APPLICATION OF TIME-LAPSE TECHNOLOGY IN ASSISTED REPRODUCTIVE TECHNOLOGY (ART) TREATMENTS

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Abstract
During human in vitro culture, a morphological microscope analysis is normally performed to select the embryo with the highest implantation potential to replace, with the hope to obtain a successful pregnancy and health live birth. 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 un-physiologic conditions as 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 fertilisation 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 analysed using an integrated software, thus 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.

Keywords: *Time-lapse monitoring, medically assisted reproduction, embryo culture, morphokinetic evaluation, embryo viability*

INTRODUCTION

Medically assisted reproduction (MAR) has advanced significantly over the past four decades, beginning with the birth of the first in vitro fertilization (IVF) baby in 1978 (Steptoe and Edwards, 1978). Since then, over 8 million IVF-conceived children have been born worldwide (Thoma et al., 2013). With rising infertility rates, many couples now require IVF, and approximately 2.5 million MAR cycles are performed globally each year, resulting in over 500,000 births. In the UK alone, IVF accounted for 3% of all births in 2016 (Human Fertilisation & Embryology Authority, 2014–2016; De Geyter et al., 2018).

Despite advancements in culture media and embryo selection, in vitro human embryo development remains suboptimal, with many high-quality embryos failing to implant (Zhao et al., 2011; Niederberger et al., 2018). More advanced culture techniques have improved embryo quality and outcomes by facilitating blastocyst-stage transfers, enhancing embryo-uterine synchronization, and reducing risks of multiple pregnancies (Gardner and Schoolcraft, 1999; Sullivan et al., 2012).

However, embryo selection methods, primarily based on morphological assessments, have not evolved significantly since the early days of IVF (Edwards et al., 1984). Morphological evaluations, which assess cleavage stage embryos and blastocyst quality, have limitations due to high variability between observers and only offer a snapshot of development (Braude, 2013; Gardner and Schoolcraft, 1999).

Time-lapse monitoring (TLM), first introduced by Lewis and Gregory (1929) and further explored by Payne et al. (1997), allows continuous monitoring of embryos throughout development without disturbing culture conditions. The system, combining an incubator, microscope, and software, maintains stable conditions while providing detailed insights into embryo development (Basile et al., 2014; Zang et al., 2010). Recent studies have linked embryo implantation potential with precise timing events during early development (Wong

et al., 2010; Meseguer et al., 2011). This review aims to explore the benefits of TLM in embryology labs and guide IVF clinics in selecting the best system for their specific needs.

CURRENT STATUS OF TIME-LAPSE TECHNOLOGY IN ART

Morphology has been the method of embryo assessment for over 40 years and represents the main approach for embryo selection during MAR treatments. However, the standard evaluation at specific time points has limitations, mainly associated to the subjectivity of the embryologist, and the missing of critical events potentially harmful to embryo's viability. Morphological assessment provides only a snapshot of embryo development in that specific time, thereby omitting what happens during the intervals between 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-b). In contrast, the TLM allows not only to assess embryo morphology and dynamic changes during the in vitro embryo development, but also provides a stable culture conditions (Basile et al., 2014; Aparicio-Ruiz et al., 2016; Meseguer et al., 2012; Wong et al., 2010). Although pioneering research on TLM has been reported since the late 1990s (Payne et al., 1997), the technology became commercially available for embryology laboratory in 2009. The large number of published articles on the use of TLM in human embryology, suggest an active application of this novel technology in the embryology laboratories worldwide. However, very little data are currently available on the global use of TLM. Scotland represents a unique country, since 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 the TLM. Besides this specific case, few papers are available reporting the worldwide use and implementation of the TLM in MAR treatments. One has been 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 use at least one time-lapse system. An analogous report has 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 an interesting information on the TLM use in two different countries, it is not enough to make a conclusion of the worldwide use of this technology. It would be interesting to obtain a global picture of the time-lapse advancements to evaluate its current use in IVF practice.

TIME-LAPSE MONITORING AND CONTINUOUS 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 will investigate whether the use of the 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-b), the early compaction stage (Iwata et al., 2014) and finally the blastocyst formation (Marcos et al., 2015, Sciorio et al., 2020-a, Sciorio et al., 2020-b). Recently, Coticchio and collaborators deeply investigated the fertilization event and illustrated several unknown characteristics, including the cytoplasmic halo (appearance and disappearance), the 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 analysed the correlation between tPNf and live birth in 159 embryos. The pronuclei morphology of 46 embryos which resulted in live birth was compared with that of 113 embryos which resulted in no live birth. The results reported that in comparison to 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). Further study noted that erratic PN movement within the cytoplasm and delayed of pronuclei fading was 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 with embryo viability and implantation potential. The conclusion was that embryos exhibiting atypical phenotypes showed a significantly lower developmental potential compared to 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 early developmental stage: including the first cytokinesis (0-33 minutes), the time interval between the end of the first mitosis and the initiation of the second, which is the duration of two-cell stage (7.8-14.3 hours), and the time interval between the second and third mitoses (0-5.8 hours-duration of the three-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 two-cell stage compared with non-implanting embryos (Lemmen et al., 2008). Using morphokinetics assessment 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 analysed 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 parameters for embryo viability and implantation (Meseguer et al., 2011-b). Subse-

Table 1. Atypical features that can be identified with time-lapse monitoring incubator

Feature	Description	Study/Reference
-Pronuclei (PN) formation -Singamy	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 et al., 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	AthaydeWirka 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 et al., 2008
Irregular chaotic division	Disordered cleavage behaviour with un-even 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., 2020-a Sciorio et al., 2020-b

quently, the same group in a retrospective multicentre study performed in ten IVF clinics compared pregnancy outcomes between time-lapse culture (n=1,390 cycles) and standard incubator (n=5,915) 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 the embryo selection. Similar results have been confirmed by the same group in a prospective randomized controlled trial two years later (Rubio et al., 2014). The introduction of more physiological culture conditions for in vitro human embryo, has driven to the routine culture and transfer of embryos at the blastocyst stage (Gardner and Schoocraft, 1999; De Vos et al., 2016). Within countries following the police of single embryo transfer, it has been witnessed an active reduction in the numbers of embryos being transferred. Additionally, the transfer of a single blastocyst prevents the many adverse medical conditions associated with multiple pregnancies (Sullivan et al, 2012; De Vos et al.,

2016). Furthermore, the blastocyst transfer provides an increased implantation rates compared to transfers at the cleavage stage, but this outcome needed to be correlated to the possible detrimental epigenetic effects associated to extended in vitro culture (Kirkegaard et al., 2012-a). In this context, the TLM has been applied to predict blastocyst formation and implantation potential based on novel morphokinetic parameters annotated at cleavage stage (Dal Canto et al., 2012). Kirkegaard and collaborators, reported that cleavage from the two to eight cell stage appears progressively earlier in embryos that will generate a blastocyst, and it will implant. The authors suggested that top quality blastocysts could be predicted within the first two days of in vitro culture by a 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) assessed the morphokinetic parameters in 7,483 embryos and they identified two feature linked to blastocyst formation: the time morula formation (81.28-96.0 hours after ICSI), and transition from five to 8-cell embryos (≤ 8.78 hours). 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 blastocyst 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., 2020-a; Sciorio et al., 2020-b). It has been reported that annotation of collapse(s) events may improve the embryo assessment at blastocyst stage. A summary of the main atypical features identified with the TLM, and some recent papers published on this approach are reported in Table 1.

CURRENT STATUS OF TIME-LAPSE TECHNOLOGY IN ART

For over 40 years, morphology has been the primary method for embryo selection during MAR treatments. However, it has limitations, such as subjectivity and missing critical developmental events. Morphological assessments offer only a snapshot of development, omitting changes between observations (Cruz et al., 2012). Additionally, embryo grading can change quickly (Wong et al., 2010; Meseguer et al., 2011). In contrast, time-lapse monitoring (TLM) allows continuous assessment of embryo morphology and dynamic changes, while providing stable culture conditions (Basile et al., 2014). Introduced to embryology labs in 2009, TLM has seen widespread adoption, though global data on its use are limited. A survey in the USA found 35 of 294 IVF labs using TLM (Dolinko et al., 2017), and a similar survey in France found 30 out of 78 clinics using it clinically (Boueilh et al., 2018). However, more comprehensive studies are needed to assess TLM's worldwide adoption.

Time-lapse monitoring and continuous embryo assessment

Identifying embryos with high implantation potential remains a challenge in MAR. TLM has helped define new concepts in embryology, such as key timing events in fertilization and early cell divisions (Wong et al., 2010; Meseguer et al., 2011). Coticchio et al. (2018) investigated fertilization timing, while Meseguer et al. (2012) showed that TLM improved pregnancy rates by 20%. Morphokinetic parameters, such as early cleavage and blastocyst formation timing, have been linked to embryo viability (Wong et al., 2010; Kirkegaard et al., 2013). Studies have also shown that embryos with atypical developmental patterns, such as chaotic cleavage or delayed pronuclei fading, have lower implantation potential (Athayde-Wirka et al., 2014). Finally, blastocyst collapse during in vitro development has been identified as a potential marker of reduced viability (Marcos et al., 2015; Sciorio et al., 2020).

POTENTIAL CORRELATION BETWEEN TLM AND EMBRYOS ANEUPLOIDY

Aneuploidy is the occurrence of a 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, miscarriages 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 where 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, 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, thus providing a cheaper, faster and less invasive evaluation approach. Therefore, several studies have correlated the morphokinetic parameters using the TLM with probability of selecting chromosomally normal embryos. It was supposed that cell division length has to be within an optimum range in order to overcome all the cellular processes preceding cytokinesis (Montag, 2013; Swain, 2013; Davies et al., 2012, Chawla et al., 2015; Campbell et al., 2013-a; Campbell et al., 2013-b). 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 stage. The author also noted that irregular divisions and an asynchronous PN disappearance were higher in abnormal embryos compared to the normal group. Chavez and colleagues (2012) investigated the relationship between genetic status and mor-

phokinetic 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 in order 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 as relevant features to embryo euploidy the time of early blastulation and the timing of full blastocyst formation (Campbell et al., 2013-a; Campbell et al., 2013-b). Basile and colleagues investigated the differences in the time of cleavage between chromosomally normal and abnormal embryos in order to identify chromosomally normal embryos. The authors showed that normal and abnormal embryos have different kinetic behaviour, 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 recently published by Reignier and co-workers. They concluded that despite several studies showed 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. Generally, all systems necessitate the use of a digital inverted microscope with a camera to collect embryo images at specific times. Some models contain 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-a; Campbell and Fishel, 2015; Chen et al., 2013). Although, all TLM systems currently available use an oil overlay on culture microdrops, there are differences in the way the embryos are cultured, and all systems needed 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 well, each holding one embryo (Campbell and Fishel, 2015; Chen et al., 2013; Racowsky et al., 2015). However, some of the culture dishes permit the sharing of culture media 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 systems use 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 other 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 the dark field technology supporting the determination of kinetic parameters, but gives limited information on the morphological features. However, other aspects influencing a decision might include the nature of the computer software used for visualisation and analysis, and the options for annotation, which may be manual or automated.

POTENTIAL BENEFIT OF TLM AND ITS IMPACT 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 pre-implantation developing embryo. Embryo culture using TLM allows culture in a stable environment, avoiding the exposure of the embryo to the un-physiologic conditions such as pH and temperature changes, or altered gases concentrations (CO_2 and O_2). The culture media used represents an important factor for the embryo culture. Over the past few decades, we have noticed several improvements in culture systems, mainly linked to media compositions. Two approaches have been suggested: the “sequential and single-step”. The first is proposed to assure the physiologic condition of the human embryo in a way that is similar to the in vivo environment, as it would move from the oviduct to the uterus (Barnes et al., 1995). In contrast, the single step media, it has been based on the concept that it is of benefit to supply all metabolic nutrients required, and 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 to the other, but the results remain inconclusive (Werner et al., 2016; Sfontouris et al., 2016). There emerges the concern of whether the use of TLM might find little parameters variations between sequential and single-step media. One of the first study to analysing the dynamics of early development between embryos cultured in single and sequential media using TLM was published by Ciray and associated. A randomized study was performed on 446 oocytes, which were divided between single and sequential media produced by the same manufacturer and cultured in the same time-lapse incubator. The result found that in single-step media, fading of PN and cleavage up to five-cell stage took place significantly earlier compared to embryos culture in sequential medium. In implanted embryos, t2 and t4 were significantly shorter with a single-step media. However, the clinical outcome rates did not differ between the two groups (Ciray et al., 2012). Similar founding was reported by Kazdar and collaborators (2017). On the other hand, other studies were not able to find 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 media in terms of clinical pregnancies adopting either with standard incubations or TLM. As mentioned before the use of TLM prevents the embryo exposure to the environmental conditions and thereby emulating the in vivo conditions. It is well reported that steady gases concentration (CO2 and O2) is extremely important for embryo development and viability (Sciorio and Smith, 2019). It has been established that oxygen concentration of the mammalian female reproductive tract is between 2% and 8% (Fischer and Bavister, 1993). Exposure of embryos to atmospheric oxygen concentration is correlated with an increased production of reactive oxygen species, which might modify embryo metabolism and gene expression (Sciorio and Smith, 2019; Fischer and Bavister, 1993; Rinaudo et al., 2006; Wale and Gardner, 2012). There is plenty amount of evidence suggesting that embryo culture in 5% rather than ambient oxygen leads to improved pregnancy outcomes (Meintjes et al., 2009; Bentekoe et al., 2012). A recent prospective randomized multicentre study performed on 1,563 oocytes, confirmed that inclusion of antioxidants to the culture media increases significantly embryo viability, implantation and ongoing pregnancy rates, possibly through the reduction of oxidative stress (Gardner et al., 2020). The advantage of lower oxygen tension level is included in the use of TLM.

FUTURE RESEARCH: EVOLUTION OF ARTIFICIAL INTELLIGENCE (AI) AND TLM

Although TLM has been proposed since the 1929 (Lewis and Gregory, 1929), the technology became commercially available 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 to the image collection are likely to come. Development of fluorescence and confocal microscopy associate to the time-lapse allowing the morphokinetic observation of organelles and chromosomes has been already 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 offer the opportunity for the evolution of artificial intelligence (AI) and the use of higher-powered computer to analyse the considerable number of images, in order to identify a specific parameter that might correlate to embryo viability and pregnancy outcomes. In that context, software programs are being used as automatic alternatives in order to standardize time-lapse annotations (Yeung et al., 2018). Unlike in 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 an exposure on thousands of embryo images and videos would later permit to identify and predict embryo quality without mediation. This might be beneficial to reduce human error and standardize the annotation, and will allow embryologists to be concentrated in different tasks. A study has been performed by Khosravi and colleagues, which used AI and TLM, and by analysing clinical data for 2,182 embryos and about 50,000 images, 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, deep learning approach has been used to automatically annotate 10,638 embryos videos from eight different IVF units across four countries. The results showed that deep learning model was able to predict foetal heartbeat pregnancy from time-lapse videos with an AUC of 0.93 (Tran et al., 2019). These are retrospective studies, and further trial 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 a 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 passing a vigorous clinical validation process.

CONCLUSIVE REMARKS

Currently, 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 get 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 to 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 affect clinical pregnancy. Presumably, in the next decade with the further advancement of AI, the TLM will develop into an establish method for embryo selection, linked to a non-invasive analytical approaches. At that stage, TLM will probably become essential for embryologists and might be routinely applied for human embryo culture in MAR treatments.

ПРИМЕНЕНИЕ ТЕХНОЛОГИИ ТАЙМ-ЛАПС В ЛЕЧЕНИИ ВСПОМОГАТЕЛЬНЫХ РЕПРОДУКТИВНЫХ ТЕХНОЛОГИЙ (ВРТ)

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Абстракт

При культивировании человеческих эмбрионов in vitro обычно проводится морфологический анализ с использованием микроскопа для выбора эмбриона с наибольшим потенциалом к имплантации с целью достижения успешной беременности и рождения здорового ребенка. Морфологическая оценка может включать количество и размер бластомеров, фрагментацию, многоядерность, степень расширения бластоцисты, а также внешний вид внутренней клеточной массы и трофэктодермы. Однако стандартная микроскопическая оценка требует удаления эмбрионов из инкубатора, что подвергает их неестественным условиям, таким как изменения pH, температуры и уровня кислорода. Кроме того, морфологическая оценка может отличаться из-за субъективности различных наблюдателей. В последнее время непрерывная культура эмбрионов с использованием мониторинга в режиме тайм-лапс (TLM) позволила эмбриологам анализировать динамические и морфокинетические события развития эмбрионов. Это позволяет эмбриологу тщательно изучать полную последовательность развития эмбриона, начиная с оплодотворения и до формирования бластоцисты. TLM обеспечивает непрерывные условия культивирования, сокращая необходимость извлечения эмбрионов из инкубатора. Система мониторинга обычно состоит из стандартного инкубатора с интегрированным микроскопом и цифровой камерой, способной собирать изображения через регулярные промежутки времени и затем преобразовывать их в видео. Эти данные можно аннотировать и анализировать с использованием встроенного программного обеспечения, что помогает эмбриологам в процессе выбора эмбрионов для переноса. Основная цель этой статьи — обсудить потенциальные преимущества и применение технологии тайм-лапс в эмбриологической лаборатории.

ԹԱՅՄ-ԼԱՓՍ ՏԵԽՆՈԼՈԳԻԱՅԻ ԿԻՐԱՌՈՒՄԸ ՎԵՐԱՐՏԱԴՐՈՂԱԿԱՆ ՕԺԱՆԴԱԿ ՏԵԽՆՈԼՈԳԻԱՆԵՐԻ (ART) ԲՈՒԺՄԱՆ ՄԵՋ

Ռոմուալդո Շիորիո
Պտղաբերության բժշկության և գինեկոլոգիական էնդոկրինոլոգիայի բաժանմունք, Կանանց, մայրերի և երեխաների դեպարտամենտ, Լոզանի համալսարանական հիվանդանոց, Լոզան, Շվեյցարիա

Ամփոփագիր

Մարդու բջիջների in vitro ցանքսերի ընթացքում սովորաբար իրականացվում է մանրադիտակային մորֆոլոգիական վերլուծություն՝ ընտրելու համար այն սաղմը, որն ունի ամենաբարձր ներպատվաստման ներուժը՝ հուսալով ստանալ հաջողակ հղիություն և առողջ ծնունդ: Մորֆոլոգիական գնահատումը կարող է ներառել բլաստոմերների քանակը և չափը, ֆրագմենտացիան, բազմաբջիջությունը, բլաստոցիստի ընդլայնման աստիճանը, ներքին բջջային զանգվածի և տրոֆէկտոդերմի տեսքը: Սակայն ստանդարտ մանրադիտակային գնահատումը ենթադրում է սաղմերը հեռացնել ինկուբատորից, ինչը նրանց ենթարկում է ոչ ֆիզիոլոգիական պայմանների՝ փոփոխված pH, ջերմաստիճանի և թթվածնի մակարդակի: Բացի դրանից, մորֆոլոգիական գնահատումը կարող է ներառել մեծ միջդիտորդական փոփոխականություն: Վերջերս շարունակական սաղմնային մշակումը թայմ-լափս մոնիտորինգի (TLM) օգտագործմամբ հնարավորություն է տվել սաղմնաբաններին վերլուծել սաղմի զարգացման դինամիկ և մորֆոկինետիկ իրադարձությունները՝ մանրակրկիտ ուսումնասիրելով սաղմի էվոլյուցիայի ամբողջական հաջորդականությունը՝ սկսած բեղմնավորումից մինչև բլաստոցիստի ձևավորումը: Հետևաբար, TLM-ն ապահովում է անխափան մշակման պայմաններ՝ նվազեցնելով սաղմերը ինկուբատորից հանելու անհրաժեշտությունը: Մոնիտորինգի համակարգը սովորաբար բաղկացած է ստանդարտ ինկուբատորից՝ ինտեգրված մանրադիտակով, որը միացված է թվային տեսախցիկի, որը պարբերաբար հավաքում է պատկերներ և այնուհետև դրանք վերամշակում տեսահոլովակի: Այս տվյալները կարող են նշվել և վերլուծվել ներկառուցված ծրագրաշարի միջոցով, ինչը սաղմնաբաններին օգնում է ընտրել սաղմեր փոխանցման համար: Այս հոդվածի հիմնական նպատակն է քննարկել TLM-ի հնարավոր առավելություններն ու կիրառումները էմբրիոլոգիայի լաբորատորիայում:

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