Time-lapse embryo imaging technology: does it improve the clinical results?

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Purpose of review
The purpose of the review is to summarize recent developments in time-lapse technologies and early embryo morphokinetics and to discuss their impact on current clinical outcomes.

Recent findings
Contemporary embryo culture and selection methodologies that are based on classical morphology are clearly limited in providing the most suitable embryo for a successful pregnancy. Noninvasive observation of embryo development by capturing the images with a time-lapse device has recently been proposed to be a better method of embryo viability assessment. Such methodologies have been shown to increase the quality and the quantity of information on the viability without disturbing the culture conditions.

Summary
Commercial availability of different time-lapse devices for human embryos facilitated the use of morphokinetics as an additional tool in human embryo selection. The application of such technologies has already shown positive results on clinical outcome by increasing our scope of traditional embryo selection, leading to higher implantation and clinical pregnancy rates. Additional benefit can come from the less-disturbed incubation environment that is created by all-in-one incubators. Such devices can also be very important research tools in order to observe and analyze the effect of different patient-specific or clinical conditions on embryo development parameters that are not available through classical embryo scoring.

Keywords
clinical outcome, embryo morphokinetics, human embryo culture and scoring, time lapse

INTRODUCTION

Traditional embryo selection methods are commonly associated with relatively low in-vitro fertilization (IVF) success rate with the clinical pregnancy rate of approximately 30% per transfer, although usually more than one embryo is transferred at a time [1]. Transferring more than one embryo furthermore increases the risk of multiple pregnancies and the associated neonatal complications and maternal pregnancy-related health problems [2▪]. Implementation of elective single embryo transfer (SET) programmes is therefore of an utmost importance [3]. Contemporary embryo culture and selection aim at developing and selecting an embryo with the highest implantation potential; however, standard selection methodologies that are based on classical morphology are clearly limited [4,5]. Unless combined with a second frozen embryo transfer cycle, the SET approach brings lower pregnancy rate and clinical outcome [6]. Researchers have long been focused on finding noninvasive embryonic markers that will improve embryo selection and make it possible to offer a SET protocol without damaging the overall IVF success. The most commonly used technique for embryo evaluation remains to be daily serial observation, however, because of the concern for the stability and safety of culture conditions, embryos cannot be observed as frequently, making the amount of information limited. Furthermore, evaluating the viability and implantation potential of embryos by looking at discontinuous frames may be very subjective, depending on the embryologist's experience.

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KEY POINTS

- Commercial availability of time-lapse devices for human embryos facilitated the use of morphokinetics as an additional valuable tool in human embryo selection.
- Currently, dynamic and morphological assessment of human embryo evaluation can consist of numerous parameters, most of which had not previously been possible to be evaluated by static culture.
- Interobserver and intraobserver variability in evaluations as well as scoring in the time-lapse embryo culture are reported to be extremely consistent and reproducible compared with static embryo culture and assessment.
- Although current results indicate positive clinical impact of time-lapse embryo culture and assessment of early embryo morphokinetics, more studies are needed in order to document and validate the true benefits of such technological advances on contemporary ART research and clinical practices.

Noninvasive observation of embryo development by capturing the images with a time-lapse device has recently been proposed to be a better method of embryo viability assessment. Such methodologies have been shown to increase the quality and the quantity of information on the viability without disturbing the culture conditions [7▪,8]. Although serial imaging of a developing cell or organism is not a new concept, the commercial availability of time-lapse devices or all-in-one incubators for human embryos facilitated the use of morphokinetics as an additional tool in human embryo selection in recent years. That is, detailed timing of each event during the course of embryo development can be a very important parameter in selecting the single embryo with the highest developmental capability in the cohort, provided that the assessment is well tolerated and does not cause any alterations in the embryo culture system [8,9].

In order to evaluate a potential impact of any invasive or noninvasive marker or a parameter on clinical efficiency, it is very important to set the correct and valid main outcome measures or endpoints. In other words, predicting blastocyst formation potential *per se* may not be an adequate endpoint for implantation because endometrial receptivity is also an independent and important parameter for a successful implantation. In this review, we aimed at summarizing and discussing the recent developments in early embryo morphokinetics, their impact on classical embryo selection and viability assessment, and finally, the possible benefits on clinical outcome.

STUDIES ON EARLY EMBRYO DEVELOPMENT AND THE EMERGENCE OF EMBRYO MORPHOKINETICS

Since the first human IVF applications, embryo viability has been one of the key factors that are associated with successful clinical outcome [10]. Many studies have so far addressed single or cumulative time points in order to evaluate the best embryo with the highest implantation potential. Although early studies initially focused on fertilization and early cleavage stages, later, with the advent of blastocyst culture, scientists or embryologists have worked on identifying noninvasive markers that are associated with the morphologies during morula/blastocyst stages as well [11–17]. However, since extending culture beyond the early cleavage stage can possess a risk of changes in culture conditions, a general solution for embryo viability assessment was to decrease the number of observations while maintaining the information as maximum as possible. For this reason, dealing with qualitative variables that give ideas about the presence or absence (early cleavage, fragmentation, compaction, etc.) rather than the time when these morphological changes occur has been widely accepted as a standard evaluation protocol worldwide.

During the last decade, improvements in image technology, advances in cell biology, and the implementation of digital video recording systems in microscopy have created the basis of today's human time-lapse embryo culture systems and have led to the complete change in the concept of human embryo culture. Historically, time-lapse embryo monitoring systems can be categorized according to their design and utilization into three groups. In the first group, an incubator environment is built around a commercially available microscope. In the second group, a specially designed time-lapse microscope system is placed in a standard incubator, and in the third group, a device with specially designed incubation and built-in microscope systems is generated, creating an all-in-one time-lapse incubation system [18,19,20▪▪,21,22].

It is important to note that the science of current time-lapse embryo culture is based on more than four decades of research in either animal or human embryos [23–33]. In 1997, Payne and colleagues [23] documented the use of time-lapse techniques in order to observe the initial events during fertilization in 38 human oocytes, and they then correlated these events with the day 3 embryo development. Ten years after this work, Mio and Maeda [24] extended the analysis period to blastocyst stage. They [24] characterized the morphokinetic events from fertilization to hatching blastocyst stage for 286 human embryos. In the same year, Lemmen and

colleagues [28] analyzed the events that occur during the first day of the development after fertilization of 102 zygotes by using a microscope with an enclosed camera system. They found a link between the early disappearance of pronuclei after fertilization, early first cleavage, and many blastomeres on day 2 of the development. This group was the first to report a link between pronucleus disappearance/early cleavage and embryo developmental potential as well as extend the analysis of interaction to include pregnancy rate. This link has recently been investigated and confirmed in human embryos on live birth outcome [34]. These first time-lapse studies have expanded our knowledge regarding the time courses and events during early embryo development by establishing early morphokinetics on human embryos; however, the use of cumulative measurement and customized algorithms in clinical embryo selection had to wait until the last decade.

THE USE OF MORPHOKINETICS IN CLINICAL EMBRYO SELECTION

Despite numerous novel gamete or embryo selection methodologies (invasive or noninvasive) having been reported in the contemporary literature, morphological evaluation of human embryos is still the most valid approach in assisted reproductive technology (ART) treatments. Classical observation of oocytes/embryos under an inverted microscope can, on the other hand, only measure or observe embryo development statically, giving a momentary information about cell number, cytoplasmic status, and embryo quality [18]. Such evaluations also create significant bias or observer-dependent subjective grading among embryologists as well as among different clinics. Such characteristics may in fact limit the probability of selecting the most viable embryo(s) for transfer during static culture. Time-lapse monitoring systems have therefore created considerable positive impact on contemporary embryo selection and culture methodologies by minimizing such variations and optimizing the culture environment.

Currently, human time-lapse incubation and analysis systems are evolving through finding new morphokinetic markers or signs that can significantly reflect the developmental potential of embryos and help the embryologist to select/deselect them during treatment such as direct cleavage from one-cell stage to three-cell stage or two-cell stage to five-cell stage [35], and searching for a possible and universal mathematical algorithm that can be simultaneously calculated and used to select/deselect the embryos in an automated manner. Although they differ in the nature of the embryo analysis, microscopy settings, and the time intervals between observations, two very recent studies have suggested the use of novel algorithms to predict embryo viability based on early morphokinetic variables that are measured with time-lapse systems [20▪▪,36]. Wong *et al.* [20▪▪] analyzed 242 frozen/thawed zygotes with time-lapse imaging in order to identify predictive parameters that are important in the successful development of a blastocyst. They have also investigated differential molecular parameters and gene expression between normal and abnormal embryo cleavage rates. According to their results, they proposed an algorithm that is based on the duration of the first cleavage, duration of the two-cell stage (time interval between the first and the second mitotic division), and the duration of the three-cell stage (time interval between the second and the third mitosis) are very important for embryonic viability toward blastocyst stage. In another report, Meseguer *et al.* [36] included in the analyses 247 embryos, which were clinically used and transferred on day 3. These are the cases in which the number of gestational sacs matched the number of transferred embryos and the cases with no pregnancy result (either 100% or 0% implantation). Consistent with the findings of Wong *et al.*, [20▪▪] they found the time interval between the first and second mitotic division, and the time interval between second set of cleavage divisions were identified as predictive markers for viability. Meseguer *et al.* [36] also identified a third parameter, the time span between intracytoplasmic sperm injection and five cell embryo formation, t5, and they concluded that t5 is the strongest marker in their predictive model. In addition to cleavage durations, Meseguer and colleagues [7▪] also identified direct cleavage from one to three blastomeres, multinucleation at the four-cell stage, and uneven blastomere size at the two-cell stage as negative factors associated with the embryo viability and implantation. Currently, dynamic and morphological assessment of human embryo evaluation can consist of numerous parameters, most of which had not previously been possible to be evaluated by static culture (Fig. 1). Compared to the high variations in embryo evaluation and grading among different embryologists during static embryo morphological assessments, interobserver and intraobserver variability in evaluations as well as scoring in the time-lapse embryo culture are reported to be extremely consistent and reproducible [37].

EMBRYO MORPHOKINETICS AND CLINICAL OUTCOME

After these two groundbreaking research studies were published, other researchers subsequently

Morphokinetic parameters	Time point definitions (relative time points from T0; Insemination time: T0)
PN appearance	Time point that the first PN is visible
PN abuttal	Time point that no visible space between the two PNs exists
PN breakdown	Time point where PNs are no longer visible
First cytokinesis	Appearance of cleavage furrow
T2 through T9	Completion of division to 2 through 9 cell-stage embryo
Tcomp	Time point that at least two blastomeres begin to compact
Tm	Time point that all blastomeres are fully compacted
Tcav	Time point that formation of blastocoel cavity is initiated through cavitation
Teb	Time point that blastocyst volume is started to expand from its original volume
Thb	Time point that an expanded blastocyst started to breach out of the zonapellucida
cc2	The duration of second cell cycle; cleavage from 2- to 3-cells
S2	The duration of cell division from 3 to 4 cells
S3	The duration of cell division from 5 to 8 cells

Schematic presentation

FIGURE 1. General parameters that are used in morphokinetic scoring of human embryos and their definitions. PN, pronucleus.

published articles investigating different embryo culture and development parameters and their effects on early morphokinetics and clinical outcome. Although recent studies are mainly focused on the possible benefits on the clinical outcome, time-lapse morphokinetics and related applications are also very valuable as clinical research tools. The technology has been increasingly used to delineate the mechanisms that were once unknown such as block to polyspermy [38].

By using a morphokinetics system and algorithm based on Wong *et al.*, Conaghan and colleagues [39] have utilized the computer-automated system as a way to improve the conventional embryo selection and found that the use of embryo morphokinetics can significantly predict the blastocyst development potential and inclusion of such systems in classical embryo selection can improve the outcome. By using a different time-lapse device, Hashimoto and colleagues [40] also found that there is a link between the timing of the early embryo cell divisions and the probability of the development of embryos into blastocyst stage. Likewise, Dal Canto and colleagues [41] have reported that there is a significant correlation with early cleavage time points and blastocyst development, expansion, and implantation. Regarding embryo selection, a multicenter study also showed that direct cleavage of zygotes from one to three or five cells is a strong negative marker for implantation [35]. The technology has also recently been used to delineate the effect of different insemination methods, culture settings, and embryo culture media compositions [42–45]. Ciray and colleagues [42] suggested that until the five-cell stage, embryos that are cultured in a single media have faster development compared to sequential culture media. However, this does not translate into overall improved clinical success rates and a more recent work on culture media failed to show any statistical differences in embryo morphokinetics in an oocyte donation programme [45].

Compared to modular and/or computer-automated time-lapse systems, systems that are composed of customized incubators with built-in cameras have also been proposed to bring additional advantages in conventional laboratory and clinical settings. Because such devices work as isolated incubators and have specific gas and humidification systems, besides advantages on embryo selection, they are also expected to protect the embryos from the external environment and minimize the stress. In a recent multicenter study, Meseguer and colleagues [22] analyzed the effect of such systems on embryo viability and showed that a commercially

available time-lapse incubator system gives superior pregnancy and implantation rates compared to standard incubators. In this comparison, the probability of achieving clinical pregnancy was +20.1% per oocyte retrieval and +15.7% per embryo transfer. However, the significance of the effect and the benefit seem to vary among different IVF laboratories, implying that embryo culture conditions and the quality of the external laboratory environment can have significant effect on clinical outcome and such devices or systems can be very beneficial on poorer laboratory or clinical environments.

Hypothetically, an optimized culture and embryo selection environment would standardize the embryo development and any other confounding factors can be traced on embryos cultured in time-lapse incubators. Starting from this idea, Munoz and colleagues [46,47] retrospectively examined the influence on key clinical parameters such as E2 concentration, dose of gonadotrophins, GnRH analog used, and the ovulation-triggering agent used during ovarian stimulations in an oocyte donation programme. Results from this study suggested that the type of the gonadotrophin did not seem to affect the embryo kinetics; however, the dose of the gonadotrophins, E2 concentration, the type of GnRH analog, or ovulation triggering agent used in the treatment protocols influence the timing of the analyzed variables. Analysis of embryos that are cultured under time-lapse conditions from obese and nonobese patients has also recently shown that female obesity does not affect the dynamic embryo quality [48]. Likewise, the effect of female smoking on early human embryo development has been studied using time-lapse systems in 135 patients and a significant impairment of smoking on early development and pregnancy rates has been documented [49].

Such studies are important not only to indicate the effects of clinical variables on embryo development but also to explain outcome differences in studies that are designed to evaluate a possible correlation between different groups studied. For example, in a recent study, Campbell and colleagues [50] have retrospectively evaluated morphokinetics and preimplantation genetic diagnosis data that were obtained after day 5 trophectoderm biopsy and small nucleotide polymorphism or array comparative genomic hybridization analysis in order to understand whether there exist morphokinetic differences among chromosomally normal and abnormal embryos. It is a well-known fact that one of the main causes of early embryo losses and decreased viability is aneuploidy [51▪▪]. Based on their results, they have established a prediction model that can be utilized in order to predict the aneuploidy level of a developing embryo [50,52]. Although there is clearly a need for improvement and should be weighed with caution, such approaches are very important and may play a significant role in the embryo selection/deselection process for the automated systems to be developed in the near future [53,54▪,55].

As mentioned above, our knowledge on the effects of external parameters such as embryo culture media, stimulation protocols, incubator conditions, as well as embryo handling techniques on the embryo viability is increasing. However, the effect and long-term consequences of in-vitro culture conditions on molecular pathways as well as early fetal growth parameters (i.e. imprinting), the risk of preterm congenital or birth malformations, are still largely unknown [56,57]. Time-lapse incubation systems and morphokinetics-included embryo selection methodologies will be expected to potentially shed light on these areas as well.

LIMITATIONS OF THE CURRENT TIME-LAPSE TECHNOLOGIES

Although the technology quickly gained an important place in contemporary ART applications, technological improvements as well as development of efficient algorithms for selecting the most viable embryos are far from being ideal at the moment. One of the main drawbacks of the current time-lapse systems is that their embryo culture components do not allow rotation of embryos, making visual observation difficult. This is especially important when there is an overlapping of blastomeres or high levels of cytoplasmic fragmentation are present. Although it does not solve the problem completely, integrating an automatic cell tracking system coupled to the time-lapse device may help and save time for the observer. Such systems are currently being developed as a part of the computerized automated embryo selection module [20▪▪,39]. The use of automated systems would also help avoid the subjectivity in the optical analysis of morphokinetic markers.

Because the involvement of microscopy and imaging technology is a must, another technical concern or area to be improved is the exposure of embryos to light and potential risks that are related to the light source or wavelength used. In general, exposure of embryos every 5–15 min to light has already been discussed previously [9,58]. Nakahara *et al.* [59] compared the effect of light exposure in a standard incubator system with a built-in camera system, and they prospectively documented that light exposure throughout embryo development did not cause any detrimental effect on fertilization as well as cleavage rates and embryo quality. When

the light exposure of the two different commercially available time-lapse systems (a modified incubator with a built-in camera system versus a time-lapse camera and recording module implemented in a standard incubator) were to be compared, the duration of illumination in the former for the image acquisition was very short (approximately 15 ms), whereas in the latter this duration became subsequently longer (approximately 1000 ms). If the total number of images taken per embryo and the complete culture are calculated, the period of illumination increases tremendously. In addition, it has been previously published that short wavelength light is more detrimental than long wavelength [60]. Considering these data, guidelines on image acquisition in terms of the wavelength used, frequency of imaging, and duration of illumination should be established in the near future.

From the mathematical or algorithmic perspective, working with time ranges or quartiles, which creates very sharp and exact limits for embryo selection, is on the other hand one of the major limitations of using this technology at the moment. Even if a much better algorithm may be developed in the near future, it is also debatable whether or not the early morphokinetic marker, which can be used up to five-cell stage where embryo development is driven primarily by the maternal genome, is representative of the correct evolution after activation of the embryonic genome itself [61].

Like any novel technology, although current results look promising in terms of better embryo selection and clinical outcome, selecting the appropriate endpoint should be of crucial importance. It should be kept in mind that if the ultimate goal in the clinic is to improve take-home baby rate, implantation rate should be considered when assessing the clinical success of any novel noninvasive technology. Additionally, more studies using selected time-lapse systems for the follow-up of live births from embryos that are cultured, analyzed, and selected are needed in order to document and validate the true benefits of such technological advances. From the clinical setting, the cost/benefit ratio is also an important consideration since current time-lapse incubation systems are very expensive and most clinics with a limited number of monthly cycles could not afford to include these devices in their investment plan.

CONCLUSION

It can be said that, with the advent of time-lapse morphokinetics, a new era of preimplantation embryology has just begun to emerge. Current technology can bring the IVF field two major potential benefits in clinical outcome: by analyzing preimplantation embryo development through not only static observations but also using novel time-lapse morphokinetics parameters, an embryo with the best implantation potential can be selected, and all-in-one time-lapse incubator systems also potentially minimize the external factors that can negatively affect the embryo viability and hence increase the overall clinical results. Continuous improvements in this technology will also emerge new connections between specific morphokinetic events and help us to define novel patient-specific or disease-specific morphokinetic markers. With the help of these novel markers, our treatment methodologies or predictive models can be further improved.

In many clinics worldwide, the use of time-lapse morphokinetics has already been shown to improve the overall clinical outcome. When the implantation potential of an embryo is more accurately diagnosed, establishing a SET policy could also minimize multiple pregnancies and associated complications.

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Conflicts of interest

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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This article is a good reference for junior embryologists or specialists. The content of the manuscript can help readers to understand the potential of this technology for clinical as well as research use.

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