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Embryo Morphokinetics Based on Time-Lapse Observation

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Abstract

Embryo incubation and evaluation are critical steps in assisted reproductive technology (ART). Conventionally, embryo assessment has been done by embryologists through removing embryos from a conventional incubator during the culture period. Over recent years, time-lapse systems (TLS) have been established which can take digital images of embryos at key points and time intervals. This technique allows embryologists to assess the embryo quality in the steady culture environment. According to TLS studies and prepared algorithm models, it seems that TLS alone or in combination with conventional morphology can be considered as a useful diagnostic tool to determine high-quality embryos and improve embryonic implantation and pregnancy rates. In addition, there were remarkable differences between embryo developmental time points and intervals regarding embryo gender, embryo fragmentation, and type of ovarian stimulation protocol. For confident conclusion, time-lapse imaging should be evaluated in further studies, and the system should be evaluated for cost/benefit ratio effectiveness in individual laboratory.

Keywords: embryo cleavage, embryo morphokinetics, embryoscope, time-lapse imaging

1. Introduction

Assisted reproductive technology (ART) may help infertile couples to realize their dream to have a child in their family, but pregnancy and live birth rates following in vitro fertilization (IVF) still remain low. It is ideal to identify viable embryos with the highest implantation potential to raise IVF success rates. In the traditional IVF practice, embryo assessments are mainly based on the morphologic observation and grade of embryologists

at each stage of oocyte and embryonic development. Some features including oocyte and embryo quality, blastomere numbers and regularity, the percentage of fragmentation, and cytoplasmic granularity have been defined as prognostic indicators of successful pregnancy. This traditional embryo assessment method may have some detrimental effects on embryo growth because frequently opening and closing of incubators often cause to the change of embryo culture environmental steadiness. In order to reduce the inter- and intra-observer difference change, the time-lapse imaging (TLI) has been introduced into in vitro fertilization (IVF) laboratory. The application of time-lapse technology to the clinical IVF laboratory has supported more detailed observations on the embryo development researches quickly.

The aim of this chapter is to determine whether TLI is useful for selection of "top quality" embryos for transfer to improve ART outcome rather than conventional morphological evaluation. The possible correlation between embryos' sex, embryo fragmentation, treatment protocols, different culture media, and embryo morphokinetics will be examined based on some new researches of TLI facilities. Furthermore, various algorithms and predictive models designed in ART cycles with TLI will be discussed.

2. Time-lapse monitoring system

The main goal in ART procedure is to improve transferred embryo implantation rate and pregnancy outcome which is influenced by many factors. A major question is how to observe embryo growth and development in in vitro culture system. Recently, a new embryo culture system with time-lapse imaging has started to be used in human IVF laboratory practice. In this automating embryology, crucial events during embryo cleavage can be monitored without removing embryo from the incubator. This technique application may protect embryos from environmental variations in temperature, pH, and humidity during embryo culture. On the other hand, the time-lapse systems (TLS) application may reduce the errors of embryo assessment which depends on embryologist's expertise and capabilities. With continuous image recording, some key events during embryo development might be recorded more completely for embryo evaluation. Another problem fronting IVF is multiple pregnancies which increased maternal and fetal complications. Worldwide, many human IVF centers or clinics tend to decrease the number of transferred embryo by elective single-embryo transfer (eSET) based on selecting high potential embryo for transfer. Time-lapse photography can help embryologist choose the most viable embryo and reduce multiple pregnancy rate. The application of TLI was initially demonstrated by Wong et al. who revealed that primary cell divisions can be considered as a tool for embryo assessment and prediction of embryo development [1]. Next, Meseguer et al. reported the association of early cleavage division timing and intervals with embryo ability for implantation [2]. Time-lapse imaging carries a noninvasive alternative to the traditional embryo morphologic assessment using developmental kinetics as well as embryo morphology and accurate observation of cellular uncommon events, such as direct cleavage to three cells, blastomere fusion, multinucleation, and fragment reabsorption [3].

2.1. Time-lapse morphokinetic parameters and embryo pregnancy potential

2.1.1. Arguments supporting predictive value of TLS

Embryo assessment by TLS combined with conventional morphologic observation may improve implantation and pregnancy rate. Adamson et al. studied 319 embryo transfer cycles which were divided to standard morphology alone and TLS and morphologic evaluation (case group). The results showed that implantation rate was 30.2 versus 19.0%, and clinical pregnancy rate was 46.0 versus 32.1%, in case and control groups, respectively (P < 0.05) [4]. In a historical cohort study, it is indicated that three embryo morphokinetics in the first 48 hours of culture including short duration of the first cytokinesis, duration of the three-cell stage, and absence of direct cleavage to three cells are associated with developing embryos to high-quality blastocysts [5]. Milewski et al. also analyzed developmental data of 1060 embryos and claimed that embryo morphokinetic parameters are related to reaching blastocyst stage and implantation capability and can be reflected in embryo quality. The most different morphokinetic parameters were median of t9 (the time from insemination to the ninth division), t8_int (the stage after the third division), and cc4 (the fourth round of cleavage) between the groups with and without chemical and clinical pregnancy [6]. In a randomized, double-blinded, controlled trial, 930 patients were divided randomly into two groups. A total of 2638 embryos monitored by TLS in case group and 2427 embryos cultured in standard incubator are considered as controls. Implantation rate (44.9 versus 37.1%) and ongoing pregnancy rate (51.4 versus 41.7%) were significantly higher in embryos monitored with TLS compared to control group, respectively. It is also reported that early pregnancy loss was meaningfully reduced in TL-monitored embryos (16.6%) compared to standard-cultured embryos (25.8%) [7]. A total of 648 embryos, resulting from 60 patients, were prospectively evaluated during culture in TLS. The embryos are cultured until Day 5 (blastocyst stage). Early cleavage division time (t2, t4, and t8) and morula (tMor), start of blastulation (tSB), blastocyst (tBL), and expanded blastocyst (tEBL) were remarkably higher in discarded embryos in comparison to blastocysts. Also, early embryo kinetic parameters are correlated to the implantation potential, but this correlation was not observed in late embryo kinetic parameters [8]. Moreover, in a prospective multicenter cohort study, 1727 embryos were evaluated in 5 IVF centers using TL monitoring in combination with Early Embryo Viability Assessment (Eeva). The main outcome was the evaluation of embryologists' skill to choose embryos using Day-3 morphology alone compared to application of both morphology and Eeva. The reported specificities for three embryologists who used morphology or morphology plus Eeva were 59.7 versus 86.3%, 41.9 versus 84.0%, and 79.5 versus 86.6%, respectively. Results showed that using Eeva in combination with Day-3 morphology significantly upgraded experienced embryologists' capability to recognize embryos which may extend to the blastocyst stage [9]. In consistence, another study used the aforementioned methodology with five embryologists and found similar results. The odds ratio by using only morphology assessment was 1.68 (95% CI = 1.29–2.19), while conventional morphology in combination with Eeva Test resulted in higher odds ratio for predicting blastocyst formation 2.57 (95% CI = 1.88–3.51). Therefore, addition of the Eeva Test to traditional embryo evaluation decreased the inconsistency among embryologists [10]. Furthermore, in a large cohort study, 9530 embryos inseminated by intracytoplasmic sperm injection (ICSI) were cultured in TL incubator. Cases were evaluated in four subgroups including "regular divisions," "viable eight

cells," "viable blastocyst," and "implanted embryos." Significant differences were reported between "regular divisions" and "viable eight cells" regarding for t2, t3, t5, cc2, cc3, s2, and s3. The timing of t5, t8, tM, cc3, and s2 was remarkably higher in "viable blastocyst" compared to the "viable eight-cell" group. Implanted embryos showed a higher rate for time of t8, tM, tB, and s2 when equated to blastocysts. The results confirmed TLS accuracy for detecting embryo development and implantation potential [11]. Similarly, in an observational study with large sample size, 7483 zygotes inseminated by ICSI were cultured in TLS. Seventeen morphokinetic parameters were evaluated, and a number of significant correlations were found between them and both blastocyst formation and implantation. The most prognostic parameters for blastocyst formation included time of morula formation (tM) and t8–t5. These parameters were less predictive for implantation potential. The parameters with the power of implantation prediction were time for expansion blastocyst (tEB) and t8–t5 [12].

2.1.2. Arguments against predictive value of TLS

In a randomized controlled trial, Park et al. compared 240 patients in a closed culture time-lapse system (TLS) with 124 patients in conventional incubator. They reported no significant differences in the number of four-cell embryos, implantation, and ongoing pregnancy rates. They also found a significant higher miscarriage rate in the TLS group [13]. These results are in line with other studies, which showed similar results in good quality embryos, embryo development, blastocyst rate, implantation, and ongoing pregnancy rates between customary culture and TLS [14–16]. In the same way, in a two-part study, poor-prognosis patients have shown no differences in Day-3 embryo quality, implantation, and clinical pregnancy rates between embryos cultured in EmbryoScope™ and conventional culture, whereas in the second portion, embryos developed in the EmbryoScope™ revealed significantly poorer quality on Day 3 compared to standard-cultured embryos [17]. Likewise, in a recent study, a total of 2092 embryos undergoing IVF cycles were evaluated by conventional morphology assessment or TLS. As results indicated, clinical pregnancy rate with transfer of Day-5 embryos was three times higher than Day-3 transfer. But clinical pregnancy rate (68 versus 63%) and implantation rate (51 versus 45%) were comparable between conventional and TLS groups, respectively [18].

In summary, new indicators based on timings and the appearance of abnormal morphological events can only be identified through time-lapse technology. Most of these markers which are detected during the early developmental embryo stages provide early and effective decision regarding embryo selection. Furthermore, it appears that kinetic parameters observed by TLS can predict blastocyst formation with high development ability. However, more studies including accurate meta-analysis should be performed to aid embryologists select embryos with high implantation potential.

2.2. Effect of gender status on embryo morphokinetics

There is a hypothesis that embryo developmental stages are different between male and female embryos and it is showed in animal studies [19, 20]. For human embryos, it is reported that male embryos grow faster than females [21, 22] but other studies negate this theory [23, 24]. Nowadays, developing of TLS for observing embryo developmental process allows monitoring

embryos exactly during early divisions. A number of 78 female and 60 male embryos were observed retrospectively. Embryos were cultured in TLS with 100% implantation and identified gender status. As results, female embryos presented earlier expansion than males. But the other key time points and intervals were as the same as blastocyst rate formation [23]. Similarly, 81 live births from successfully treated ART cycles evaluated, respectively. Results indicated that female status is related to late cleavage (t8), morula (tM), and blastocyst-stage morphokinetic parameters. The authors concluded that some expanded blastocyst-stage morphokinetic variables are correlated with female embryo gender [25]. Otherwise, in another study 176 male and 161 female embryos were evaluated, and there were remarkable differences between embryo developmental time points and intervals regarding gender. The authors designed a model according to the time of second synchrony and morula formation with four subgroups to predict the probability of an embryo being female [26]. It seems that further studies with larger samples are needed to confirm the association between embryo morphokinetics and gender status.

2.3. Fragmentation impact on embryo morphokinetics

Fragmentation is a common pattern in early embryo development stages. In conventional morphological embryo assessment, high fragmented embryos are considered inappropriate for transfer or cryopreservation due to low implantation potential [27]. An increase and decrease in the number and volume of fragments as well as reabsorption and lysis may take place during the embryo culture period, and these events could not be detected without using time-lapse system. Application of time-lapse incubator and embryoscope in recent years provides an opportunity to get more information of embryonic growth at different time points rather than morphologic evaluation at particular time point. Stensen et al. evaluated 1943 oocytes and 372 embryos using the PolScope instrument and TL imaging, respectively. It is reported that embryos with <10% fragmentation (low degree) at 42–45 hours after insemination were originated from oocytes with an early presence of the meiotic spindle, quick first mitosis, late start of the second mitosis, and a smaller period of the third mitosis. However, embryos with high fragmentation (>50% fragmentation) were resulted from oocytes with late appearance of meiotic spindle (36.5 hours after human chorionic gonadotropin (hCG) injection), delayed start of the first mitosis (29.8 hours after insemination), early initiation of the second mitosis (36.4 hours after insemination), and a longer interval of the third mitotic cell cycle [28]. It is reported before that fragmentation during early embryo developmental stages is related to mitotic errors [27]. In Stensen's study, it is confirmed that highly fragmented embryos at the time of the first mitotic cell division could not reabsorb fragmentation and considered as embryos with the high level of fragmentation during morphological assessment. According to the aforementioned data, a correlation was noticed between fragmentation and progress of the meiotic and the mitotic cell cycles among in vitro-derived embryos.

2.4. Effect of ovarian stimulation protocol on embryo morphokinetics

The quality of oocytes and embryos is affected by several factors in in vitro cycles. One of these elements is treatment protocol which is applied for ovarian stimulation in ART cycles and is dependent to patient's condition and clinician's decision. It is confirmed that gonadotropin

type which is used for ovarian control, increase hormone levels in follicular fluid as well as apoptosis in cumulus cells [29]. Recently, the effect of different drugs and dosage used in ovarian stimulation on embryo quality has been evaluating via TLS. Munoz and colleagues monitored 2817 embryos in oocyte donation cycles by TLS retrospectively. They reported that embryos derived from cycles stimulated by gonadotropin-releasing hormone (GnRH) antagonist + GnRH agonist divided faster than embryos originated from patients who treated with GnRH agonist + human chorionic gonadotropin (hCG). But the difference was not significant between groups excluding the first developmental stage [30]. In addition, the other retrospective study examined 739 embryos by TLS and compared the embryo morphokinetics among patients who triggered by hCG versus GnRHa using TLS. They found developmental delay in embryos originated from hCG-triggered cycles compared to embryos derived from cycles triggered by GnRHa [31]. Conversely, there was no significant difference between three studied groups by TLS regarding embryo morphokinetics. The patients were treated with only recombinant Follicle-stimulating hormone (rFSH), only human menopausal gonadotropin (HMG), and a combination of FSH and HMG. The time points and time intervals in rFSH group reported close-to-ideal timing with no significant difference [32]. According to limited mentioned studies, it can be concluded that the type of ovarian stimulation protocol affects embryo developmental kinetics in different patterns.

2.5. Effect of the type of culture media on embryo morphokinetics

It is proven that culture media components and conditions are important for optimal embryo development. These days the effect of culture media on embryo morphokinetics is visible accurately by using TL monitoring system. Animal research using TLS confirmed the effect of different culture media with varied components on blastocyst formation [33]. In a cohort study, 532 human embryos were cultured in 2 different media, Global as single-step medium and Sage Cleavage as sequential medium. Embryos are monitored by TLS regarding two cells (t2), three cells (t3), four cells (t4), and five cells (t5) as the same as the length of the second cell cycle (cc2) and the synchrony in the division from two to four cells (s2). There were no significant differences between embryos regarding mentioned time points between two culture media. The implantation and pregnancy rates were comparable between groups [34]. The results of a randomized clinical trial on 1356 embryos were similar to the mentioned study. All zygotes were divided into two single steps and sequential media randomly and monitored in TLS until Day 5. The percentages of good quality blastocyst on Day 5 were equivalent between two groups. However, the number of good quality embryos on Day 3 was significantly higher among embryos cultured in single-step medium. Eleven morphokinetic parameters were evaluated, and only four parameters (t7, t8, t3c4c—time between the first observation of 3 and 4 completely separated blastomeres, and t5c7c—time between the first observation of 5 and 7 completely separated blastomeres) differ significantly between embryos cultured in altered media. The authors concluded that single-step culture medium is as nutrient as sequential media for blastocyst development [35]. Costa-Borges et al. compared embryo morphokinetics in single culture media with and without medium renewal on Day 3 of culture. The results revealed no significant differences in good quality blastocysts, blastocyst formation rate, embryo early and late morphokinetics as well as clinical pregnancy, take-home baby rate, and perinatal outcomes [36].

According to the restricted mentioned study, it seems that single-step and sequential media have equivalent effect on embryo morphokinetics. However, additional researches are needed for confirmation of this theory.

2.6. Morphokinetic algorithms for prediction of embryo implantation potential

Developing of TLS provides the opportunity to observe continuous embryo development. Embryologists can select the most viable embryo using a new scoring system based on embryo morphokinetics. It seems that a globally accepted algorithm is needed to predict embryo implantation potential. For the first time, Meseguer et al. introduced a hierarchical model using early embryo morphokinetics. They divided 247 evaluated embryos to 6 subsections, and 4 of these groups were further subdivided into two subcategories. Based on the findings, the multivariable model was designed to categorize embryos according to their implantation potential [37]. Four years later validation of this model was evaluated in a retrospective study by Freour et al. They calculated the implantation rate matching to each subgroup designed by Meseguer's model and analyzed the same data in subgroups according to the day of embryo transfer. The findings did not show the same sensitivity of Meseguer's model for prediction of implantation rate according to morphokinetic subgroups [38]. For creating a time-lapse deselecting model, 270 embryos transferred on Day 3 with known implantation data (KID) were analyzed based on both qualitative and quantitative parameters retrospectively. In addition, 66 KID embryos were evaluated subsequently for validation of the model. Qualitative deselection parameters were described as poor conventional morphology on Day 3, abnormal cleavage patterns detected by time-lapse monitoring, and less than eight cells at 68 hours post-insemination. Quantitative parameters were the time from pronuclear fading (PNF) to five-cell stage and duration of three-cell stage. In conclusion this deselecting method reported as a reliable tool for embryo selection [39]. In a retrospective multicentric study, 1664 intracytoplasmic sperm injection (ICSI) cycles were analyzed. Of them 799 were used to generate an algorithm, and 865 cycles were applied to exam its predictive value in the second study phase. The timing to two cells (t2), three cells (t3), four cells (t4), and five cells (t5) as well as the length of the second cell cycle (cc2 = t3–t2) and the synchrony in the division from two to four cells (s2 = t4–t3) were studied, and both implantation and clinical pregnancy rates were investigated. Three parameters of t3, cc2, and t5 are related to implantation. According to these data, embryos were categorized to four subgroups. In the second phase of the study, the algorithm was authenticated among 1620 transferred embryos. In this phase, embryos were classified based on the algorithm, and significant differences in implantation rate were found between the different subgroups. The authors claimed that aforementioned algorithm is a powerful tool for embryo selection in TLS [40].

Milewski et al. create two predictive models for blastocyst development [41] and the transferred embryo implantation ability [42]. They evaluated the embryo morphokinetic parameters between embryos developed to blastocyst, and embryos did not reach blastocyst stage [41] as well as between implanted and non-implanted embryos [42]. Based on the findings and using statistical analysis, two models were generated which presented TLS as a good predictive instruments for embryo implantation but not as high as the model for blastocyst for-

mation. Recently, Petersen et al. presented an appropriate algorithm for Day 3 of transferred embryos which is not dependent on culture conditions and fertilization method. The data was gathered retrospectively from a record of 3275 KID embryos transferred on Day 3 performed in 24 clinics. The new algorithm (KIDScore) was developed based on the six TLS parameters including one morphological and five morphokinetic events. Embryos were allocated to five categories, which predict the embryos' implantation potential. The algorithm was validated using a discrete data set of embryos cultured until Day 5 to examination of its ability to predict blastocyst formation. It is concluded that KIDScore could be considered as a "generally applicable Day-3 algorithm" which can be useful in "different clinical settings" [43]. Steadily, the effectiveness of the six embryo selection algorithms (ESAs) [15, 40, 44–47] was observed among 884 IVF or ICSI cycles. Validity of each ESA for detecting embryo implantation potential was determined using specificity, sensitivity, positive and negative predictive value (PPV and NPV), area under the receiver operating characteristic curve (AUC), and likelihood ratio (LR) regarding implantation rate in each model. Results showed the necessity of development in predictive algorithms according to the patients' characteristics, treatment protocols, and environmental factors. They believed that current ESAs may not work properly during application in other clinics [48]. Dominguez et al. combined TLS and proteomics to design a new model for the best embryo selection. They evaluated seven proteins in embryo culture media and find a correlation between them and embryo morphokinetics. The most relevant parameters were interleukin (IL-6) and cc2 among proteins and embryo time points, respectively. According to this relationship, an algorithm was considered for estimation embryo implantation rate. Embryos in the existence of IL-6 and 5–12 hours cc2 had remarkable implantation rate compared to other embryos [3].

Declared data showed that using embryo morphologic variables in combination with key time events leads to generate cumulative score models. It seems that these predictive algorithms integrate different variables and could not be easily adjusted to provide a globally accepted model. It is necessary to design in-house models which are specified for the same patients and conditions.

2.7. Different studies based on time-lapse observation

With the recent progress of TLS, a new opportunity is provided to study embryo developmental process accurately. TL monitoring offers a possibility to have exact observation on both early and late embryo morphokinetics and their correlation to embryo origin, fertilization methods, genetic abnormalities, and observer variability. In this section we review some of these studies.

To evaluate the influence of embryo origin by means of treatment-related factors on embryo morphokinetics, a cohort study was established among 1507 embryo from 243 patients. The results showed that blastocyst-stage embryos are more influenced by patients' characteristics than cleavage-stage embryos. Patients' age and dose of FSH have a positive correlation with delayed blastocyst development. It is also shown that embryos fertilized by ICSI have a significant faster first cleavage division than IVF originate embryos [49].

Minasi et al. examined that morphokinetic parameters of 928 blastocysts underwent preimplantation genetic screening (PGS). They reported no significant difference among euploid and aneuploid embryos regarding time-lapse morphokinetics and concluded that morphokinetic parameters can be used in combination with, not instead of, PGS for detecting embryo ploidy status [50]. Conversely, in a retrospective cohort study, a total of 460 embryos were cultured in TLS and biopsied on Day 3. Comparative genomic hybridization (CGH) microarray was performed for detecting aneuploidy. The result showed that some kinetic parameters including tPNF, t2, t5, cc2, and cc3 differ significantly among normal and aneuploid embryos [51].

Regarding the effect of intercellular communication on embryo development, a study was conducted on 765 good quality four-cell embryos. Four-cell embryos were investigated for intercellular contact point (ICCP) on Day 2 after insemination. The results showed that embryos with less than six ICCPs at the termination of four-cell stage have a decreased implantation potential when compared to those reaching six ICCPs by the end of four-cell stage (5 versus 38.5%). They concluded that discarding of embryos with poor morphology, abnormal cleavage, and fewer than six ICCPs at the four-cell stage results in a meaningful improvement in implantation rate [52].

To define the impact of fertilization methods on embryo morphokinetic, Bodri et al. evaluated 500 expanded blastocysts incubated in TLS retrospectively. The result indicated that IVF-fertilized embryos have a significant delay in early embryo developmental stages (pronucleus fading to t4) compared to embryos inseminated by ICSI, whereas IVF-fertilized embryos developed faster during blastocyst expansion stage. They reported a 1.5-hour time difference between standard IVF embryo and ICSI-fertilized embryo [53]. However, a definite conclusion needs further assessment with more studied cases.

For the first time, Sundvall et al. evaluate inter- and intra-observer inconsistency of time-lapse explanations. Three observers performed self-directed interpretations on time-lapse recordings on 158 fertilized embryos. Totally, the correlation was high for all of the examined parameters. Results showed close and strong interobserver agreement. The highest correlation was found for the timing of pronucleus breakdown, the completion of blastocyst hatching, and the appearance and disappearance of the first nucleus after the first division. There was also a perfect agreement for all cleavage stages. Two binary parameters including multinucleation and evenness of blastomeres at two-cell stage presented reasonable agreement. Intra-observer variability evaluation demonstrated comparable results for most parameters. The authors indicated that embryo morphokinetic factors can be used certainly for embryo viability prediction, even the recording interpret by a trainee operator [54].

2.8. Conclusion

Embryo selection criteria based on the current morphological evaluation do not associate with a high implantation or pregnancy rate. During the recent years, different studies based on a TLS have delivered new knowledge on embryo development proposing embryologists the chance to improve embryo evaluation and selection. Analysis of human embryo morphokinet-

ics provides an improvement of implantation potential prediction at both early and late cleavage stages as well as prognosis potential to reach the blastocyst stage. Furthermore, TLS plays a key role in progress of SET policy to minimize multiple pregnancy and related complication. Moreover, embryo time-lapse monitoring reduces inter- or intra-observer variability. "Teleembryology" may be considered as another advantage of TLS that allows monitoring embryos remotely via the Internet from any location. In addition TL monitoring collects large amount of data including recorded and stored images and videos which can be analyzed retrospectively. However, several studies presented positive outcomes and clinical validity of TLS; there are some limitations regarding this instrument. Some arguments remain regarding embryo exposure to light during image acquisition (every 5–15 min). It is a preference to plan guidelines on image capturing in terms of light wavelength, duration of lightening, and frequency of imaging. Likewise, there are restrictions in embryo rotation which make difficulties in the visual observation especially in the presence of cytoplasmic fragmentation or overlapping blastomeres. On the other hand, an important limitation in morphokinetic assessment is that the human embryo morphology is not a good figure of the chromosomal status. It is well known that embryos with good morphology may have aneuploidy, whereas suboptimal embryos may be euploid [21, 55]. Therefore, TLS should be applied in conjunction with PGS for detecting genetic abnormalities. Moreover, a number of confounding factors are recommended to effect timing of morphokinetic parameters including, oxygen tension, ovarian stimulation protocol, fertilization methods, type of culture media, smoking, and advanced age which should be considered in TLS researches.

Recently, some algorithms which are designed according to embryo morphokinetics suggested predicting embryo implantation and pregnancy potential. It states that the time points in these models are overlapped and the algorithms lose their predictive value when externally applied. Future properly designed study is needed to plan a common classification for key time points and time intervals that are accepted worldwide.

In conclusion, implantation rate should be considered as the first outcome and take-home baby rate as the final outcome to evaluating success of this new technology. Some researchers believed that embryo selection via TLS should remain an experimental policy due to lack of evidence-based medicine to sufficiently assess the safety and effectiveness of this equipment. However, it is important to know that TLS is a powerful noninvasive technology for the study of embryo development which offers a wide-range document of morphological and dynamic parameters about each embryo. The system should be evaluated for cost/benefit ratio effectiveness in individual laboratory.

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References

[1] Wong CC, Loewke KE, Bossert NL, Behr B, De Jonge CJ, Baer TM, et al. Non-invasive imaging of human embryos before embryonic genome activation predicts development to the blastocyst stage. Nature Biotechnology. 2010;**28**(10):1115-1121

[2] Meseguer M, Rubio I, Cruz M, Basile N, Marcos J, Requena A. Embryo incubation and selection in a time-lapse monitoring system improves pregnancy outcome compared with a standard incubator: A retrospective cohort study. Fertility and Sterility. 2012;**98**(6):1481-1489 e10

[3] Dominguez F, Meseguer M, Aparicio-Ruiz B, Piqueras P, Quinonero A, Simon C. New strategy for diagnosing embryo implantation potential by combining proteomics and time-lapse technologies. Fertility and Sterility. 2015;**104**(4):908-914

[4] Adamson GD, Abusief ME, Palao L, Witmer J, Palao LM, Gvakharia M. Improved implantation rates of day 3 embryo transfers with the use of an automated time-lapse-enabled test to aid in embryo selection. Fertility and Sterility. 2016;**105**(2):369-375 e6

[5] Kirkegaard K, Kesmodel US, Hindkjaer JJ, Ingerslev HJ. Time-lapse parameters as predictors of blastocyst development and pregnancy outcome in embryos from good prognosis patients: A prospective cohort study. Human Reproduction. 2013;**28**(10):2643-2651

[6] Milewski R, Czerniecki J, Kuczynska A, Stankiewicz B, Kuczynski W. Morphokinetic parameters as a source of information concerning embryo developmental and implantation potential. Ginekologia Polska. 2016;**87**(10):677-684

[7] Rubio I, Galan A, Larreategui Z, Ayerdi F, Bellver J, Herrero J, et al. Clinical validation of embryo culture and selection by morphokinetic analysis: A randomized, controlled trial of the EmbryoScope. Fertility and Sterility. 2014;**102**(5):1287-1294, e5

[8] Desai N, Ploskonka S, Goodman LR, Austin C, Goldberg J, Falcone T. Analysis of embryo morphokinetics, multinucleation and cleavage anomalies using continuous time-lapse monitoring in blastocyst transfer cycles. Reproductive Biology and Endocrinology: RB&E. 2014;**12**:54

[9] Conaghan J, Chen AA, Willman SP, Ivani K, Chenette PE, Boostanfar R, et al. Improving embryo selection using a computer-automated time-lapse image analysis test plus day 3 morphology: Results from a prospective multicenter trial. Fertility and Sterility. 2013;**100**(2):412-419 e5

[10] Diamond MP, Suraj V, Behnke EJ, Yang X, Angle MJ, Lambe-Steinmiller JC, et al. Using the Eeva Test adjunctively to traditional day 3 morphology is informative for consistent embryo assessment within a panel of embryologists with diverse experience. Journal of Assisted Reproduction and Genetics. 2015;**32**(1):61-68

[11] Herrero J, Tejera A, Albert C, Vidal C, de los Santos MJ, Meseguer M. A time to look back: Analysis of morphokinetic characteristics of human embryo development. Fertility and Sterility. 2013;**100**(6):1602-1609, e1-e4

[12] Motato Y, de los Santos MJ, Escriba MJ, Ruiz BA, Remohi J, Meseguer M. Morphokinetic analysis and embryonic prediction for blastocyst formation through an integrated time-lapse system. Fertility and Sterility. 2016;**105**(2):376-384, e9

[13] Park H, Bergh C, Selleskog U, Thurin-Kjellberg A, Lundin K. No benefit of culturing embryos in a closed system compared with a conventional incubator in terms of number of good quality embryos: Results from an RCT. Human Reproduction. 2015;**30**(2):268-275

[14] Nakahara T, Iwase A, Goto M, Harata T, Suzuki M, Ienaga M, et al. Evaluation of the safety of time-lapse observations for human embryos. Journal of Assisted Reproduction and Genetics. 2010;**27**(2-3):93-96

[15] Cruz M, Garrido N, Herrero J, Perez-Cano I, Munoz M, Meseguer M. Timing of cell division in human cleavage-stage embryos is linked with blastocyst formation and quality. Reproductive Biomedicine Online. 2012;**25**(4):371-381

[16] Kirkegaard K, Hindkjaer JJ, Grondahl ML, Kesmodel US, Ingerslev HJ. A randomized clinical trial comparing embryo culture in a conventional incubator with a time-lapse incubator. Journal of Assisted Reproduction and Genetics. 2012;**29**(6):565-572

[17] Wu YG, Lazzaroni-Tealdi E, Wang Q, Zhang L, Barad DH, Kushnir VA, et al. Different effectiveness of closed embryo culture system with time-lapse imaging (EmbryoScope(TM)) in comparison to standard manual embryology in good and poor prognosis patients: A prospectively randomized pilot study. Reproductive Biology and Endocrinology: RB&E. 2016;**14**(1):49

[18] Goodman LR, Goldberg J, Falcone T, Austin C, Desai N. Does the addition of time-lapse morphokinetics in the selection of embryos for transfer improve pregnancy rates? A randomized controlled trial. Fertility and Sterility. 2016;**105**(2):275-285, e10

[19] Alomar M, Tasiaux H, Remacle S, George F, Paul D, Donnay I. Kinetics of fertilization and development, and sex ratio of bovine embryos produced using the semen of different bulls. Animal Reproduction Science. 2008;**107**(1-2):48-61

[20] Bermejo-Alvarez P, Roberts RM, Rosenfeld CS. Effect of glucose concentration during in vitro culture of mouse embryos on development to blastocyst, success of embryo transfer, and litter sex ratio. Molecular Reproduction and Development. 2012;**79**(5):329-336

[21] Alfarawati S, Fragouli E, Colls P, Stevens J, Gutierrez-Mateo C, Schoolcraft WB, et al. The relationship between blastocyst morphology, chromosomal abnormality, and embryo gender. Fertility and Sterility. 2011;**95**(2):520-524

[22] Hentemann MA, Briskemyr S, Bertheussen K. Blastocyst transfer and gender: IVF versus ICSI. Journal of Assisted Reproduction and Genetics. 2009;**26**(8):433-436

[23] Serdarogullari M, Findikli N, Goktas C, Sahin O, Ulug U, Yagmur E, et al. Comparison of gender-specific human embryo development characteristics by time-lapse technology. Reproductive Biomedicine Online. 2014;**29**(2):193-199

[24] Weston G, Osianlis T, Catt J, Vollenhoven B. Blastocyst transfer does not cause a sex-ratio imbalance. Fertility and Sterility. 2009;**92**(4):1302-1305

[25] Bodri D, Kawachiya S, Sugimoto T, Yao Serna J, Kato R, Matsumoto T. Time-lapse variables and embryo gender: A retrospective analysis of 81 live births obtained following minimal stimulation and single embryo transfer. Journal of Assisted Reproduction and Genetics. 2016;**33**(5):589-596

[26] Bronet F, Nogales MC, Martinez E, Ariza M, Rubio C, Garcia-Velasco JA, et al. Is there a relationship between time-lapse parameters and embryo sex? Fertility and Sterility. 2015;**103**(2):396-401 e2

[27] Chavez SL, Loewke KE, Han J, Moussavi F, Colls P, Munne S, et al. Dynamic blastomere behaviour reflects human embryo ploidy by the four-cell stage. Nature Communications. 2012;**3**:1251

[28] Stensen MH, Tanbo TG, Storeng R, Abyholm T, Fedorcsak P. Fragmentation of human cleavage-stage embryos is related to the progression through meiotic and mitotic cell cycles. Fertility and Sterility. 2015;**103**(2):374-381, e4

[29] Requena A, Cruz M, Agudo D, Pacheco A, Garcia-Velasco JA. Type of gonadotropin during controlled ovarian stimulation affects the endocrine profile in follicular fluid and apoptosis rate in cumulus cells. European Journal of Obstetrics, Gynecology, and Reproductive Biology. 2016;**197**:142-146

[30] Munoz M, Cruz M, Humaidan P, Garrido N, Perez-Cano I, Meseguer M. The type of GnRH analogue used during controlled ovarian stimulation influences early embryo developmental kinetics: A time-lapse study. European Journal of Obstetrics, Gynecology, and Reproductive Biology. 2013;**168**(2):167-172

[31] Gurbuz AS, Gode F, Uzman MS, Ince B, Kaya M, Ozcimen N, et al. GnRH agonist triggering affects the kinetics of embryo development: A comparative study. Journal of Ovarian Research. 2016;**9**:22

[32] Munoz M, Cruz M, Humaidan P, Garrido N, Perez-Cano I, Meseguer M. Dose of recombinant FSH and oestradiol concentration on day of HCG affect embryo development kinetics. Reproductive Biomedicine Online. 2012;**25**(4):382-389

[33] Morbeck DE, Krisher RL, Herrick JR, Baumann NA, Matern D, Moyer T. Composition of commercial media used for human embryo culture. Fertility and Sterility. 2014;**102**(3):759-766 e9

[34] Basile N, Morbeck D, Garcia-Velasco J, Bronet F, Meseguer M. Type of culture media does not affect embryo kinetics: A time-lapse analysis of sibling oocytes. Human Reproduction. 2013;**28**(3):634-641

[35] Hardarson T, Bungum M, Conaghan J, Meintjes M, Chantilis SJ, Molnar L, et al. Noninferiority, randomized, controlled trial comparing embryo development using media developed for sequential or undisturbed culture in a time-lapse setup. Fertility and Sterility. 2015;**104**(6):1452-1459 e1-e4

[36] Costa-Borges N, Belles M, Meseguer M, Galliano D, Ballesteros A, Calderon G. Blastocyst development in single medium with or without renewal on day 3: A prospective

cohort study on sibling donor oocytes in a time-lapse incubator. Fertility and Sterility. 2016;**105**(3):707-713

[37]   Meseguer M, Herrero J, Tejera A, Hilligsoe KM, Ramsing NB, Remohi J. The use of morphokinetics as a predictor of embryo implantation. Human Reproduction. 2011;**26**(10): 2658-2671

[38]   Freour T, Le Fleuter N, Lammers J, Splingart C, Reignier A, Barriere P. External validation of a time-lapse prediction model. Fertility and Sterility. 2015;**103**(4):917-922

[39]   Liu Y, Chapple V, Feenan K, Roberts P, Matson P. Time-lapse deselection model for human day 3 in vitro fertilization embryos: The combination of qualitative and quantitative measures of embryo growth. Fertility and Sterility. 2016;**105**(3):656-662 e1

[40]   Basile N, Vime P, Florensa M, Aparicio Ruiz B, Garcia Velasco JA, Remohi J, et al. The use of morphokinetics as a predictor of implantation: A multicentric study to define and validate an algorithm for embryo selection. Human Reproduction. 2015;**30**(2):276-283

[41]   Milewski R, Kuc P, Kuczynska A, Stankiewicz B, Lukaszuk K, Kuczynski W. A predictive model for blastocyst formation based on morphokinetic parameters in time-lapse monitoring of embryo development. Journal of Assisted Reproduction and Genetics. 2015;**32**(4):571-579

[42]   Milewski R, Milewska AJ, Kuczynska A, Stankiewicz B, Kuczynski W. Do morphokinetic data sets inform pregnancy potential? Journal of Assisted Reproduction and Genetics. 2016;**33**(3):357-365

[43]   Petersen BM, Boel M, Montag M, Gardner DK. Development of a generally applicable morphokinetic algorithm capable of predicting the implantation potential of embryos transferred on Day 3. Human Reproduction. 2016;**31**(10):2231-2244

[44]   Azzarello A, Hoest T, Mikkelsen AL. The impact of pronuclei morphology and dynamicity on live birth outcome after time-lapse culture. Human Reproduction. 2012;**27**(9): 2649-2657

[45]   Campbell A, Fishel S, Bowman N, Duffy S, Sedler M, Hickman CF. Modelling a risk classification of aneuploidy in human embryos using non-invasive morphokinetics. Reproductive Biomedicine Online. 2013;**26**(5):477-485

[46]   Chamayou S, Patrizio P, Storaci G, Tomaselli V, Alecci C, Ragolia C, et al. The use of morphokinetic parameters to select all embryos with full capacity to implant. Journal of Assisted Reproduction and Genetics. 2013;**30**(5):703-710

[47]   Dal Canto M, Coticchio G, Mignini Renzini M, De Ponti E, Novara PV, Brambillasca F, et al. Cleavage kinetics analysis of human embryos predicts development to blastocyst and implantation. Reproductive Biomedicine Online. 2012;**25**(5):474-480

[48]   Barrie A, Homburg R, McDowell G, Brown J, Kingsland C, Troup S. Examining the efficacy of six published time-lapse imaging embryo selection algorithms to predict implantation to demonstrate the need for the development of specific, in-house morphokinetic selection algorithms. Fertility and Sterility. 2017; 107(3):613-621

[49] Kirkegaard K, Sundvall L, Erlandsen M, Hindkjaer JJ, Knudsen UB, Ingerslev HJ. Timing of human preimplantation embryonic development is confounded by embryo origin. Human Reproduction. 2016;**31**(2):324-331

[50] Minasi MG, Colasante A, Riccio T, Ruberti A, Casciani V, Scarselli F, et al. Correlation between aneuploidy, standard morphology evaluation and morphokinetic development in 1730 biopsied blastocysts: A consecutive case series study. Human Reproduction. 2016;**31**(10):2245-2254

[51] Chawla M, Fakih M, Shunnar A, Bayram A, Hellani A, Perumal V, et al. Morphokinetic analysis of cleavage stage embryos and its relationship to aneuploidy in a retrospective time-lapse imaging study. Journal of Assisted Reproduction and Genetics. 2015;**32**(1):69-75

[52] Liu Y, Chapple V, Feenan K, Roberts P, Matson P. Clinical significance of intercellular contact at the four-cell stage of human embryos, and the use of abnormal cleavage patterns to identify embryos with low implantation potential: A time-lapse study. Fertility and Sterility. 2015;**103**(6):1485-1491 e1

[53] Bodri D, Sugimoto T, Serna JY, Kondo M, Kato R, Kawachiya S, et al. Influence of different oocyte insemination techniques on early and late morphokinetic parameters: Retrospective analysis of 500 time-lapse monitored blastocysts. Fertility and Sterility. 2015;**104**(5):1175-1181 e1-e2

[54] Sundvall L, Ingerslev HJ, Breth Knudsen U, Kirkegaard K. Inter- and intra-observer variability of time-lapse annotations. Human Reproduction. 2013;**28**(12):3215-3221

[55] Ziebe S, Lundin K, Loft A, Bergh C, Nyboe Andersen A, Selleskog U, et al. FISH analysis for chromosomes 13, 16, 18, 21, 22, X and Y in all blastomeres of IVF pre-embryos from 144 randomly selected donated human oocytes and impact on pre-embryo morphology. Human Reproduction. 2003;**18**(12):2575-2581