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Use of time-lapse monitoring in medically assisted reproduction treatments: a mini-review

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Review

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Summary

During human *in vitro* culture, a morphological microscope analysis is normally performed to select the best embryo to transfer, with the hope of obtaining a successful pregnancy. The morphological evaluation may combine number and size of blastomeres, fragmentation, multinucleation, blastocyst expansion, inner-cell mass and trophectoderm appearance. However, standard microscopy evaluation involves the removal of the embryos from the incubator, exposing them to changes in pH, temperature, and oxygen level. Additionally, morphological assessments might include high inter-observer variability. Recently, continuous embryo culture using time-lapse monitoring (TLM) has allowed embryologists to analyse the dynamic and morphokinetic events of embryo development and, based on that, the embryologist is able to scrutinize the complete sequence of embryonic evolution, from fertilization to the blastocyst formation. Therefore, TLM allows an uninterrupted culture condition, reducing the need to remove embryos from the incubator. The monitoring system is normally composed of a standard incubator with an integrated microscope coupled to a digital camera, which is able to collect images at regular times, and subsequently processed into video. These data can be annotated and analyzed using an integrated software, therefore this allows embryologists to facilitate the process of embryo selection for transfer. The main aim of this paper is to discuss the potential benefits and uses of the TLM in the embryology laboratory.

Introduction

Medically assisted reproduction (MAR) has evolved considerably over the past 4 decades, with several key advances and historical achievements (Steptoe and Edwards, 1978). Over 8 million *in vitro* fertilization (IVF) children have been born since 1978 when the first IVF baby was announced (Thoma *et al.*, 2013). In recent years, the number of couple facing infertility issues has increased steadily, many of whom will ultimately need IVF treatments. Worldwide, approximately 2.5 million MAR cycles are performed, resulting in over 500,000 deliveries annually. In the UK, IVF babies account for about 3% of all babies born in 2016 (Human Fertilisation and Embryology Authority, 2018; De Geyter *et al.*, 2018). However, *in vitro* development of the human embryo is still suboptimal, and many good quality embryos fail to implant and generate a viable pregnancy (Zhao *et al.*, 2011; Niederberger *et al.*, 2018).The advent of more complex and physiological culture medium, had facilitated the generation of better quality embryos, and allows embryo transfer at the blastocyst stage.

Extending embryo culture enables the selection of embryos at a more advanced stage, and improves both uterine and embryonic synchronicity, therefore resulting in better pregnancy outcome rates (Gardner and Schoolcraft, 1999; De Vos *et al.*, 2016). In addition, transfer of a single blastocyst avoids many adverse medical conditions for mother and baby associated with multiple pregnancies (Sullivan *et al.*, 2012). However, the embryologist's ability to select the best embryo for transfer has not changed much since the birth of Louise Brown (Steptoe and Edwards, 1978).

From the beginnings of IVF, it was noted that the grade of embryo development was associated with successful pregnancy (Edwards *et al.*, 1984). To predict embryo developmental competence and implantation potential, embryos are traditionally selected for transfer based on morphological evaluation, which provides a snap-shot of embryo development. The characteristics investigated for cleavage stage embryos may include the number and size of blastomeres, fragmentation and multinucleation (De los Santos *et al.*, 2016). For blastocyst assessment, the most widely used grading is that originally proposed by Gardner and Schoolcraft (1999). It is an alphanumeric system and, although does not cover all aspects of blastocyst morphology, has been very effective in classifying the appearance and compactness of the inner-cell mass, the cohesiveness of trophectoderm (TE) cells and degree of expansion of the blastocoels cavity (Gardner and Schoolcraft, 1999). However, morphological assessment has restricted the ability to predict implantation potential due to high inter-observer variability (Braude, 2013).

Embryo development is a dynamic process in which the morphology changes significantly in a short time (Lemmen *et al.*, 2008). Although TLM was first reported by Lewis and

Gregory (1929) and Payne and colleagues (1997), it has been introduced only recently into the embryology laboratory and has allowed scientists to analyse the dynamic process and the whole sequence of embryo development from fertilization to blastocyst formation. The time-lapse system combines three components: an incubator, a microscope and a software program. The association of those elements provides continuous embryos monitoring (Meseguer *et al.*, 2012; Basile *et al.*, 2014; Aparicio-Ruiz *et al.*, 2016) and, in parallel, maintains a stable and uninterrupted culture environment, which avoids the need to move embryos outside the incubator, exposing them to non-physiological conditions including fluctuating temperatures, humidity, pH and gas concentrations (Zhang *et al.*, 2010). Wong and collaborators (2010) found that development of human embryos to the blastocyst stage was linked to key timing events in the early embryo division such as the duration of the first cleavage, and the length of the interval between the second and third divisions.

In 2011, Meseguer and co-workers reported that embryo implantation was associated with specific cell division timing parameters, introducing the term 'morphokinetics' (Meseguer *et al.*, 2011). This review paper is not intended to provide scientific evidence of TLM, which has been recently investigated by Armstrong and colleagues (2019), the main objective will be to illustrate the different available TLM systems and to divulge and evaluate the potential benefits of the TLM in the embryology laboratory in order to advise IVF clinics to select the most suitable system appropriate to their circumstances.

Present status of time-lapse monitoring

Morphology has been the method of embryo assessment for over 40 years and represents the main approach for embryo selection during MAR cycles. However, the standard evaluation at specific time points has limitations, mainly associated with the subjectivity of the embryologist, and the missing critical events potentially harmful to the embryo's viability. Morphological assessment provides only a snap-shot of embryo development in that specific time, thereby omitting what happens during the intervals between the two observations (Cruz *et al.*, 2012). Furthermore, the embryo grade may change notably within a short amount of time (Wong *et al.*, 2010; Meseguer *et al.*, 2011). In contrast, the TLM allows the embryologist not only to analyze embryo morphology and dynamic changes during the *in vitro* embryo development, but also provides stable culture conditions (Wong *et al.*, 2010; Meseguer *et al.*, 2012; Basile *et al.*, 2014; Aparicio-Ruiz *et al.*, 2016).

Although pioneering research on TLM has been reported since 1929 and in the late 1990s (Lewis and Gregory, 1929; Payne *et al.*, 1997), the technology became commercially available for embryology laboratory only in 2009. The large numbers of published articles on the use of TLM in human embryology suggest an active application of this novel technology in embryology laboratories worldwide. However, at this time, very few data are available on the global use of TLM. Scotland is a unique country, as the government had provided funding to permit all public assisted conception units (NHS-Scotland, National Health Service https://www.scot.nhs.uk) within the state to invest in TLM. In addition to this specific case, few papers are available reporting the worldwide use and implementation of the TLM in MAR treatments. One paper was published by Dolinko and colleagues (2017), and showed the results of a survey of 294 IVF units in the USA.

The authors reported that only 162 units responded, with 35 laboratories announcing that they used at least one time-lapse system. An analogous report have been published by a French team, Boueilh and co-workers (2018). Amongst the 78 respondents, 30 centres reported using TLM clinically. Although these surveys provide interesting information on TLM use in two different countries, it is not enough to make a conclusion of the worldwide use of this technology. It would be really interesting to obtain a global picture of the time-lapse approach to evaluate its current use in IVF practice.

Time-lapse monitoring and embryo assessment: from fertilization to blastocyst formation

The identification of the embryo with the best implantation potential and high viability to generate a successful pregnancy remains an elusive goal in MAR cycles. Here, in this paragraph, we will investigate whether the use of TLM and morphokinetic embryo assessment might help in achieving this goal. Time-lapse observations have been used to define new or poorly described concepts of human embryology such as the fertilization process (Coticchio *et al.*, 2018), the duration of the first three cell cycles (Wong *et al.*, 2010, Meseguer *et al.*, 2011), the early compaction stage (Iwata *et al.*, 2014) and finally blastocyst formation (Marcos *et al.*, 2015, Sciorio *et al.*, 2020a, 2020b).

Recently, Coticchio and collaborators thoroughly investigated the fertilization event and illustrated several unknown characteristics including the cytoplasmic halo (appearance and disappearance), pronuclei (PN) fading, time from PN fading (tPNf) and the first cleavage, and those novel features were used to predict embryo quality on day-3 (Coticchio *et al.*, 2018). Another prospective study analyzed the correlation between tPNf and live birth in 159 embryos. The pronuclei morphology of 46 embryos that resulted in live birth was compared with that of 113 embryos that resulted in no live birth. The results reported that, in comparison with embryos resulting in no live birth, tPNf occurred significantly later in embryos resulting in live birth and never earlier than 20 h 45 min (Azzarello *et al.*, 2012). A further study noted that erratic PN movement within the cytoplasm and delayed pronuclei fading were indicative of compromised embryo development (Athayde Wirka *et al.*, 2014). The authors in this retrospective multicentre trial identified four atypical phenotypes, including abnormal syngamy, abnormal first cytokinesis, abnormal cleavage and chaotic cleavage, and correlated their relationship with embryo viability and implantation potential. The conclusion was that embryos exhibiting atypical phenotypes showed a significantly lower developmental potential compared with the control group (Athayde Wirka *et al.*, 2014). Wong and co-workers (2010) suggested that blastocyst stage could be predicted with high sensitivity by the timing of the early developmental stage: including the first cytokinesis (0–33 min), the time interval between the end of the first mitosis and the initiation of the second, which is the duration of 2-cell stage (7.8–14.3 h), and the time interval between the second and third mitoses (0–5.8 h duration of the 3-cell stage).

Lemmen and colleagues established that embryos resulting in successful pregnancies displayed not only a significantly higher cleavage synchrony but also a higher synchrony in nuclear appearance at the 2-cell stage compared with non-implanting embryos (Lemmen *et al.*, 2008). Using morphokinetics assessment it has been possible to demonstrate associations between various cleavage stage events and the embryo's ability to reach the blastocyst stage (Wong *et al.*, 2010; Cruz *et al.*, 2012).

Meseguer and collaborators analyzed large data sets on transferred embryos generated by ICSI, and the results displayed that

the timing of the cleavage to five cells was the most predictive parameter for embryo viability and implantation (Meseguer *et al.*, 2011). Subsequently, the same group in a retrospective multicentre study performed in 10 IVF clinics compared pregnancy outcomes between time-lapse culture ($n = 1390$ cycles) and a standard incubator ($n = 5915$) and they reported a relative 20% improvement in pregnancy rates in the TLM (Meseguer *et al.*, 2012). The authors associated the elevated clinical pregnancy obtained in the TLM group to a combination of both stable culture conditions and the use of morphokinetic parameters for embryo selection.

Similar results were confirmed by the same group in a prospective randomized controlled trial 2 years later (Rubio *et al.*, 2014). The introduction of more physiological culture conditions for *in vitro* human embryos, has resulted in the routine culture and transfer of embryos at the blastocyst stage (Gardner and Schoolcraft, 1999; De Vos *et al.*, 2016). Within countries undertaking only single embryo transfer, an active reduction in the numbers of embryos being transferred has been witnessed.

Additionally, the transfer of a single blastocyst prevents many adverse medical conditions associated with multiple pregnancies (Sullivan et al, 2012; De Vos *et al.*, 2016). Furthermore, blastocyst transfer provides increased implantation rates compared with transfers at the cleavage stage, but this outcome needs to be correlated with the possible detrimental epigenetic effects associated with extended *in vitro* culture (Kirkegaard *et al.*, 2012). In this context, TLM has been applied to predict blastocyst formation and implantation potential based on novel morphokinetic parameters noted at cleavage stage (Dal Canto *et al.*, 2012).

Kirkegaard and collaborators reported that cleavage from the 2- to 8-cell stages appears progressively earlier in embryos that will generate a blastocyst and will implant. The authors suggested that top quality blastocysts could be predicted within the first 2 days of *in vitro* culture by the short duration of the first cleavage, and duration of the 3-cell stage (Kirkegaard *et al.*, 2013). In the same way, Hashimoto and collaborators (2012) showed that better quality blastocysts presented significantly shorter times for synchrony between the 3- and 4-cell stages. Recently, Motato and colleagues (2016) analyzed the morphokinetic parameters in 7483 embryos and identified two features linked to blastocyst formation: the time of morula formation (81.28–96.0 h after ICSI), and the time of transition from 5- to 8-cell embryos (≤ 8.78 h). Finally, spontaneous blastocyst collapse during *in vitro* embryo development has been suggested as a novel marker of embryo viability and implantation potential. Retrospective studies have reported that blastocysts exhibiting collapse during development are less likely to implant and generate a pregnancy compared with embryos that do not (Marcos *et al.*, 2015, Sciorio *et al.*, 2020a, 2020b). It has been reported that annotation of collapse(s) events may improve the embryo assessment at blastocyst stage. Summaries of the main atypical features identified with the TLM are given in Tables 1 and Table 2 including some recent papers published on the time-lapse technology.

Correlation between TLM and embryos aneuploidy

Aneuploidy is the occurrence of the wrong number of chromosomes in a cell, for example 45 or 47 chromosomes instead of the normal 46. Aneuploidy is an important concern in *in vitro* human embryos obtained from MAR treatments: the transfer of aneuploid embryos may result in implantation failure, miscarriage or birth of an offspring with a range of potential abnormalities (Sciorio and Dattilo, 2020).

The conventional procedure to investigate aneuploidy in human embryos is termed preimplantation genetic testing for aneuploidy (PGT-A), previously called preimplantation genetic screening (PGS), which consists in an IVF cycle in which embryos are biopsied and screened for chromosomal abnormalities prior to replacement into the uterus. The procedure was first introduced by Handyside (Handyside *et al.*, 1990). However, PGT-A is an expensive technology and is not allowed in some countries, and there remains some debate regarding its cost–effectiveness, the invasiveness of the procedure and the clinical efficiency (Sermon *et al.*, 2016; Sciorio and Dattilo, 2020).

It has been hypothesized that TLM could be used to identify embryo aneuploidy, therefore providing a cheaper, faster and less invasive evaluation approach. Several studies have correlated morphokinetic parameters using TLM with the probability of selecting chromosomally normal embryos. It was supposed that cell division length has to be within an optimum range to overcome all the cellular processes preceding cytokinesis (Davies *et al.*, 2012; Campbell *et al.*, 2013a, 2013b; Montag, 2013; Swain, 2013; Chawla *et al.*, 2015).

Davies and co-workers (2012) found that aneuploidy embryos showed delays on the first two cleavages as well as prolonged transitions between 2- and the 4-cell stages. The author also noted that irregular divisions and an asynchronous PN disappearance were higher in abnormal embryos compared with the normal group. Chavez and colleagues (2012) investigated the relationship between genetic status and morphokinetic parameters, and demonstrated that euploid embryos have definite timing at the first cell divisions up to the 4-cell stage.

Chawla and associates (2015) assessed several morphokinetic features including timings of the extrusion of second polar body, pronuclei appearance and fading, time of first division, second and third cleavages duration in 460 embryos to discriminate abnormal embryos. The results showed that morphokinetic parameters differed significantly for euploid and aneuploidy embryos (Chawla *et al.*, 2015). Campbell and collaborators using the TLM tried to develop a model to identify embryos aneuploidies. They found the time of early blastulation and the timing of full blastocyst formation were relevant features for embryo euploidy (Campbell *et al.*, 2013a, 2013b).

Basile and colleagues investigated the differences in the time of cleavage between chromosomally normal and abnormal embryos to identify chromosomally normal embryos. The authors showed that normal and abnormal embryos had different kinetic behaviours and, based on that, they proposed an algorithm as a non-invasive tool to increase the likelihood of selecting genetically normal embryos (Basile *et al.*, 2014). A comprehensive review on the value of TLM as a tool to identify and select euploid embryos has been published recently by Reignier and co-workers. They concluded that, despite several studies showing significant differences in morphokinetic parameters between euploid and aneuploid embryos, none of them found adequate evidence to recommend the clinical use of TLM in identifying embryo aneuploidies. Consequently, selection of embryos using time-lapse technology should not be considered as a replacement for PGT-A (Reignier *et al.*, 2018).

Different TLM systems

At present, there are several commercially available time-lapse systems. During the selection process of a TLM model, the clinic should take in consideration some practical aspects, including size and space of each system, the cost and the laboratory workload.

Table 1. Atypical phenotypes observed with time-lapse monitoring

Feature	Description	Study/Reference
Pronuclei (PN) formation Syngamy	Wrong PN movement in the cytoplasm	Coticchio *et al.*, 2018 Azzarello *et al.*, 2012
Appearance of two PN Pronuclei reappearance	Asynchronous appearance and disappearance of PN Pronuclei fading and reappearance	Coticchio *et al.*, 2018
Pronuclei size	Difference in pronuclear areas before pronuclear fading	Otsuki *et al.*, 2017
PN fragmentation PN fusion	Formation of micronuclei A pronucleus formed by the fusion of two preexisting pronuclei	Mio and Maeda, 2008 Coticchio *et al.*, 2018
Unipolar cleavage furrow Tripolar cleavage furrow Pseudofurrows	Appearance of cleavage furrow on one site of the zygote Appearance of three cleavage furrows Zygote presenting oolemma ruffling before cytokinesis	Wong *et al.*, 2010 Athayde Wirka *et al.*, 2014
Absent cleavage Reverse cleavage	Arrest at zygote stage Fusion of two cells into one blastomere	Barrie *et al.*, 2017 Desai *et al.*, 2014
Direct cleavage	Cleavage of zygote to three cells or one blastomere divides to three cells	Athayde Wirka *et al.*, 2014 Barrie *et al.*, 2017 Meseguer *et al.*, 2011
Blastomere movement	Blastomere and cytoplasm movement before division	Ezoe *et al.*, 2019
Multinucleation	Blastomere with more than one nucleus	Desai *et al.*, 2014 Hashimoto *et al.*, 2016
Internalization of cellular fragments	Fragments reabsorbed into one blastomere	Mio and Maeda, 2008
Irregular chaotic division	Disordered cleavage behaviour with uneven cleavages and fragmentation	Athayde Wirka *et al.*, 2014 Barrie *et al.*, 2017 Meseguer *et al.*, 2011
Early compaction	Formation of tight junctions between blastomeres in day 3 embryos	Iwata *et al.*, 2014
Cell exclusion	Exclusion of one or more blastomeres from the morula formation	Coticchio *et al.*, 2019
Spontaneous blastocyst collapse	Collapse of blastocyst with complete disappearance of blastocoel cavity	Marcos *et al.*, 2015 Sciorio *et al.*, 2020a Sciorio *et al.*, 2020b

Generally, all systems necessitate the use of a digital inverted microscope with a camera to collect embryo images at specific times. Some models contain an incubator equipped with a built-in camera, while other systems comprise a camera that is placed in a traditional large-box incubator (Kirkegaard *et al.*, 2012; Chen *et al.*, 2013; Campbell and Fishel, 2015).

Although all TLM systems available at this time use an oil overlay on culture microdrops, there are differences in the way the embryos are cultured, and all systems need a specific culture dish, supplied by the manufacturer. Some models provide an individual culture set-up, in which the dish has a designed number of wells, each holding one embryo (Chen *et al.*, 2013; Campbell and Fishel, 2015; Racowsky *et al.*, 2015). However, some culture dishes permit sharing of culture medium between compartments and are designed for group culture, allowing exchange of soluble components. This may represent an important concern when deciding which specific model to purchase. However, each system uses a different light source and differs in the way the embryos are brought into the field of view, some without movement of the embryos versus others in which there is a constant movement of the culture dish. Few systems use bright field technology allowing the assessment of both kinetic parameters and embryo morphology.

Other models apply dark field technology supporting the determination of kinetic parameters, but give limited information on the morphological features. However, other aspects influencing a decision might include the nature of the computer software used for visualization and analysis, and the options for annotation, which may be manual or automated. Technical characteristics of some TLM systems available at this time are reported in Table 3.

Time-lapse monitoring and its effect on embryo culture

Human embryo culture is associated with numerous physical and chemical stressors (Wale and Gardner, 2016), which might create a hostile environment for the preimplantation developing embryo. Embryo culture using TLM allows culture in a stable environment, avoiding the exposure of the embryo to the non-physiological conditions such as pH and temperature changes, or altered gases concentrations (CO_2 and O_2). The culture medium used represents an important factor for embryo culture.

Over the past few decades, we have noticed several improvements in the culture systems, mainly linked to medium composition. Two approaches have been suggested: the 'sequential and the single-step'. The first is proposed to ensure that the physiological conditions of the human embryo are similar to that of the *in vivo* environment, when it would move from the oviduct to the uterus (Barnes *et al.*, 1995).

In contrast, the single-step medium is based on the concept that it is of benefit to supply all metabolic nutrients required, and that the embryo will use them according to its demand (Summers *et al.*, 1995). Several studies have been performed in conventional incubators with the aim to establish which system is superior, but the results remain inconclusive (Sfontouris *et al.*, 2016; Werner *et al.*, 2016). The concern emerges of whether the use of TLM might find small parameters variations between sequential and single-step media.

Table 2. Some studies published from 2010 that have used time-lapse technology

Study	Aim/Description
Azzarello *et al.*, 2012	Pronuclei (PN) development in embryos after ICSI
Athayde Wirka *et al.*, 2014	Identification of atypical embryo phenotypes by time-lapse, and correlation with embryo development
Aparicio-Ruiz *et al.*, 2016	To correlate morphokinetic parameters with blastocyst formation, quality, implantation and ongoing pregnancy rates
Aguilar *et al.*, 2014	Time correlation between fertilization events and embryo implantation
Armstrong *et al.*, 2019	Time-lapse Cochrane review
Basile *et al.*, 2014	Elaborate with use of time-lapse an algorithm to increase the probability of noninvasively selecting a chromosomally normal embryo
Boueilh *et al.*, 2018	Evaluation of time-lapse imaging used in French IVF units
Coticchio *et al.*, 2018	Time-lapse analysis and novel aspects of human fertilization and new morphokinetic parameters of embryo viability
Cruz *et al.*, 2012	Correlation between embryo division kinetics and blastocyst formation
Chavez *et al.*, 2012	Precise cell cycle parameter timing is observed in all euploid embryos to the 4-cell stage, whereas only 30% of aneuploid embryos exhibit parameter values within normal timing windows
Campbell *et al.*, 2013a	Develop a model to categorize the risk of embryo aneuploidy based on morphokinetic parameters
Campbell *et al.*, 2013b	Evaluate the effectiveness of the previously established, morphokinetic-based aneuploidy risk classification model
Chawla *et al.*, 2015	To analyze differences in morphokinetic parameters of euploid and aneuploid embryos utilizing time-lapse imaging and genetic analysis
Chen *et al.*, 2013	Time-lapse review
Dal Canto *et al.*, 2012	Analyze cleavage timings in relationship to blastocyst formation and implantation
Fréour *et al.*, 2013	Evaluate of embryo morphokinetic parameters and female smoking status
Hashimoto *et al.*, 2012	Assess the development kinetics of embryos and their ability to develop to blastocyst
Iwata *et al.*, 2014	Analyze the timing of initiation of compaction in human embryos
Kirkegaard *et al.*, 2013	Duration of the first cytokinesis, duration of the 3-cell stage and direct cleavage to three cells predicted development of a high-quality blastocyst
Meseguer *et al.*, 2011	Generate and evaluate an embryo selection tool based on morphokinetics
Meseguer *et al.*, 2012	Compare pregnancy outcomes in an incubator with time-lapse versus tissue culture chamber
Motato *et al.*, 2016	To correlate morphokinetic parameters with blastocyst formation and implantation
Munõz *et al.*, 2013	Evaluate if type of GnRH analogue used during controlled ovarian stimulation influences early embryo developmental kinetics
Montag 2013	Time-lapse review: attempts to correlate timings with embryonic aneuploidy
Racowsky *et al.*, 2015	Time-lapse review
Rubio *et al.*, 2012	Analyze implantation rate of embryos with cleavage from 2 to 3 cells in less than 5 h
Sciorio *et al.*, 2020a; Sciorio *et al.*, 2020b; Marcos *et al.*, 2015	Time-lapse imaging showed that blastocyst collapse(s) event is associated to low implantation potential
Sundvall *et al.*, 2013	To assess the variability of time-lapse annotations
Swain 2013	Review of studies that attempt to correlate timings with embryo aneuploidy
Wong *et al.*, 2010	Prediction of embryo potential to blastocyst stage using morphokinetic parameters

One of the first studies to analyzing the dynamics of early development between embryos cultured in single and sequential medium using TLM was published by Ciray and associates. A randomized study was performed on 446 oocytes, which were divided between single and sequential medium produced by the same manufacturer and cultured in the same time-lapse incubator. The result found that in single-step medium, fading of PN and cleavage up to 5-cell stage took place significantly earlier compared with embryos cultured in sequential medium. In implanted embryos, t2 and t4 were significantly shorter with the single-step medium. However, the clinical outcome rates did not differ between the two groups (Ciray *et al.*, 2012). Similar findings were reported by Kazdar and collaborators (2017).

Conversely, other studies were not able to found any morphokinetic differences between the two approaches (Basile *et al.*, 2013; Sfontouris *et al.*, 2017). Therefore, current data have been unable to show a clear superiority of either single-step nor sequential medium in terms of clinical pregnancies adopting either with

Table 3. Comparisons of technical characteristics of some TLM systems at this time available

Some models available	EmbryoScope	EmbryoScope+ [Plus]	Evea	Geri®	Primo Vision	Esco Miri
Design	Integrated incubator microscope	Integrated incubator microscope	Applicable in standard incubator	Integrated incubator microscope	Applicable in standard incubator	Integrated incubator microscope
Number of embryo in one dish	12	16	12	16	9 or 16	14
Number of patients per system	6	15	4	6	6	6
Embryo culture	Single	Single	Group	Single	Group	Single
Number of focal planes	9	11	1	11	11	No limit
Frequency of images	10 min	10 min	5 min	5 min	5 min	5 min
Light/Illumination	Red LED	Red LED	Dark field	Red LED	Green LED	Red LED
Registration	CE medical device class IIa FDA	CE medical device class IIa FDA	CE and Canada approved FDA pending	FDA pending	CE medical device class IIa	CE medical device class IIa FDA pending

standard incubations or TLM. As mentioned previously, the use of TLM prevents embryo exposure to environmental conditions and thereby emulates *in vivo* conditions. It has been well reported that steady gas concentrations (for CO_2 and O_2) are extremely important for embryo development and viability (Sciorio and Smith, 2019). It has been established that the oxygen concentration of the mammalian female reproductive tract is between 2 and 8% (Fischer and Bavister, 1993).

Exposure of embryos to atmospheric oxygen concentrations is correlated with an increased production of reactive oxygen species, which might modify embryo metabolism and gene expression (Fischer and Bavister, 1993; Rinaudo *et al.*, 2006; Wale and Gardner, 2012; Sciorio and Smith, 2019). There is large amounts of evidence suggesting that embryo culture in 5%, rather than ambient, oxygen leads to improved pregnancy outcomes (Meintjes *et al.*, 2009; Bontekoe *et al.*, 2012). A recent prospective randomized multicentre study performed on 1563 oocytes, confirmed that inclusion of antioxidants in the culture medium increased embryo viability, implantation and ongoing pregnancy rates significantly, possibly through the reduction of oxidative stress (Gardner *et al.*, 2020). The advantage of lower oxygen tension levels is included in the use of TLM.

Future research

Although TLM has been proposed since the 1929 (Lewis and Gregory, 1929), the technology became commercially available only about a decade ago, therefore in comparison with other technical advancements made in cell biology, time-lapse might be considered in its childhood, and, as such, the technology applied could be further improved. Looking to the future, it will be expected that some developments correlated with image collection are likely to become available.

Development of fluorescence and confocal microscopy associated with the time-lapse and allowing the morphokinetic observation of organelles and chromosomes has already been proposed (Holubcova *et al.*, 2015; Patel *et al.*, 2015), as well as fluorescence live-cell imaging of human embryos (Hashimoto *et al.*, 2016). Furthermore, one concern of TLM is the difficulty to assess and interpret the huge amount of data collected, which offers the opportunity for evolution of artificial intelligence (AI) and the use of higher-powered computers to analyze the considerable amount of images, and to identify a specific parameter that might correlate with embryo viability and pregnancy outcomes.

In that context, software programs are being used as automatic alternatives to standardize time-lapse annotations (Yeung *et al.*, 2018). Unlike other medical fields, ART has not yet explored the advantages of AI for automated embryo evaluation and selection. It has been hypothesized that an AI approach trained through exposure to thousands of embryo images and videos would later permit identification and prediction of embryo quality without mediation. This might be beneficial to reduce human error and standardize the annotation, and will allow embryologists concentrate on different tasks.

A study was performed by Khosravi and colleagues that used AI and TLM and, by analyzing clinical data for 2182 embryos and about 50,000 images, they developed a model that was able to predict blastocyst quality with an AUC of >0.98 (Khosravi *et al.*, 2019). In another recent retrospective trial, a deep learning approach has been used to automatically annotate 10,638 embryo videos from eight different IVF units across four countries. The results showed that deep learning model was able to predict fetal heartbeat pregnancy from time-lapse videos with an AUC of 0.93 (Tran *et al.*, 2019). These are retrospective studies, and further trials, including prospective randomized controlled trials, are required to evaluate the clinical significance of AI in IVF. However these trials demonstrated that the deep learning model and AI have an high level of predictability of embryo viability and implantation (Khosravi *et al.*, 2019; Tran *et al.*, 2019). Of course, before the AI approach would be clinically used, it will be required to pass vigorous clinical validation process.

Conclusion

At this time, despite extensive advancement achieved in MAR worldwide, most IVF units still perform embryo selection based on standard morphological evaluation, which has several limitations. Novel objective criteria should be included in the selection

process of embryos to be transferred in IVF cycles. In that context, the introduction of TLM provides new morphokinetic features during *in vitro* culture, allowing embryologists to obtain new insights into key stages of embryo development, and therefore improve the selection process. Detection of atypical embryo phenotypes has proven to be essential for the process of deselecting embryos with a poor prognosis for transfer, which might result in a negative pregnancy.

Based on current technology, continuous TLM might bring a safe and steady embryo culture environment, which has allowed embryologists to identify unknown or undetectable aspects of development, including direct cleavage into three cells, which negatively affects clinical pregnancy. Presumably, in the next decade with the further advancement of AI, TLM will develop into an established method for embryo selection, linked to a non-invasive analytical approaches. At this future stage, TLM will probably become essential for embryologists and might be routinely applied for human embryo culture in MAR treatments.

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