regression and the calculation of the area under the curve from a receiver operating characteristic curve analysis, a predictive model was built for implantation in which derived regression coefficients can be used prospectively in an algorithm to rank embryos for selection.

Graduated (cumulative) embryo scoring

Fisch et al. (2001) have proposed an algorithm for predicting blastocyst formation and pregnancy rates based on the presence of early developmental milestones of embryo development. The graduated embryo score is a system for embryo evaluation (total score up to 100) based on individual culture for each embryo, allowing sequential microscopic assessments of developmental stages starting on days 1–3 of embryo culture. The parameters that were evaluated included nuclear alignment along the PN axis, regular cleavage and degree of fragmentation at the first cell division as well as the blastomere number, symmetry and fragmentation on day 3. Higher scores were correlated with higher rates of blastocyst formation as well as higher pregnancy rates (**Table 8**).

Despite the above promising studies suggesting benefit to graduated scoring for embryo selection, again there is no consensus as to whether this truly enhances ranking and selection of embryos compared with single-stage evaluations.

Automated morphological assessment of embryos using digital imaging

Assessment of embryo morphology is a subjective process and, due to the subjective nature of the evaluations, drift in scoring and lack of definitive ways to assess the specific characteristics is likely associated with intra-observer variability (i.e. within the same embryologist) and inter-observer variability (i.e. among different embryologists) (Arce et al., 2006; Baxter Bendus et al., 2006; Paternot et al., 2009). The evaluation time has to be as short as possible to prevent embryo exposure to pH and temperature shifts as well as excessive light exposure, which have adverse effects on embryo development and quality (Umaoka et al., 1992; Garrisi et al., 1993).

Multilevel computerized images enable assessment over an unlimited time period and allow a more detailed evaluation of the embryo. Early studies that involved embryo imaging, typically evaluated the embryo at specific time points (days 1, 2 and 3), specifically including only stages of early development (Nagy et al., 1994; Lundin et al., 2001; Fenwick et al., 2002). Newer technologies have enabled assessment of the same embryo by multiple observers using imaging systems which capture one image or a series of sequential images of the embryos again at specific time points (Arce et al., 2006; Lemmen et al., 2008). Such analyses have identified specific kinetic markers of embryo quality.

Paternot et al. (2011b) evaluated embryo morphology on consecutive days of early development, using a

computer-assisted scoring system that recorded image sequences of embryos. Sequential images allowed focusing on multiple planes within the embryo, compared with 2D images which limit assessment to one focal plane only. However, this technology was not fully automatic and an embryologist was needed to define the boundaries of the cells within the embryos. The embryos were then evaluated semi-automatically; the diameters of the zygotes as well as that of the blastomeres on days 2 and 3 were drawn manually and the total volume of the zygote/embryo was calculated by the computer. The software was programmed to calculate the number and size of the blastomeres and the degree of fragmentation. Blastomeres were distinguished from fragments based on their size (Johansson et al., 2003; Hnida et al., 2005). According to previous data showing that the total volume of the embryo does not change during the first days of development (Hnida and Ziebe, 2004), the difference in the cytoplasmic volume of the zygote and the embryo (the sum of the volume of the individual blastomeres) was calculated by the computer system and was interpreted as fragmentations. Use of a computer-assisted scoring system was better for predicting implantation and live birth than standard day-3 scoring and, importantly, the mean time that the embryos were out of the incubator for the multi-images was significantly shorter compared with the usual day-3 scoring (Paternot et al., 2011a).

Novel techniques for evaluating oocyte/embryo viability

Polarized microscopy (Polscope)

A novel polarized microscopy system coupled with image-processing software has allowed non-invasive visualization of the meiotic spindle and the different layers of the zona pellucida in human oocytes (Keefe et al., 2003; Montag and van der Ven, 2008). The system is based on birefringence — an optical property that derives from the molecular property of macromolecules in cellular features such as membranes, microtubules, microfilaments and other cytoskeletal structures. Birefringent structures that are illuminated as the microtubules in spindles can be visualized as bright structures (Keefe et al., 2003). While at least one study has shown that use of polarized microscopy for analysis of human oocytes increases prediction of developmental competence (Moon et al., 2003), the method is not routinely applied as it remains unclear whether application of this technique results in improved embryo selection and higher pregnancy rates (Wang et al., 2001).

Time-lapse evaluation

Concerns regarding the effects of handling the embryos outside the controlled incubator environment, and the limited information obtained from a few static observations, have led to development of new systems for time-lapse imaging acquisition. Moreover if these systems permit selection of the superior embryo(s) before or at the cleavage stage, their use will reduce the need for blastocyst transfer and will thus address concerns regarding possible epigenetic modifications induced by prolonged culture (reviewed by Batcheller et al., 2011), as well as data suggesting that the risk of preterm birth and major congenital malformations may be higher after blastocyst than cleavage-stage transfer (Källén et al., 2010). Such systems enable continuous documentation of early embryo growth without disturbing the culture environment (Lemmen et al., 2008). Gathering information about the dynamic pattern of embryo development might give useful information for embryo selection (Cruz et al., 2011; Montag et al., 2011).

As a proof-of-principle safety study, Lemmen et al. (2008) undertook a study to confirm that development of 2PN zygotes was similar to their siblings during culture in a time-lapse system (Nikon Diaphot 300 microscope with camera in a closed system) as compared with the standard incubator. Although their findings confirmed no deleterious effect of culturing in the time-lapse system, to date, there are no follow-up studies showing that this system improves embryo selection. Likewise, another proof-of-principle safety study has confirmed that using the EmbryoScope does not impair the development or implantation rates of embryos cultured in this time-lapse imaging system compared with those cultured in standard incubators (Cruz et al., 2011). Again, however, there are no prospective randomized trial data yet available showing that embryos selected from time-lapse imaging have significantly improved implantation rates compared with those selected after conventional morphological evaluation with an inverted microscope.

Three other important recent studies with time-lapse imaging systems have generated data that may support future routine application of such systems for improved embryo selection. In the first of these studies, Wong et al. (2010) correlated time-lapse videography imaging of supernumerary IVF embryos from the zygote to the blastocyst stage, with gene expression profiles of the developing embryos. A total of 242 embryos were evaluated: 100 were cultured from zygote to days 5–6 and the remaining embryos were removed at various stages for quantitative real-time PCR gene analysis. In this study, 33–53% of the cultured embryos formed blastocysts. Retrospective analysis showed that three parameters predicted development to the blastocyst stage with >93% accuracy: (i) duration of the first cytokinesis; (ii) time interval between the end of the first cleavage and initiation of the second cleavage (from 1- to 2-cell embryo); and (iii) synchronicity of the blastomeres in the second cleavage division, from 2- to 4-cell embryo). Gene expression analysis of embryos that appeared to develop normally according to imaging revealed specific patterns which were correlated with the developmental stage. The three kinetic markers specified above have led to derivation of an algorithm for prediction of blastocyst formation. However, as none of the embryos in this study were transferred, it is unclear whether the embryos exhibiting these markers would implant at a higher rate than those selected by conventional morphological evaluations.

In the second recent study with an automated time-lapse imaging system, Meseguer et al. (2011) investigated the correlation between timing of various developmental milestones, morphological patterns and implantation. A total of 247 embryos were included and were incubated in a tri-gas IVF incubator with a built-in camera that

automatically acquired images every 15 min. Embryo morphology was evaluated on days 2 and 3 based on acquired digital images and was based on the number, symmetry and fragmentation of the blastomeres, as well as the presence of multinucleated cells and degree of compaction. Embryos were transferred based on their final morphology and a retrospective analysis of the acquired images for each embryo was performed in order to define parameters that were associated with implantation. These authors also found a correlation between the duration of the second cell cycle and the synchrony of the second and third cell divisions and implantation potential. However, as these analyses were also retrospective, it is currently unknown what utility these developmental kinetic markers have for prospective embryo selection.

In the third recent study, Hashimoto et al. (2012) used a time-lapse system to provide an additional investigation of the relevance of developmental kinetics to formation of morphologically normal blastocysts. These investigators observed that embryos with a high potential to develop to high scoring blastocysts can be selected at 2–3 days of culture based on the time required to complete the second and third mitotic divisions. Again, however, these data were collected retrospectively.

Concluding remarks

Minimal requirements for embryo selection should include standardization, ease of assessment, objectivity, minimal harm to the embryo and a high correlation with pregnancy rates. Automated time-lapse imaging systems have the potential to meet these requirements. Preliminary safety studies have been performed demonstrating comparable development of embryos cultured in these specialized systems compared with standard incubators, and multivariable algorithms for selection have been developed from retrospective analyses. Much hope is now being pinned on these systems (Kiessling, 2010; reviewed by Reijo Pera, 2011) and prospective trials are currently underway. Such trials must be undertaken in multiple clinics with a broad spectrum and heterogeneity of infertility patients, as it is likely that different multivariable models will be required for different patient groups. Only after completion of such trials will it be known whether time-lapse analysis does improve implantation rates.

More than 25 years ago, Cummins et al. (1986) developed a formula for scoring the growth rate of human embryos based on assessments performed with the human eye. Even if algorithms derived from automated imaging improve upon this very early work, it seems likely that other screening tools will be used in tandem, whether these are profiling approaches (genomic, proteomic or metabolomic) or targeted approaches to specific molecules. Although available data do not support the utility of either proteomic (Katz-Jaffe and Gardner, 2008) or metabolomic (Vergouw et al., 2012) approaches for improved embryo selection, novel high-throughput genome-wide approaches for aneuploidy screening hold great promise in this arena (reviewed by Treff, 2012). Such approaches include a validated quantitative real-time PCR-based assay for comprehensive chromosome screening of all 24 chromosomes in trophectoderm biopsy specimens (Treff and Scott, 2012). Importantly, this comprehensive chromosome screening method has a 4-h turnaround so the blastocysts can be transferred fresh and, in a case-control study, was shown to improve pregnancy rates and reduce miscarriage rates (Forman et al., 2012). Nevertheless, prospective randomized trial data are needed to confirm clinical utility and to identify those patient populations for which the technology is applicable. Clearly, considerable challenges lay ahead as effective classification systems for ranking embryos continue to be developed. With these in hand, transfer of the single, most developmentally competent embryo in every cohort will move clinicians closer towards maximizing pregnancy rates, while minimizing the risk of twins for each patient.

References

Alikani, M., Cohen, J., Tomkin, G., Garrisi, G.J., Mack, C., Scott, R.T., 1999. Human embryo fragmentation in vitro and its implications for pregnancy and implantation. Fertil. Steril. 71, 836–842.

Alikani, M., Calderon, G., Tomkin, G., Garrisi, J., Kokot, M., Cohen, J., 2000. Cleavage anomalies in early human embryos and survival after prolonged culture in-vitro. Hum. Reprod. 15, 2634–2643.

Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Reprod. Biomed. Online 22, 632–646.

Ambroggio, J., Gindoff, P.R., Dayal, M.B., Khaldi, R., Peak, D., Frankfurter, D., Dubey, A.K., 2011. Multinucleation of a sibling blastomere on day 2 suggests unsuitability for embryo transfer in IVF-preimplantation genetic screening cycles. Fertil. Steril. 96, 856–859.

Arce, J.C., Ziebe, S., Lundin, K., Janssens, R., Helmgaard, L., Sørensen, P., 2006. Interobserver agreement and intraobserver reproducibility of embryo quality assessments. Hum. Reprod. 21, 2141–2148.

Balaban, B., Urman, B., Sertac, A., Alatas, C., Aksoy, S., Mercan, R., 2000. Blastocyst quality affects the success of blastocyst-stage embryo transfer. Fertil. Steril. 74, 282–287.

Balaban, B., Urman, B., Isklar, A., Alatas, C., Aksoy, S., Mercan, R., Mumcu, A., Nuhoglu, A., 2001. The effects of pronuclear morphology on embryo quality parameters and blastocyst transfer outcome. Hum. Reprod. 16, 2357–2361.

Balaban, B., Yakin, K., Urman, B., 2006. Randomized comparison of two different blastocyst grading systems. Fertil. Steril. 85, 559–563.

Batcheller, A., Cardozo, E., Maguire, M., DeCherney, A.H., Segars, J.H., 2011. Are there subtle genome-wide epigenetic alterations in normal offspring conceived by assisted reproductive technologies? Fertil. Steril. 96, 1306–1311.

Baxter Bendus, A.E., Mayer, J.F., Shipley, S.K., Catherino, W.H., 2006. Interobserver and intraobserver variation in day 3 embryo grading. Fertil. Steril. 86, 1608–1615.

Boiso, I., Veiga, A., Edwards, R.G., 2002. Fundamentals of human embryonic growth in vitro and the selection of high-quality embryos for transfer. Reprod. Biomed. Online 5, 328–350.

Carrillo, A.J., Lane, B., Pridman, D.D., Risch, P.P., Pool, T.B., Silverman, I.H., Cook, C.L., 1998. Improved clinical outcomes for in vitro fertilization with delay of embryo transfer from 48 to 72 h after oocyte retrieval: use of glucose- and phosphate-free media. Fertil. Steril. 69, 329–334.

Ceyhan, S.T., Jackson, K.V., Racowsky, C., 2009. Selecting the most competent embryo. In: Carrell, D.T., Racowsky, C.,

Schlegel, P.N., Voorhis, B.J. (Eds.), Biennial Review of Infertility. Humana Press, LLC, New York, pp. 143—169.

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

Cummins, J.M., Breen, T.M., Harrison, K.L., Shaw, J.M., Wilson, L.M., Hennessey, J.F., 1986. A formula for scoring human embryo growth rates in in vitro fertilization: its value in predicting pregnancy and in comparison with visual estimates of embryo quality. J. In Vitro Fert. Embryo Transf. 3, 284—295.

Cutting, R., Morroll, D., Roberts, S.A., Pickering, S., Rutherford, A., 2008. Elective single embryo transfer: guidelines for practice, British Fertility Society and Association of Clinical Embryologists. Hum. Fertil. 11, 131—146.

Desai, N.N., Goldstein, J., Rowland, D.Y., Goldfarb, J.M., 2000. Morphology evaluation of human embryos and derivation of and embryo quality scoring system specific for day 3 embryos: a preliminary study. Hum. Reprod. 15, 2190—2196.

Dokras, A., Sargent, I.L., Ross, C., Gardner, R.L., Barlow, D.H., 1991. The human blastocyst: its morphology and HCG secretion in vitro. Hum. Reprod. 6, 1143—1151.

Dokras, A., Sargent, I.L., Barlow, D.H., 1993. Human blastocyst grading: an indicator of developmental potential? Hum. Reprod. 8, 2119—2127.

Edwards, R.G., Purdy, J.M., Steptoe, P.C., Walters, D.E., 1981. The growth of human preimplantation embryos in vitro. Am. J. Obstet. Gynecol. 141, 408—416.

Fenwick, J., Platteau, P., Murdoch, A.P., Herbert, M., 2002. Time from insemination to first cleavage predicts developmental competence of human preimplantation embryos in vitro. Hum. Reprod. 17, 407—412.

Fisch, J., Rodriguez, H., Ross, R., Overby, G., Sher, G., 2001. The graduated embryo scoring system (GES) predicts blastocyst formation and pregnancy rates from cleavage-stage embryos. Hum. Reprod. 16, 1970—1975.

Forman, E.J., Tao, X., Ferry, K.M., Taylor, D., Treff, N.R., Scott, R.T., 2012. Single embryo transfer with comprehensive chromosome screening results in improved ongoing pregnancy rates and decreased miscarriage rates. Hum. Reprod. 27, 1217—1222.

Fujimoto, V.Y., Browne, R.W., Bloom, M.S., Sakkas, D., Alikani, M., 2011. Pathogenesis, developmental consequences, and clinical correlations of human embryo fragmentation. Fertil. Steril. 15, 1197—1204.

Gardner, D.K., Schoolcraft, W.B., 1999a. In vitro culture of human blastocysts. In: Jansen, R., Mortimer, D. (Eds.), Toward Reproductive Certainty: Fertility and Genetics Beyond 1999. Parthenon Publishing, London, pp. 378—388.

Gardner, D.K., Schoolcraft, W.B., 1999b. Culture and transfer of human blastocysts. Curr. Opin. Obstet. Gynecol. 11, 307—311.

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

Garrisi, G.J., Chin, A.J., Dolan, P.M., Nagler, H.M., Vasquez-Levin, M., Navot, D., Gordon, J.W., 1993. Analysis of factors contributing to success in a program of micromanipulation-assisted fertilization. Fertil. Steril. 59, 366—374.

Giorgetti, C., Terriou, P., Auquier, P., Hans, E., Spach, J.L., Salzmann, J., Roulier, R., 1995. Embryo score to predict implantation after in-vitro fertilization: based on 957 single embryo transfers. Hum. Reprod. 10, 2427—2431.

Hardarson, T., Hanson, C., Sjogren, A., Lundin, K., 2001. Human embryos with unevenly sized blastomeres have lower pregnancy and implantation rates: indications for aneuploidy and multinucleation. Hum. Reprod. 16, 313—318.

Hashimoto, S., Kato, N., Saeki, K., Morimoto, Y., 2012. Selection of high-potential embryos by culture in poly (dimethylsiloxane) microwells and time-lapse imaging. Fertil. Steril. 97, 332—337.

Hesters, L., Prisant, N., Fanchin, R., Méndez Lozano, D.H., Feyereisen, E., Frydman, R., Tachdjian, G., Frydman, N., 2008. Impact of early cleaved zygote morphology on embryo development and in vitro fertilization-embryo transfer outcome: a prospective study. Fertil. Steril. 89, 1677—1684.

Hill, G.A., Freeman, M., Bastias, M.C., Rogers, B.J., Herbert, C.M., Osteen, K.G., Wentz, A.C., 1989. The influence of oocyte maturity and embryo quality on pregnancy rate in a program for in vitro fertilization-embryo transfer. Fertil. Steril. 52, 801—806.

Hnida, C., Ziebe, S., 2004. Total cytoplasmic volume as biomarker of fragmentation in human embryos. J. Assist. Reprod. Genet. 21, 335—340.

Hnida, C., Agerholm, I., Ziebe, S., 2005. Traditional detection versus computer-controlled multilevel analysis of nuclear structures from donated human embryos. Hum. Reprod. 20, 665—671.

Holte, J., Berglund, L., Milton, K., Garello, C., Gennarelli, G., Revelli, A., Bergh, T., 2007. Construction of an evidence-based integrated morphology cleavage embryo score for implantation potential of embryos scored and transferred on day 2 after oocyte retrieval. Hum. Reprod. 22, 548—557.

Jackson, K.V., Ginsburg, E.S., Hornstein, M.D., Rein, M.S., Clarke, R.N., 1998. Multinucleation in normally fertilized embryos is associated with an accelerated ovulation induction response and lower implantation and pregnancy rates in in vitro fertilization-embryo transfer cycles. Fertil. Steril. 70, 60—66.

Johansson, M., Hardarson, T., Lundin, K., 2003. There is a cutoff limit in diameter between a blastomere and a small anucleate fragment. J. Assist. Reprod. Genet. 20, 309—313.

Jones, G., Trounson, A., Lolatgis, N., Wood, C., 1998. Factors affecting the success of human blastocyst development and pregnancy following in vitro fertilization and embryo transfer. Fertil. Steril. 70, 1022—1029.

Jurisicova, A., Varmuza, S., Casper, R.F., 1996. Programmed cell death and human embryo fragmentation. Mol. Hum. Reprod. 2, 93—98.

Källén, B., Finnström, O., Lindam, A., Nilsson, E., Nygren, K.G., Olausson, P.O., 2010. Blastocyst versus cleavage stage transfer in in vitro fertilization: differences in neonatal outcome? Fertil. Steril. 94, 1680—1683.

Katz-Jaffe, M.G., Gardner, D.K., 2008. Symposium: innovative techniques in human embryo viability assessment. Can proteomics help to shape the future of human assisted conception? Reprod. Biomed. Online 17, 497—501.

Keefe, D., Liu, L., Wang, W., Silva, C., 2003. Imaging meiotic spindles by polarization light microscopy: principles and applications to IVF. Reprod. Biomed. Online 7, 24—29.

Kiessling, A.A., 2010. Timing is everything in the human embryo. Nat. Biotechnol. 28, 1025—1026.

Kligman, I., Benadiva, C., Alikani, M., Munné, S., 1996. The presence of multinucleated blastomeres in human embryos is correlated with chromosomal abnormalities. Hum. Reprod. 11, 1492—1498.

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

Lundin, K., Bergh, C., Hardarson, T., 2001. Early embryo cleavage is a strong indicator of embryo quality in human IVF. Hum. Reprod. 16, 2652—2657.

Magli, M.C., Gianaroli, L., Ferraretti, A.P., Lappi, M., Ruberti, A., Farfalli, V., 2007. Embryo morphology and development are dependent on the chromosomal complement. Fertil. Steril. 87, 534—541.

Meseguer, M., Herrero, J., Tejera, A., Hilligsøe, K.M., Ramsing, N.B., Remohí, J., 2011. The use of morphokinetics as a predictor of embryo implantation. Hum. Reprod. 26, 2658–2671.

Montag, M., van der Ven, H., 2001. Evaluation of pronuclear morphology as the only selection criterion for further embryo culture and transfer: results of a prospective multicentre study. Hum. Reprod. 16, 2384–2389.

Montag, M., van der Ven, H., 2008. Symposium: innovative techniques in human embryo viability assessment. Oocyte assessment and embryo viability prediction: birefringence imaging. Reprod. Biomed. Online 17, 454–460.

Montag, M., Liebenthron, J., Köster, M., 2011. Which morphological scoring system is relevant in human embryo development? Placenta 32, S252–S256.

Moon, J., Hyun, C., Lee, S., Son, W., Yoon, S., Lim, J., 2003. Visualization of the metaphase II meiotic spindle in living human oocytes using the Polscope enables the prediction of embryonic developmental competence after ICSI. Hum. Reprod. 18, 817–820.

Munné, S., 2006. Chromosome abnormalities and their relationship to morphology and development of human embryos. Reprod. Biomed. Online 12, 234–253.

Munné, S., Alikani, M., Cohen, J., 1994. Monospermic polyploidy and atypical embryo morphology. Hum. Reprod. 9, 506–510.

Nagy, Z.P., Liu, J., Joris, H., Bocken, G., Desmet, B., Van Ranst, H., Vankelecom, A., Devroey, P., Van Steirteghem, A.C., 1994. Time-course of oocyte activation, pronucleus formation and cleavage in human oocytes fertilized by intracytoplasmic sperm injection. Hum. Reprod. 9, 1743–1748.

Nagy, Z.P., Dozortsev, D., Diamond, M., Rienzi, L., Ubaldi, F., Abdelmassih, R., Greco, E., 2003. Pronuclear morphology evaluation with subsequent evaluation of embryo morphology significantly increases implantation rates. Fertil. Steril. 80, 67–74.

Paternot, G., Devroe, J., Debrock, S., D'Hooghe, T.M., Spiessens, C., 2009. Intra- and inter-observer analysis in the morphological assessment of early-stage embryos. Reprod. Biol. Endocrinol. 29, 105.

Paternot, G., Wetzels, A.M., Thonon, F., Vansteenbrugge, A., Willemen, D., Devroe, J., Debrock, S., D'Hooghe, T.M., Spiessens, C., 2011a. Intra- and interobserver analysis in the morphological assessment of early stage embryos during an IVF procedure: a multicentre study. Reprod. Biol. Endocrinol. 9, 1–5.

Paternot, G., Debrock, S., D'Hooghe, T., Spiessens, C., 2011b. Computer-assisted embryo selection: a benefit in the evaluation of embryo quality? Reprod. Biomed. Online 23, 347–354.

Pellestor, F., Girardet, A., Andréo, B., Arnal, F., Humeau, C., 1994. Relationship between morphology and chromosomal constitution in human preimplantation embryo. Mol. Reprod. Dev. 39, 141–146.

Perez, G.I., Tao, X.J., Tilly, J.L., 1999. Fragmentation and death (a.k.a. apoptosis) of ovulated oocytes. Mol. Hum. Reprod. 5, 414–420.

Puissant, F., Van Rysselberge, M., Barlow, P., Deweze, J., Leroy, F., 1987. Embryo scoring as a prognostic tool in IVF treatment. Hum. Reprod. 2, 705–708.

Racowsky, C., Combelles, C., Nureddin, A., Pan, Y., Finn, A., Miles, L., Gale, S., O'Leary, T., Jackson, K.V., 2003. Day 3 and day 5 morphological predictors of embryo viability. Reprod. Biomed. Online 6, 323–331.

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

Racowsky, C., Vernon, M., Mayer, J., Ball, G.D., Behr, B., Pomeroy, K.O., Wininger, D., Gibbons, W., Conaghan, J., Stern, J.E., 2010. Standardization of grading embryo morphology. Fertil. Steril. 94, 1152–1153.

Racowsky, C., Stern, J.E., Gibbons, W.E., Behr, B., Pomeroy, K.O., Biggers, J.D., 2011. National collection of embryo morphology data into Society for Assisted Reproductive Technology Clinic Outcomes Reporting System: associations among day 3 cell number, fragmentation and blastomere asymmetry, and live birth rate. Fertil. Steril. 95, 1985–1989.

Reijo Pera, R.A., 2011. Non-invasive imaging of human embryos to predict developmental competence. Placenta 32, S264–S267.

Richter, K., Harris, D., Daneshmand, S., Shapiro, B., 2001. Quantitative grading of a human blastocyst: optimal inner cell mass size and shape. Fertil. Steril. 76, 1157–1167.

Rienzi, L., 2005. Significance of morphological attributes of the early embryo. Reprod. BioMed. Online 10, 669–681.

Rienzi, L., Ubaldi, F., Iacobelli, M., Ferrero, S., Minasi, M.G., Martinez, F., Tesarik, J., Greco, E., 2002. Day 3 embryo transfer with combined evaluation at the pronuclear and cleavage stages compares favourably with day 5 blastocyst transfer. Hum. Reprod. 17, 1852–1855.

Rienzi, L., Ubaldi, F., Minasi, M.G., Iacobelli, M., Martinez, F., Tesarik, J., Greco, E., 2003. Blastomere cytoplasmic granularity is unrelated to developmental potential of day 3 human embryos. J. Assist. Reprod. Genet. 20, 314–317.

Saldeen, P., Sundstrom, P., 2005. Nuclear status of four-cell preembryos predicts implantation potential in in vitro fertilization treatment cycles. Fertil Steril. 84, 584–589.

Scott, L.A., Smith, S., 1998. The successful use of pronuclear embryo transfers the day following oocyte retrieval. Hum. Reprod. 13, 1003–1013.

Scott, L., Alvero, R., Leondires, M., Miller, B., 2000. The morphology of human pronuclear embryos is positively related to blastocyst development and implantation. Hum. Reprod. 15, 2394–2403.

Shoukir, Y., Campana, A., Farley, T., Sakkas, D., 1997. Early cleavage of in-vitro fertilized embryos to the 2-cell stage: a novel indicator of embryo quality and viability. Hum. Reprod. 12, 1531–1536.

Skiadas, C.C., Racowsky, C., 2007. Development rate, cumulative scoring and embryonic viability. In: Elder, K., Cohen, J. (Eds.), Human Preimplantation Embryo Selection. Taylor and Francis, Colchester, UK, pp. 101–121.

Steer, C., Mills, C., Tan, S., Campbell, S., Edwards, R.G., 1992. The cumulative embryo score: a predictive embryo scoring technique to select the optimal number of embryos to transfer in an in-vitro fertilization and embryo transfer program. Hum. Reprod. 7, 117–119.

Stephenson, E., Braude, P., Mason, C., 2007. International community consensus standard reporting derivation of human embryonic stem cell lines. Regen. Med. 2, 349–362.

Tesarik, J., Greco, E., 1999. The probability of abnormal preimplantation development can be predicted by a single static observation on pronuclear stage morphology. Hum. Reprod. 14, 1318–1323.

Torello, M.J., Ardoy, M., Calderon, G., Cuadros, J., Herrer, R., Moreno, JM., Ortiz, A., Prados, F., Rodriguez, L, Ten, J., 2005. Criterios ASEBIR de vaoracion morfologica de Oocitos, Embriones tempranos y Blastocistos. ASEBIR Congress, Zaragoza.

Treff, N.R., 2012. Genome-wide analysis of human preimplantation aneuploidy. Semin. Reprod. Med. 30, 283–288.

Treff, N.R., Scott, R.T., 2012. Methods for comprehensive chromosome screening of oocytes and embryos: capabilities, limitations, and evidence of validity. J. Assis. Reprod. Genet. 29, 381–390.

Umaoka, Y., Noda, Y., Nakayama, T., Narimoto, K., Mori, T., Iritani, A., 1992. Effect of visual light on in vitro embryonic development in the hamster. Theriogenology 38, 1043–1054.

Van Royen, E., Mangelschots, K., Neubourgh, D., Valkenburg, M., Van de Meerssche, M., Ryckaert, G., Eestermans, W., Gerris, J.,

1999. Characterization of a top quality embryo, a step towards single-embryo transfer. Hum. Reprod. 14, 2345—2349.

Van Royen, E., Mangelschots, K., Vercruyssen, M., De Neubourg, D., Valkenburg, M., Ryckaert, G., Gerris, J., 2003. Multinucleation in cleavage stage embryos. Hum. Reprod. 18, 1062—1069.

Van Soom, A., Mateusen, B., Leroy, J., De Kruif, A., 2003. Assessment of mammalian embryo quality: what can we learn from embryo morphology? Reprod. Biomed. Online 7, 664—670.

Vergouw, C.G., Kieslinger, D.C., Kostelijk, E.H., Botros, L.L., Schats, R., Hompes, P.G., Sakkas, D., Lambalk, C.B., 2012. Day 3 embryo selection by metabolomic profiling of culture medium with near-infrared spectroscopy as an adjunct to morphology: a randomized controlled trial. Hum. Reprod. 27, 2304—2311.

Vernon, M., Stern, J.E., Ball, G.D., Wininger, D., Mayer, J., Racowsky, C., 2011. Utility of the national embryo morphology data collection by the Society for Assisted Reproductive Technologies (SART): correlation between day-3 morphology grade and live-birth outcome. Fertil. Steril. 95, 2761—2763.

Wang, W., Meng, L., Hackett, R., Keefe, D., 2001. Developmental ability of human oocytes with or without birefringent spindles imaged by Polscope before insemination. Hum. Reprod. 16, 1464—1468.

Wittemer, C., Bettahar-Lebugle, K., Ohl, J., Rongières, C., Nisand, I., Gerlinger, P., 2000. Zygote evaluation: an efficient tool for embryo selection. Hum. Reprod. 15, 2591—2597.

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

Ziebe, S., Petersen, K., Lindenberg, S., Andersen, A.G., Gabrielsen, A., Andersen, A.N., 1997. Embryo morphology or cleavage stage: how to select the best embryos for transfer after in-vitro fertilization. Hum. Reprod. 12, 1545—1549.

Ziebe, S., Lundin, K., Janssens, R., Helmgaard, L., Arce, J.C.MERIT (Menotrophin vs Recombinant FSH in vitro Fertilisation Trial) Group, 2007. Influence of ovarian stimulation with HP-hMG or recombinant FSH on embryo quality parameters in patients undergoing IVF. Hum. Reprod. 22 (9), 2404—2413.

Zollner, U., Zollner, K.P., Hartl, G., Dietl, J., Steck, T., 2002. The use of a detailed zygote score after IVF/ICSI to obtain good quality blastocysts: the German experience. Hum. Reprod. 17, 1327—1333.

Zollner, U., Zollner, K.P., Steck, T., Dietl, J., 2003. Pronuclear scoring: time for international standardization. J. Reprod. Med. 48, 365—369.

Declaration: The authors report no financial or commercial conflicts of interest.

Received 11 June 2012; refereed 16 October 2012; accepted 16 October 2012.