ALIVE

ART LEARNING INITIATIVES FOR EXPERTS

Issue 6

Time Lapse Imaging in Art

part 1

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contents

abbreviations

ART	Assisted Reproductive Technology
ASRM	American Society for Reproductive Medicine
CI	Conventional Incubator
ESHRE	European Society of Human Reproduction and Embryology
EEVA	Early Embryo Viability Assessment
IVF	In Vitro Fertilization
ICSI	Intracytoplasmic Sperm Injection
PGS	Pre-implantation Genetic Screening
PN	Pronucleus
RCT	Randomized Controlled Trial
TLS	Time-lapse Monitoring Systems
TLI	Time-lapse Incubator
TLC	Time-Lapse Video Cinematography

expert insights

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Although Assisted Reproductive Technology (ART) has given a great solace to millions of couples worldwide, problems such as low success rate and high multiple pregnancy rates still exist.

Even today, the most conventional method used to select embryos has been based on morphological characteristics. However, infertility specialists are constantly yearning to implement new technologies/strategies to improve embryo selection and thereby increase the success rate after embryo transfer.

The last 2 decades have seen the advent of new technology called Time-lapse technology (TLT) which helps the continuous surveillance of the embryo development and optimal culture conditions using three basic elements - an incubator, an optical microscope and a specifically designed software program.

This technology minimizes the pressure on the embryologists and circumvents the morphological analysis of the embryo to select the embryo with the highest implantation potential.

This issue was drafted to know in depth about the role of Time lapse imaging in ART and provides information right from the role of incubators, culture conditions and safety, importance of humidity in embryo culturing to the various advantages offered by the Time-lapse technology. The information will be discussed in two parts.

introduction

1. *Fishel S, Campbell A, Montgomery S, et al. Live births after embryo selection using morphokinetics versus conventional morphology: a retrospective analysis. Reprod Biomed Online. 2017;35(4):407-416.*
2. *Adolfsson E, Porath S, Andershed AN. External validation of a time-lapse model; a retrospective study comparing embryo evaluation using a morphokinetic model to standard morphology with live birth as endpoint. JBRA Assist Reprod. 2018;22(3):205-214.*
3. *Silver DH, Feder M, Gold-Zamir Y et al. Data-Driven Prediction of Embryo Implantation Probability Using IVF Time-lapse Imaging. Medical Imaging with Deep Learning 2020;1-6.*

The success of *in vitro* fertilization (IVF) during the last three decades has evolved from aspirational live birth rates of around 15% to rates of between 35% and 60%.
Several factors like the female age, advances in follicular stimulation regimens, multiple embryo transfer and improvements in the culture and selection of the human embryo for transfer have been attributed to this success.
The outcome of the infertility treatment is often determined by the successful culture, evaluation and selection of embryos.
Although manual morphological annotation and quality assessment of embryos *in vitro* remains the gold standard for predicting IVF success, efforts to standardize and improve prediction accuracy have become increasingly computational in the last 2-3 decades.
An approach from the morphological assessment of embryos to the modern level of computational assessment is discussed herewith.

incubators

1. *Practical Manual of In Vitro Fertilization: Advanced Methods and Novel Devices, Zsolt Peter Nagy, Alex C. Varghese, Ashok Agarwal, (Eds.). 2012, XXIII, in CO_2 and Low O_2 Incubators by Meintjes M. p 61-71.*
2. *Swain JE. Decisions for the IVF laboratory: comparative analysis of embryo culture incubators. Reprod Biomed Online. 2014;28(5):535-547.*
3. *Piane LD, Molinari E, Salvagno F et al. Metabolomics in reproductive medicine: general principles and applications to the study of gametes, embryos and follicular fluid. J Reprod Stem Cell Biotechnol.2011; 2(1):14-28.*
4. *Fawzy M, AbdelRahman MY, Zidan MH et al. Humid versus dry incubator: a prospective, randomized, controlled trial. Fertil Steril. 2017;108(2):277-283.*

Embryo incubators can be considered as the heart of any IVF laboratory. Understanding the strengths and weaknesses of the incubators is invaluable as a tool and essential to optimize clinical IVF outcomes.

Features of an ideal incubator

1. Culture conditions as close to natural
2. Correct pH, correct humidity, adjustable O_2
3. Uninterrupted culture
4. Monitored culture

Desirable features

1. Assessment of embryo quality
2. Metabolomics assessment
3. Non invasive Pre-implantation Genetic Testing

Incubators have undergone several improvements, with designs varying from the conventional CO2 to time-lapse cinematography, including triple gas (low oxygen) and benchtop models.

2

Evolution of incubators

1. *Swain JE. Decisions for the IVF laboratory: comparative analysis of embryo culture incubators. Reprod Biomed Online. 2014;28(5):535-547.*
2. *Swain JE. Practical pH for the IVF Laboratory. Accessed from website https://journals.sagepub.com/doi/pdf/10.1177/205891581200300205 as on 01.06.2020.*
3. *Higdon HL 3rd, Blackhurst DW, Boone WR. Incubator management in an assisted reproductive technology laboratory. Fertil Steril. 2008;89(3):703-710.*
4. *Bavister B. Oxygen concentration and preimplantation development. Reprod Biomed Online. 2004;9(5) :484-486.*
5. *Van Montfoort APA, Arts EGJM, Wijnandts L, et al. Reduced oxygen concentration during human IVF culture improves embryo utilization and cumulative pregnancy rates per cycle. Hum Reprod Open. 2020;2020(1):hoz036.*
6. *Geri®: Focusing on What Matters, the Embryo. Accessed from website https://hcp.merckgroup.com/en/fertility/technologies/Geri.html as on 01.06.2020.*
7. *EEVA. Accessed from website https://hcp.merckgroup.com/en/fertility/technologies/Eeva.html. as on 01.06.2020.*

Ever since the treatment of infertility via IVF was envisaged, one of the main challenges was to establish an extra-corporal or an *in vitro* culture for the gamete and embryos.

Key environmental variables to consider within the culture system include pH of the culture medium, temperature, media osmolality and air quality.

All these variables are impacted by the laboratory incubator and thus incubator selection and management is critical to ensure success of an IVF program.

Science has progressed way ahead of the inverted glass domes that were used as incubators initially. The CO_2 concentration was determined to be around 6% - both for the embryos as well as to maintain the pH of the culture media.

Humidity was also considered to be an important factor, and both the factors of CO_2 concentration and humidity, along with the temperature maintenance to around 37^0C was achieved by the manufacture of the **box incubator**.

But the box incubator had its inherent drawbacks. The temperature fall after every door opening was more than optimal and the recovery had a lot to be desired. The fall of CO_2 concentration was also not optimally controlled by the built-in purge of the gases.

Incubator oxygen concentration was always a debate. The initial embryos were cultured at atmospheric concentration and they proved to be detrimental, but by the turn of the century the trend shifted to low O_2 concentration.
The O_2 that was removed from the incubator gases was replaced by an extra load of N_2.
Many RCTs and meta-analysis proved that low O_2 concentration was the need of the hour.

A recently published meta-analysis showed that 5% oxygen resulted in more good-quality embryos that were suitable for freezing.
Swain et al., showed that advances in technology has thus resulted in multiple incubator types with varying capabilities and differing methods of regulating their internal environment (Table 1).
The selection of an appropriate culture incubator for the IVF laboratory has become a complex process.

Table 1. Incubator technology variables that should be considered when evaluating and selecting a unit for the laboratory

Gas type	CO_2 sensor	O_2 sensor	Temperature control[a]	Design[b]	Humidity control	Contamination control[a,c]
CO_2-only	Infrared	Zirconium	Air jacket	Benchtop	Yes[d]	Heat
Low O_2 mixer	Thermal conductivity	Galvanic (fuel-cell)	Water jacket	Two-chamber	No	UV
Low O_2 premixed cylinder			Direct heat	Multichamber		H_2O_2
				Other (i.e. timelapse imaging)		Copper alloy
				Small box		External HEPA
				Large box		

HEPA = high-efficiency particulate absorption.
[a]May be influenced by presence/absence of an internal fan.
[b]Other novel designs exist, but these are general terms to refer to the most commonly used incubators in the IVF laboratory; actual volumes will vary from unit to unit.
[c]Ease of removing inner parts and/or wiping interior is also important to consider.
[d]Some units bubble gas through a water pan to expedite rehumidification.

Table adapted from Reprod Biomed Online. 2014;28(5):535-547.

More recently, **benchtop/topload** units of varying sizes/configurations designed specifically for clinical IVF with extremely small chambers (~0.31–0.5L) are being used which have the advantage of an almost instantaneous recovery of the pre-set culture conditions, thus preventing damage to the gametes & embryos. It has the option of choosing between the pre-mixed gas mixtures or adjusting the concentration individual gases at the operator level.
Now the only impediment to a perfect culture system was the need of intermittent checking of the embryos to see the optimal growth.

This was overcome by the development of the **Time Lapse incubator**.
The earlier incubators were either of the dry type, with no humidity or they were systems that were placed in the box incubator *per se.* Both these options did not present the ideal culture system, despite the advantage of uninterrupted culture.
This was overcome with the advent of the more advanced Time lapse incubators which provided incubation in a humidified environment and the add-on of monitored uninterrupted culture.

Advanced innovative benchtop incubator is integrated with continuous embryo monitoring capabilities; designed to provide an individualized and undisturbed incubation environment, helping to achieve optimal culture conditions to improve embryo quality and viability.
The components are updated to enable the capture of dark-field images and the integration of the automated evaluation of early embryo development to improve embryo assessment.

Time Lapse technology in IVF

1. *Castelló D, Motato Y, Basile N, et al. How much have we learned from time-lapse in clinical IVF? Mol Hum Reprod. 2016;22(10):719-727.*
2. *Chen M, Wei S, Hu J, et al. Does time-lapse imaging have favorable results for embryo incubation and selection compared with conventional methods in clinical in vitro fertilization? A meta-analysis and systematic review of randomized controlled trials. PLoS ONE.2017; 12(6): e0178720.*
3. *Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Hum Reprod. 2011;26(6):1270 -1283.*
4. *Milewski R and Ajduk A. Time-lapse imaging of cleavage divisions in embryoquality assessment. Reproduction (2017);154 R37-R53.*
5. *Lundin K, Park H. Time-lapse technology for embryo culture and selection. Ups J Med Sci. 2020 Feb 25:1-8. doi: 10.1080/03009734.2020.1728444. [Epub ahead of print].*
6. *Raudonis V, Paulauskaite-Taraseviciene A, Sutiene K et al. Towards the automation of early-stage human embryo development detection. Biomed Eng Online. 2019;18(1):120.*
7. *Armstrong S, Vail A, Mastenbroek S et al. Time-lapse in the IVF-lab: how should we assess potential benefit? Hum Reprod. 2015;30(1):3-8.*

Assisted Reproductive Technology (ART) has resulted in the birth of over 5 million babies worldwide. Despite achievement of significant improvements in ART, two major problems still persist, the first one being low success rate and the second one is high multiple pregnancy rates. In ART, the cultivation of fertilized oocytes and the subsequent selection of embryos is a vital step. The most conventional method used for decades to select embryos has been based on morphological characteristics.

According to the Istanbul consensus workshop on embryo assessment, the manual selection criteria for fertilized oocytes and embryos are as follows (Table 2).

Table 2. Timing of observation of fertilized oocytes and embryos and expected stage of development at each point (Instanbul Consensus)

Developmental Day	Event/Observation	Definition of Event	Timeline of Occurrence (Hours post insemination)
Day 1	Fertilization Check	Pronuclei Stage	17 ± 1
	Syngamy Check	Expect 50% in syngamy (upto 20% may be at the 2 cell stage)	23 ± 1
	Early cleavage check	2 Cell stage	26 ± 1h post ICSI 28 ± 1h post IVF
Day 2	Day 2 Embryo Assessment	4 Cell	44 ± 1
Day 3	Day 3 Embryo Assessment	8 Cell	68 ± 1
Day 4	Day 4 Embryo assessment	Morula	92 ± 2
Day 5	Day 5 Embryo assessment	Blastocyst	116 ± 2

ICSI, intracytoplasmic sperm injection.

Table adapted from Hum Reprod. 2011;26(6):1270 -1283.

A figure outlines the different timings and corresponding stages of human embryo development. It shows the appearance of a blastocoel cavity, embryonic divisions and helps to identify the abnormal morphology in human embryos.
Link (https://rep.bioscientifica.com/view/journals/rep/154/2/REP-17-0004.xml ; Figure 1)

Embryo handling throughout ART process involves transferring them between dishes and assessing them at specified times. This happens outside the incubator which can affect the culture media (pH, temperature) and embryo environment eventually leading to stress on the embryo, thus affecting its development and quality.
To circumvent these issues, Time-lapse monitoring systems (TLS) or Time-lapse technology (TLT) is being used of late, and combines three basic elements: an incubator, an optical microscope and a software program.
Integrating these elements, continuous surveillance is provided while optimal culture conditions are maintained.
Typically such a system consists of three main components:

(1) a light source
(2) microscope optics and
(3) a video camera.

Usually, red light at 650 nm is used to illuminate an embryo, which is cultivated in a specially designed culturing dish, called a culture coin. Microscope optics magnify the embryo cells by 20 times. The TL system is equipped with a camera that allows the capture of embryo images. The TL system uses a special mirror (prism) that concentrates light and directs it to the embryo and camera sensor (Figure 1).

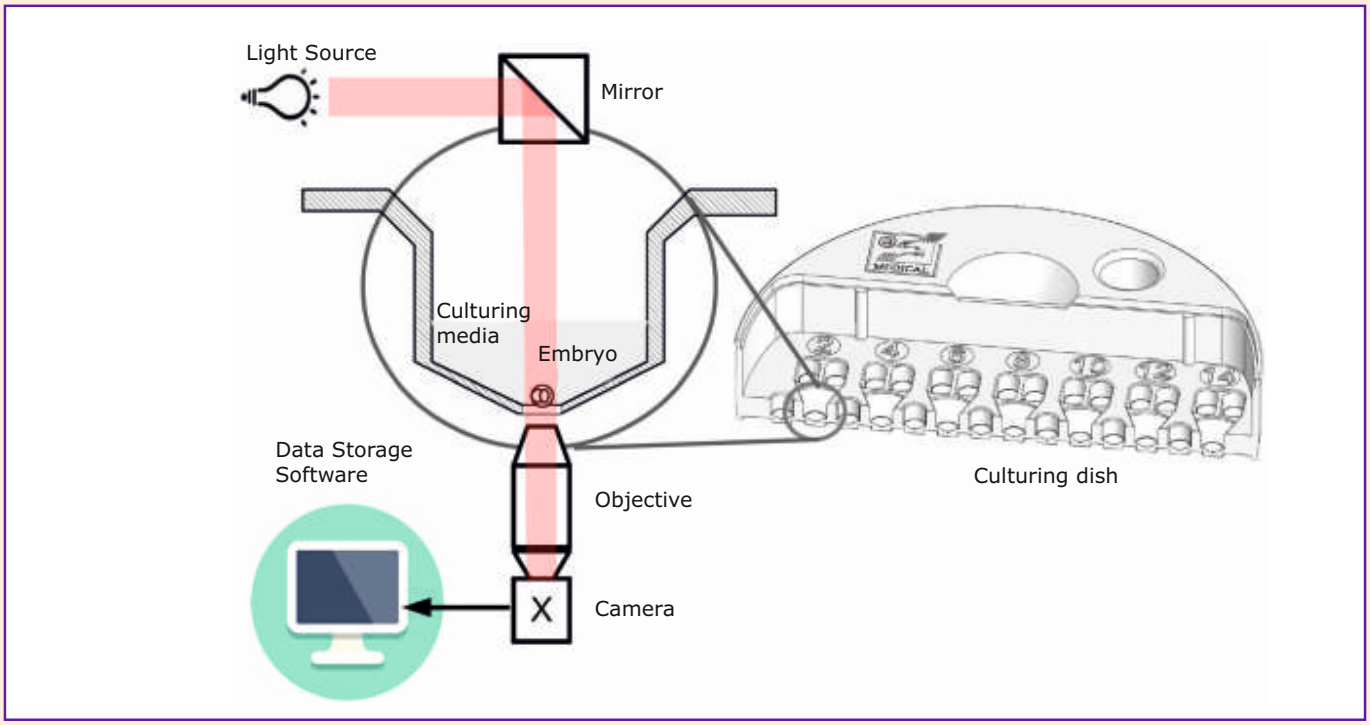

Figure 1. Scheme of Time lapse system.

Figure taken from Biomed Eng Online. 2019;18(1):120.

TL system takes digital images of embryos at set time intervals. The system can either be installed into an existing embryo incubator or can exist as a combined time-lapse incubation system. With the use of special software, images can be compiled to create a time-lapse sequence of embryo development avoiding the need to remove embryos from the incubator for morphological assessment.

Time lapse technology: A brief note on origin

1. *Albani E, Sinchetto M, Parisi I et al. Comparison among different Time-Lapse systems: what has changed in the laboratory? 2015 November-December; 2(6): 246–251. Accessed from website https://www.cce-online.org/materiale_cic/926_2_6/7858_comparison/article.htm as on 25.04.2020.*
2. *Castelló D, Motato Y, Basile N et al. How much have we learned from time-lapse in clinical IVF? Mol Hum Reprod. 2016;22(10):719-727.*
3. *Mio Y, Maeda K. Time-lapse cinematography of dynamic changes occurring during in vitro development of human embryos. Am J Obstet Gynecol 2008;199:660.e1–660.e5.*
4. *Pribenszky C, Mátyás S, Kovács P et al. Pregnancy achieved by transfer of a single blastocyst selected by time-lapse monitoring. Reprod Biomed Online. 2010 Oct;21(4):533-536.*

Time-lapse technology has been the quest for researchers for many years.

Payne et al., in 1997 developed Time-Lapse video cinematography (TLC) to overcome the limitations of intermittent observation.

For the first time, the sequence and the timing of the human fertilization process, novel ooplasm wave and novel cytoplasmic flare were observed.
This technique provided high-resolution, continuous imaging, in which the various components of the cell could be distinguished and monitored during the recording period.

Later, Lemmen et al., in 2008, for the first time correlated a higher cell number on day 2 with the early pro-nuclei disappearance and 1st cleavage.

Mio and Maeda in 2008, described the kinetics of events until blastocyst stage including the fertilization process and the development from a 2-cell embryo to a hatched blastocyst.

In 2010, Wong et al., studied the three parameters that collectively predicted blastocyst formation before day 3 and the correlation of the single cell/embryo gene expression profiles morphology phenotypes with aberrant gene expression pattern.

Pribenszky et al., in 2010 reported the first live birth after time-lapse assessment of a five embryo cohort up to blastocyst stage.

Finally, in 2012 Hashimoto et al., analyzed two parameters related to the formation of high-scoring blastocysts.

Time-Lapse Technology: Humidity, Culture Conditions and Safety

1. Chen M, Wei S, Hu J, et al. Does time-lapse imaging have favorable results for embryo incubation and selection compared with conventional methods in clinical in vitro fertilization? A meta-analysis and systematic review of randomized controlled trials. PLoS ONE.2017; 12(6): e0178720.

The most potential advantage of the TLT is the decreased frequency of handling and exposure of embryos to suboptimal conditions. This eliminates the risks of stress from temperature changes, high oxygen exposures and pH changes in the culture medium and thus provides improved culture conditions.

1. Importance of Humidity in Embryo Culturing

1. Consensus Group C. 'There is only one thing that is truly important in an IVF laboratory: everything' Cairo Consensus Guidelines on IVF Culture Conditions. Reprod Biomed Online. 2020;40(1):33-60.
2. Fawzy M, AbdelRahman MY, Zidan MH et al. Humid versus dry incubator: a prospective, randomized, controlled trial. Fertil Steril. 2017;108(2):277-283.
3. Simopoulou M, Sfakianoudis K, Rapani A, et al. Considerations Regarding Embryo Culture Conditions: From Media to Epigenetics. In Vivo. 2018;32(3):451-460.

Earlier, the CO_2 regulation in incubators was mediated using thermal conductivity sensors and humidity was an essential aspect for good performance.
However, the more recent shift to infrared sensors has removed this requirement suggesting the use of moisture-free incubator.
There was also a concern that humidity could negatively impact the growth of embryos due to the likely increased overgrowth of microorganisms in the incubator.

However, a prospective, double-blind, randomized, controlled trial by Fawzy et al., showed that human embryos cultivated *ex vivo* in a dry incubator had statistically significantly decreased implantation and clinical and ongoing pregnancy rates (Table 3).
The researchers opined that higher ongoing pregnancy rate in the humid culture may indicate that there is an effect beyond embryo development.

Table 3. Clinical outcomes in the dry vs. humid culture groups by intention-to-treat analysis

Outcome rates	Dry culture (n = 147)	Humid culture (n = 150)	Odds ratio (95% CI)	p value
Clinical pregnancy	63/147 (43)	85/150 (57)	0.57 (0.36–0.91)	.017
Ongoing pregnancy	54/147 (37)	78/150 (52)	0.54 (0.34–0.85)	.008
Implantation	69/257 (27)	93/258 (36)	0.65 (0.45–0.95)	.025

Note: Data presented as proportions, n (%), unless specified otherwise. Logistic regression analysis was used for between-group data comparisons. Data presentations are proportions (rate difference) and 95% confidence interval (CI). Chemical pregnancy indicates a positive pregnancy test with no gestational sac identified 15 days after the test.

Table adapted from Fertil Steril. 2017;108(2):277-283.

Temperature and humidity work hand in hand to secure stability in culture. Appropriate levels of humidity ensure a controlled environment otherwise there could be a disturbance in culture osmolality which can negatively affect embryo development.
The issue of optimal temperature is vast and very well documented literature supports the current level to be set at 37°C.

2. Combining optimal culture conditions (humidified) with time lapse system

1. *Perez Albala S. Aparicio-Ruiz B, Albert C et al. Embryo culture conditions under high humidity significantly enhances blastocysts formation and quality according to an automatic time-lapse algorithm. Poster P-260. Accessed from website https://www.ivi-rmainnovation.com/wp-content/uploads/2019/05/Embryo-culture-conditions-under-high-humidity-significantly-enhances-blastocysts-formation-and-quality-according-to-an-automatic-time-lapse-algorithm.pdf as on 02.06.2020.*
2. *Del Gallego R, Albert C, Marcos J et al. Humid Vs. Dry embryo culture conditions on embryo development: a continuous embryo monitoring assessment. P-636 Wednesday, October 10, 2018. Accessed from website https://www.fertstert.org/article/S0015-0282(18)31604-2/pdf as on 02.06.2020.*
3. *Holmes R, Weinberg J, Kalaghan L et al. P-405 - Comparison of humidified versus non-humidified incubation with sequential culture media in a time-lapse incubator using sibling oocyte splits. ASRM2019. Accessed from website https://asrm.confex.com/asrm/2019/meetingapp.cgi/Paper/3558 as on 02.06.2020.*
4. *Gallego RD, Remohí J, Meseguer M. Time-lapse imaging: the state of the art. Biol Reprod. 2019;101(6):1146-1154.*
5. *Cruz M, Gadea B, Garrido N, et al. Embryo quality, blastocyst and ongoing pregnancy rates in oocyte donation patients whose embryos were monitored by time-lapse imaging. J Assist Reprod Genet. 2011;28(7):569–573.*
6. *Nakahara T, Iwase A, Goto M et al. Evaluation of the safety of time-lapse observations for human embryos. J Assist Reprod Genet. 2010;27(2-3):93-96.*
7. *Takenaka M, Horiuchi T, Yanagimachi R. Effects of light on development of mammalian zygotes. Proc Natl Acad Sci USA. 2007;104(36):14289-14293.*
8. *Ottosen LD, Hindkjaer J, Ingerslev J. Light exposure of the ovum and preimplantation embryo during ART procedures. J Assist Reprod Genet. 2007;24(2-3):99-103.*
9. *Pomeroy KO and Reed ML. The effect of light on embryos and embryo culture. J Reprod Stem Cell Biotechnol 2013;3(2):46-54.*
10. *Insua MF, Cobo AC, Larreategui Z et al. Obstetric and perinatal outcomes of pregnancies conceived with embryos cultured in a time-lapse monitoring system. Fertil Steril. 2017; 108:498–504.*

Of late, many studies are published with embryo culture conditions under humidity observed with a time lapse system.

In a retrospective study from Spain, researchers analyzed 3001 embryos from 361 patients in the first part of the study and in the second part, 116 patients with 1016 embryos were randomly distributed under high or low humidity in a continuous embryo monitoring incubator.

Embryos were generated by ICSI and incubated in a Time-lapse incubator that used automatic cell-tracking software.

The results showed a direct correlation with higher blastocyst rates and better good quality embryos in humidity chambers compared to dry chambers.

The blastocyst rate in humidity chambers was 77.2% versus 70.9% in dry chambers and percentage of good quality blastocysts 42.8% (humidity) vs. 35.8% (dry) (p=0.022; Figure 2).

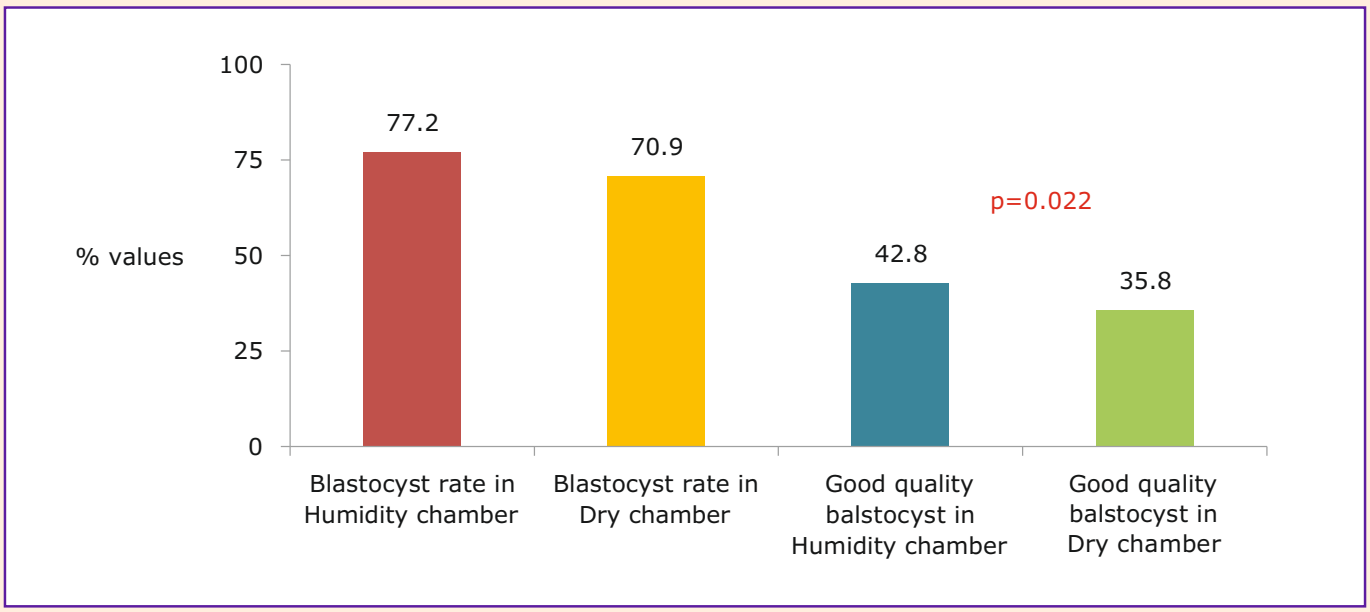

Figure 2. Higher blastocyst rates and better good quality embryos in humidity chambers compared to dry chambers.

Del Gallego et al., in a prospective, randomized study which included a total of 1,734 embryos from 176 patients showed a clear improvement on embryo development and subsequent IVF outcome in a humidified atmosphere of a **time-lapse incubator.**

A significantly higher blastocyst rate ($p<0.05$) was found in embryos cultured under humid conditions (HC) (74.5% vs. 69.2%) compared to dry conditions (DC).

There was also a clear trend observed towards a higher number of pregnancies (HC = 83.3% vs.

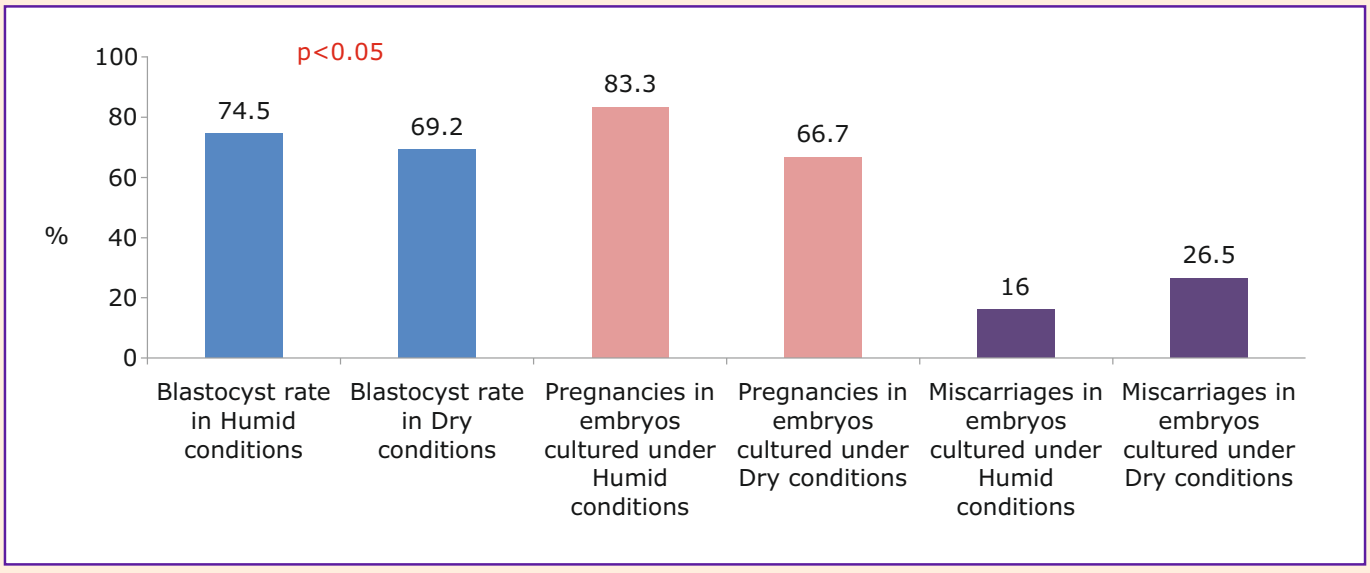

Figure 3. Improved embryo development and subsequent IVF outcome in Humid conditions vs. Dry conditions.

DC = 66.7%) and less miscarriages (HC = 16% vs. DC = 26.5%; Figure 3.) in the HC group.

In another prospective, randomized study presented at the American Society of Reproductive Medicine 2019 Scientific Congress by Holmes et al., the use of a **humidified chamber** yielded significantly more good quality blastocysts on day 5, 6 and overall by day 7 than non-humidified chambers (Table 4).

The study compared outcomes following sibling zygote splits in identical culture conditions, within the same incubator, utilizing a time-lapse system and both dry and humidified culture.

Table 4. Comparison of Humidified vs. Non-humidified incubation

	Humidified	Non - Humidified
Oocyte#	105	110
% Fertilization	79.0%	81.8%
% Good Cleavage Rate	79.5%	76.7%
% Total Blastocysts on D5	48.2%	43.3%
% Blastocysts ≥ 3BB D5	24.1%	14.4%
% Total Blastocysts D6	62.7%	54.4%
% Blastocysts ≥ 3BB D6	49.4%	25.6%
% Total Blastocysts D7	62.7%	60.0%
% Blastocysts ≥ 3BB D7	50.6%	31.1%

Table adapted from https://asrm.confex.com/asrm/2019/meetingapp.cgi/Paper/3558.

Published studies have also shown that the embryo culture conditions were improved by TLSs and their safety has been confirmed with no differences or detrimental effects on embryos cultured in these devices.

Cruz et al., conducted a study to evaluate whether incubation conditions with a time lapse incubator was comparable to standard laboratory incubation in 478 embryos by comparing embryo quality, development and ongoing pregnancy rates.

The study results showed that time-lapse monitoring did not affect the embryo quality, blastocyst development (Table 5) or viability compared to standard incubator.

Table 5. Blastocyst rate, proportion of frozen and transferred embryos incubated in Time lapse incubator vs. the standard incubator

	Blastocyst	CI95%	Frozen	CI95%	Transferred	CI95%
Time lapse incubator (n=238)	54.8 (n=130)	47.5–62.1	7.6 (n=18)	2.8–12.4	21.0 (n=50)	15.9–26.9
Standard incubator (n=240)	50.6 (n=121)	44.3–56.9	10.9 (n=26)	7–14.8	24.1 (n=58)	18.7–29.5
p	ns		ns		ns	

Table adapted from J Assist Reprod Genet. 2011;28(7):569–573.

Studies have shown that light is one of the factors of embryonic environment and that its effects should not be ignored.

Visible light (400-700 nm wavelengths) is an unnatural stress factor to preimplantation embryos cultured *in vitro*.

The wavelengths of 400 to 500 nm of Blue light (near-ultraviolet) are considered to be potentially damaging to cells (gametes and embryos).

A green bypass filter may be prudent when viewing gametes and embryos.

A retrospective study by Insua et al., demonstrated no detrimental effects on obstetric or perinatal outcomes when a Time-lapse incubator was used rather than a conventional incubator (CI).

The delivery rate was 49.3% (TLS) vs. 40.0% (CI) but multiple deliveries were higher in the TLS group: 31.0% (67 of 216) vs. 24.7% (40 of 162) in the CI group.

what are the different advantages of the Time-Lapse technology?

1. Machtinger R and Racowsky C. Morphological systems of human embryo assessment and clinical evidence. Reprod Biomed Online. 2013;26(3):210-21.

The minimal requirements for embryo selection should include standardization, ease of assessment, objectivity, minimal harm to the embryo and a high correlation with pregnancy rates. Automated Time-lapse imaging systems have the potential to meet these requirements.

Time-Lapse technology is an answer for several shortfalls of the conventional embryo evaluation procedures. Some advantages of TL technology are discussed herewith.

1. Overcoming the Limitations of Conventional Embryo Assessment/Scoring

1. *Lundin K, Park H. Time-lapse technology for embryo culture and selection. Ups J Med Sci. 2020 Feb 25:1-8. doi: 10.1080/03009734.2020.1728444. [Epub ahead of print].*
2. *Machtinger R and Racowsky C. Morphological systems of human embryo assessment and clinical evidence. Reprod Biomed Online. 2013;26(3):210-21.*
3. *Kovacs P. Embryo selection: the role of time-lapse monitoring. Reproductive Biology and Endocrinology 2014, 12:124.*
4. *Chen M, Wei S, Hu J, et al. Does time-lapse imaging have favorable results for embryo incubation and selection compared with conventional methods in clinical in vitro fertilization? A meta-analysis and systematic review of randomized controlled trials. PLoS ONE.2017; 12(6): e0178720.*
5. *Boueilh T, Reignier A, Barriere P et al. Time-lapse imaging systems in IVF laboratories: a French national survey. J Assist Reprod Genet. 2018;35(12):2181-2186.*
6. *Basile N and Meseguer M. Time-lapse technology: evaluation of embryo quality and new markers for embryo selection. Expert Rev. Obstet. Gynecol.2012; 7(2); 175-190.*
7. *Kovacs P. Time-lapse embryoscopy: Do we have an efficacious algorithm for embryo selection?. Journal of Reproductive Biotechnology and Fertility. 2016; 5:1-12.*
8. *Conaghan J, Chen AA, Willman SP et al. Improving embryo selection using a computer-automated time-lapse image analysis test plus day 3 morphology: results from a prospective multicenter trial. Fertil Steril. 2013;100(2):412-419.*
9. *Quang LN, Tram LTB, Thuy NHM et al. Comparison of clinical outcome of frozen embryo transfer after embryo selection based on morphokinetic versus morphologic criteria for freezing. Biomed. Res. Ther.2018; 5(12):2910-2917.*

Earlier, the common strategy was to select the 'good-quality' embryos (looking at number of cells, degree of fragmentation, and multinucleation) at the cleavage stage, transfer 2-3 embryos, and cryopreserve the rest of the good-quality embryos, while discarding the 'non-good-quality' (judged from morphology and cleavage) embryos.
A figure shows the estimated 'growth' curve of development of human embryos in culture used initially way back in the 1980s to define embryo development.
Link https://www.rbmojournal.com/article/S1472-6483(12)00700-6/fulltext; Figure 1

However, now, the standardized criteria for grading embryos have been proposed by two organizations, the Society for Assisted Reproductive Technology system and the Alpha Executive and ESHRE special Interest Group of Embryology system.

According to the morphologic characteristics, embryos can be scored at various stages (pronuclear, cleavage, blastocyst). However, by this approach it is believed that approximately 20-40% of the embryos identified only will implant.
Thus, the conventional embryo selection methods have relatively low clinical pregnancy rate of approximately 30% per transfer.

In addition, the static morphological evaluation of embryos has several limitations which are as follows:

- Embryo exposure to suboptimal culture conditions.
- Limited predictive value for ploidy status and implantation.
- High inter- or intra-observer agreement.

- Several critical stages in between observations that may go unnoticed.

On the contrary, Time-Lapse technology offers the following advantages:

- Continuous monitoring of embryonic development (Figure 4).
- Close follow up of embryos from fertilization up until the transfer.
- Live image tracking of embryo morphology.
- No need to remove the embryos from the optimal culture conditions.
- No change in environment of embryos.
- Detailed knowledge about the kinetic and morphologic changes/abnormalities an embryo undergoes *in vitro.*
- Increased learning of 'embryo behaviour' (such as irregular cleavages etc.).
- Study of embryo development in different settings, such as different culture media and patient populations.
- Possibility to perform studies comparing oxygen levels, temperature, pH of the culture medium.
- Precise timing of kinetic events (cell divisions, duration and synchrony of the cell cycles, fragmentation, timing of compaction, blastocyst formation, and expansion and blastocyst dynamics).
- Correlation of these timings/intervals with various stages of embryonic development, implantation, and live birth.
- Morphokinetic parameters can be used to build algorithms that can help to choose the fittest embryo for transfer.
- Training of embryologists in assessing embryo quality, as well as the validation of different scoring systems.
- Removes observers' bias.
- Enhances learning skills of average embryologist.
- Standardization of laboratory is seen.
- Improves the lab quality.

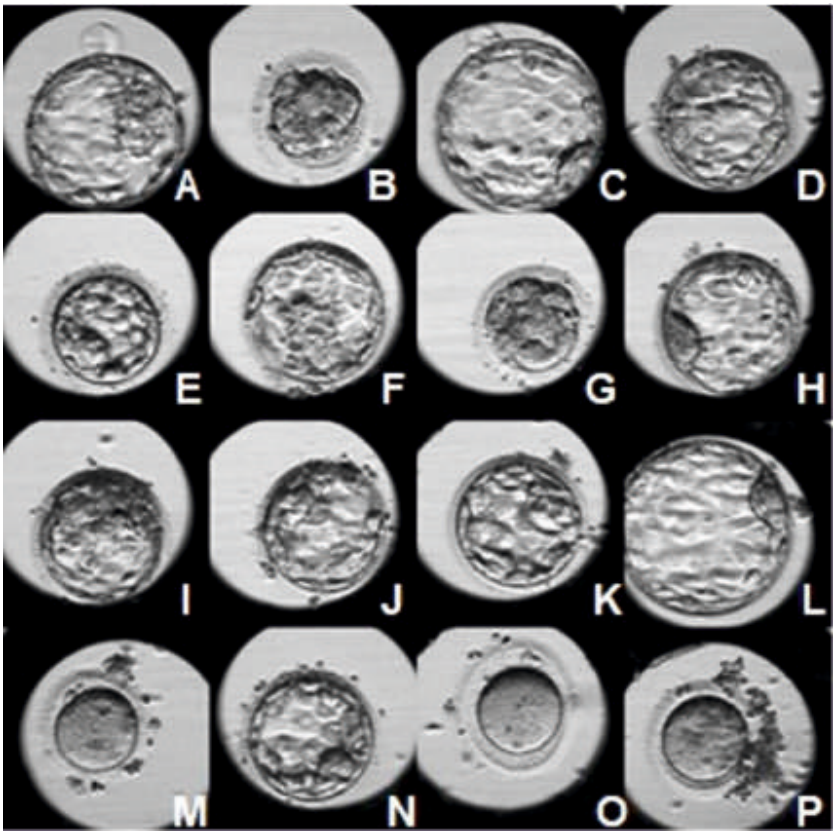

Figure 4. Embryos were monitored by a Time-lapse system. Figure 4A, C, D, E, F, H, I, J, K, L, N: embryos reached the blastocyst stage.
Figure 4B: embryo was blocked at morula stage.
Figure 4M, 4O, 4P: oocytes were not fertilized.

Figure taken from Biomed. Res. Ther.2018; 5(12):2910-2917.

2. Use of Morphokinetic parameters for embryo assessment

1. *Milewski R and Ajduk A. Time-lapse imaging of cleavage divisions in embryoquality assessment. Reproduction (2017);154 R37–R53.*
2. *Quang LN, Tram LTB, Thuy NHM et al. Comparison of clinical outcome of frozen embryo transfer after embryo selection based on morphokinetic versus morphologic criteria for freezing. Biomed. Res. Ther.2018; 5(12):2910-2917.*
3. *Kovacs P. Time-lapse embryoscopy: Do we have an efficacious algorithm for embryo selection?. Journal of Reproductive Biotechnology and Fertility. 2016; 5:1-12.*

Morphokinetic parameters include absolute timings of successive embryonic divisions, as well as relative timings, i.e. periods between divisions, reflecting either duration of the cell cycle or synchronisation of the cleavage rounds.
Time-lapse systems record the data of embryo development (morphokinetics), including the precise determination of the onset, duration, and interval between cell divisions.

Time lapse also allows quantifying the dynamic morphologic characteristics of a developing human embryo, such as pronuclei (PN) and nucleus formation and disappearance, fragmentation, and size distribution of blastomeres.

The number of nuclei in a blastomere, degree of fragmentation, and occurrence of irregular cleavages can also be assessed including the detection of direct or reverse cleavage divisions.

Data also can be obtained for the spatial arrangement of the blastomeres, which may also be predictive of the human embryo's developmental potential.
The recorded data can be used to classify and select the potential embryos with support of software.

These morphokinetic parameters provide information for reproductive heuristic models – algorithms that can predict chances of achieving a blastocyst stage, implantation, biochemical or clinical pregnancy, or a full term development by each examined embryo including distinguishing euploid from aneuploid embryos.

Some definitions of the kinetic TL parameters are listed in the box.

t_0: time of insemination during IVF or mid-time of injection of the oocyte cohort during ICSI
ICSI: intra-cytoplasmic sperm injection
tPNf: time to pronuclear fading
t_2, t_3 . . . t_8: time to the 2-, 3- . . . 8-cell stage
tx-ty: time between the x-cell to y-cell stage
S_1: time from appearance of the cleavage furrow to the completion of first cell division
S_2: time from the 3- to 4-cell stage
S_3: time from the 5- to 8-cell stage
tM: time to morula stage
tSB: time to start of blastulation
*t*EB: time to expanded blastocyst

Box. Definitions of kinetic TL parameters.

Adapted from Journal of Reproductive Biotechnology and Fertility. 2016; 5:1-12.

3. Automated embryo developmental annotations

1. *Khosravi P, Kazemi E, Zhan Q et al. Deep learning enables robust assessment and selection of human blastocysts after in vitro fertilization. NPJ Digit Med. 2019;2:21.*
2. *Conaghan J, Chen AA, Willman SP et al. Improving embryo selection using a computer-automated time-lapse image analysis test plus day 3 morphology: results from a prospective multicenter trial. Fertil Steril. 2013;100(2):412-419.*
3. *Geri Assess® 2.0 Annotation Accuracy. Accessed from website https://www.geneabiomedx.com/getattachment/Resources/Technotes/Downloads/Geri-Assess%C2%AE-2-0-A-consistent-approach-to-selectin/QRTM213-Geri-Assess-2-0-Annotation-Accuracy.pdf?lang=en-AU&ext=.pdf as on 02.06.2020.*

Although TLT represents a step toward more objective embryo evaluation, there is always a scope for inter- and intra-evaluator variation among embryologists.
Consequently, automated embryo developmental annotations may add objectivity to the process of embryo selection and thus lead to an improvement of the IVF process.

A prospective, multicenter clinical study Conaghan et al., evaluated whether non-invasive, computer-automated test could improve the prediction of usable blastocyst formation by D3 that could help embryologists for D3 embryo selection.

The researchers used the test termed Eeva (Early embryo viability assessment), which was enabled by dark-field, time-lapse imaging and cell-tracking software algorithms.

The results showed that the Eeva prediction and cell-tracking software results indicated a high probability of usable blastocyst formation.

Compared with morphology evaluation alone, Eeva significantly improved the specificity (84.2% vs. 52.1%; $p<0.0001$) and positive predictive value, PPV (54.1% vs. 34.5%; $p<0.01$) of usable blastocyst predictions in the development phase (Table 6).

The experienced embryologists were able to significanlty predict the usable blastocyst by addition of Eeva to traditional D3 morphology and there was also a reduced variability among embryologists.

Table 6. Usable blastocyst prediction performance (% specificity and % PPV) of Eeva compared with D3 morphology for two independent data sets in the development and test phases

	No. of patients	No. of embryos	Specificity, %	PPV, %
Morphology (development phase)	45	292	52.1	34.5
Eeva (development phase)	45	292	84.2	54.1
Eeva (test phase)	74	941	84.7	54.7

Table adapted from Fertil Steril. 2013;100(2):412-419.

A performance testing of a latest Time lapse system was done to assess the accuracy of automated developmental event annotations and was tested by comparing them to manual annotations.

Over 3,700 events from >400 embryos from 69 patients were included in the tests.

The key developmental events and observations and their definitions automatically annotated by the TLS are shown in table 7.

The results showed that the latest software could perform automated annotations of critical embryo developmental events and observations to the level that can support clinics in their embryo assessments.

Table 7. Key developmental events and observations and their definitions automatically annotated by an advanced TLS

Developmental Day	Event/ Observation	Definition of Event	Time in hours
Day 1	PN Appearance	The first frame where appearance of at least one visible PN is identified	1-28
	PN Disappearance	The first frame where disappearance of all visible PNs is identified	17-30
Day 2	2-cell division	The first frame where 2 discrete membrane-separated blastomeres is identified	20-40
Day 3	3-cell division	The first frame where 3 discrete membrane-separated blastomeres is identified	30-48
Day 4	4-cell division	The first frame where 4 discrete membrane-separated blastomeres is identified	32-54
Day 5	5-cell division	The first frame where 5 discrete membrane-separated blastomeres is identified	38-68
	6-cell division	The first frame where 6 discrete membrane-separated blastomeres is identified	46-78
	Morula Transition	The first frame where appearance of cellular compaction and blurring of distinctive individual cell membranes is identified	64-100
	Early Blastocyst Transition	The first frame where appearance of blastocyst cavitation is identified	86-126
	Expanded Blastocyst Transition	The first frame where appearance of clearly expanded blastocyst with a minimum diameter of 167 µm is identified	86-192
	Hatching Blastocyst Transition	The first frame where appearance of cellular hatching, shown as trophoblast cells extruding from the zona pellucida, is identified	86-192

Table adapted from Geri Assess® 2.0 Annotation Accuracy.

feedback form

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Satisfaction Score for ALIVE Newsletter Time Lapse Imaging in ART Part 1: Issue 6; July 2020

Rating Scale	Poor ----------Excellent (Please circle the appropriate rating)									
Scientific content	1	2	3	4	5	6	7	8	9	10
Relevance of the topic	1	2	3	4	5	6	7	8	9	10
Impact on my daily practice	1	2	3	4	5	6	7	8	9	10
Innovation	1	2	3	4	5	6	7	8	9	10
Overall level of satisfaction	1	2	3	4	5	6	7	8	9	10

What aspects of the Newsletter issue 6 did you find particularly interesting and/or informative?

_____

Please suggest topics/areas that you would like to be covered in future issues of the Alive Newsletter?

_____

How can the subsequent Newsletter issues be improved?

_____

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