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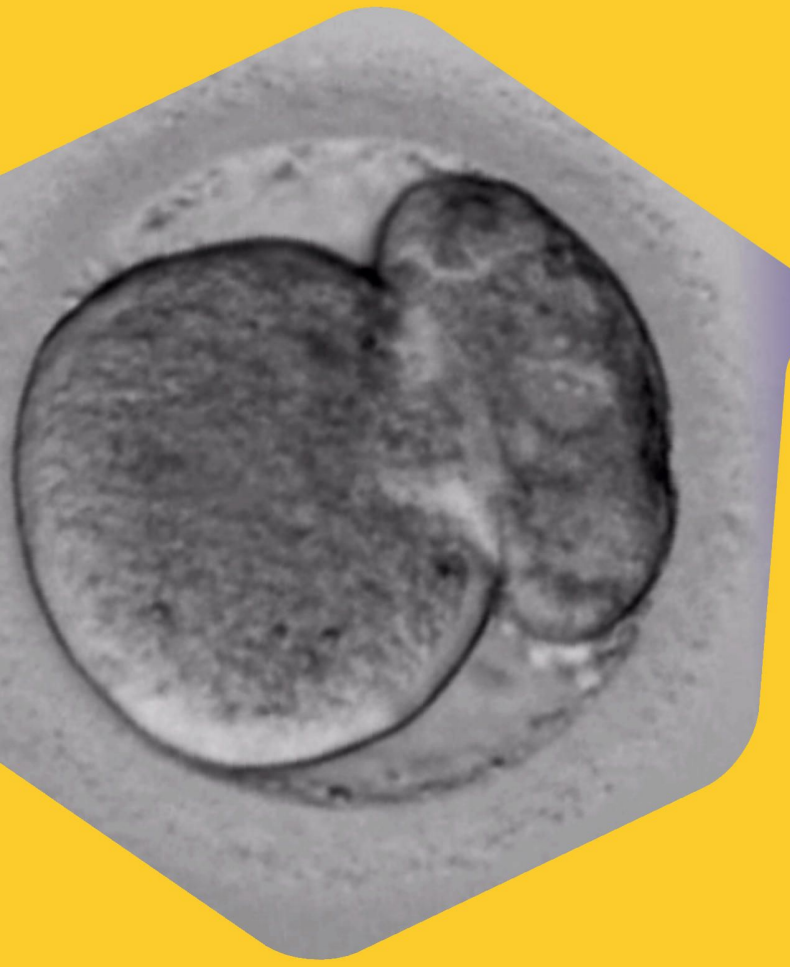
ART LEARNING INITIATIVES FOR EXPERTS

Issue 7



Time-lapse imaging in ART

Part 2

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MERCK

contents

abbreviations

ART	Assisted Reproductive Technology
CI/SC	Conventional incubator / standard 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
TLI	Time-lapse incubator / imaging
TLC	Time-Lapse video cinematography

expert insights

Dr. G. A. Rama Raju

Founder & Director,
Krishna IVF Clinic,
Visakhapatnam, India.

Dr. Manish Banker

Medical Director,
Nova IVF Fertility,
Ahmedabad.

Dr. (Col)Sandeep Karunakaran

Clinical Head & Senior Consultant
Oasis Fertility, Hyderabad.

The success rates of IVF have phenomenally improved over the last decades when looked back to the first birth of Louise Joy Brown on 25th July 1978.

It is a very well accepted fact that embryo evaluation and selection is fundamental in clinical IVF.

Although morphological quality assessment of embryos *in vitro* remains the gold standard for predicting IVF success rates, advance in research has slowly ushered in the use of more advanced Time-lapse Imaging (TLI) technology that helps embryologists with immense data enabling them to increase the success rate after embryo transfer.

Clinical data have shown that morphokinetic embryo selection improves ongoing pregnancy and live birth rates and reduces early pregnancy loss. However, research also states that Time-lapse Imaging technology is not accepted world over as there are shortcomings with respect to a universally accepted algorithm or a current national guidance, recommendation or policy for the use of TLI technology.

Despite all these discussions, TLI probably offers the safest and most stable embryo culture environment based on current technology.

It is only in the last decade that TLI technology has been introduced into human IVF as a routine procedure and already continued embryo monitoring has allowed embryologists to identify several previously unknown or undetectable aspects of development thus delivering a significant clinical impact.

This issue was drafted to know the role of Clinical efficacy of Time-lapse system vs. Preimplantation genetic diagnosis/screening (PGD)/(PGS) for selection of good quality embryos and compare the use of Time-lapse system over conventional incubator methods for embryo selection, pregnancy rates and improved perinatal outcomes. A brief note on the future of TLI technology is also discussed.

A peek from the last issue 6..

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.
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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. Eava. Accessed from website https://hcp.merckgroup.com/en/fertility/technologies/Eeva.html as on 3.11.2020.

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.
There is a continuous evolution of the incubators from the inverted glass domes to the latest incubator types with varying capabilities and differing methods of regulating their internal environment even with low oxygen.
Of late, there is an advent of the more advanced Time-lapse incubators providing incubation in a humidified environment with an add-on of uninterrupted culture monitoring.
The incubator components 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: potential advantages

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.
2. Kovacs P. Time-lapse embryoscopy: Do we have an efficacious algorithm for embryo selection?. Journal of Reproductive Biotechnology and Fertility. 2016; 5:1-12.
3. Lundin K and Park H. Time-lapse technology for embryo culture and selection. Ups J Med Sci. 2020;125(2): 77-84. doi: 10.1080/03009734.2020.1728444.
4. 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.

The most potential advantage of the Time-lapse Technology (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.

Time-lapse technology also offers the following advantages:

- Continuous monitoring of embryonic development.
- 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.

Time-lapse vs. PGD/PGS in selecting good quality embryo (euploid embryo)

1. *Yang Z, Zhang J, Salem SA et al. Selection of competent blastocysts for transfer by combining time-lapse monitoring and array CGH testing for patients undergoing preimplantation genetic screening: a prospective study with sibling oocytes. BMC Med Genomics. 2014;7:38.*
2. *Patel DV, Shah PB, Kotdawala AP et al. Morphokinetic behavior of euploid and aneuploid embryos analyzed by time-lapse in embryoscope. J Hum Reprod Sci 2016;9:112-118.*
3. *Chen HF, Chen SU, Ma GC et al. Preimplantation genetic diagnosis and screening: Current status and future challenges. J Formos Med Assoc. 2018;117(2):94-100.*
4. *Basile N, Nogales Mdel C, Bronet F et al. Increasing the probability of selecting chromosomally normal embryos by time-lapse morphokinetics analysis. Fertil Steril. 2014;101(3):699-704.*
5. *Mandawala AA, Harvey SC, Roy TK et al. Time-lapse embryo imaging and morphokinetic profiling: Towards a general characterisation of embryogenesis. Anim Reprod Sci. 2016;174:2-10.*
6. *Yang Z, Liu J, Collins GS et al. Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis IVF patients: results from a randomized pilot study. Mol Cytogenet. 2012;5:24.*

Aneuploidy and Euploid embryos

Technology advancement has always yearned for the selection of the most competent embryos with the highest potential of implantation for transfer.

Published data has clearly confirmed that the main cause of embryo arrest, miscarriage, implantation failure, pregnancy loss or birth defects is the presence of numerical chromosome abnormality or aneuploidy. The most common abnormality in *in vitro* fertilized embryos is aneuploidy which increases with maternal age.

Aneuploidy of human embryos might result due to the premature division and nondisjunction of chromatids during meiosis along with several contributory factors such as paternal, mitotic or meiotic errors.

Chromosomes containing fragments/micronuclei may contribute to aneuploidy that may have negative consequences on the normal development process.

It is thus important and necessary to avoid the selection of aneuploid embryos during the IVF procedure.

Preimplantation genetic diagnosis (PGD) was initially applied to the screening of X-linked disorders and later extended to aneuploidy screening with the use of fluorescence *in situ* hybridization (FISH).

While PGD helps to prevent the transmission of monogenic inherited disorders in families afflicted with the diseases to the future offsprings, preimplantation genetic screening (PGS) screens embryo with aneuploidy in order to enhance the implantation rates as well as live birth rates.

The PGD is a well-established clinical practice but PGS is still under debate and its acceptance by the clinical community is still evolving.

PGS as an invasive technology has legal and social hurdles and clinics also may not have sufficient capacity as it is highly expensive to perform the technique.

On the contrary, Time-lapse technology has provided a potential non-invasive alternative to PGS, whereby, certain morphokinetic parameters are used to identify ploidy and hence, prenatally select euploid embryos for transfer.

A randomized pilot study by Yang et al., showed that there was a significant increase in the clinical pregnancy rate with lower miscarriage rates and no twin pregnancies in first time IVF patients with good-prognosis whose embryos were screened for aneuploidy compared to the control group whose embryos were selected for transfer based on morphology alone.

Clinical efficacy of Time-lapse system vs. PGD/PGS for selection of good quality embryos

1. *Yang Z, Zhang J, Salem SA et al. Selection of competent blastocysts for transfer by combining time-lapse monitoring and array CGH testing for patients undergoing preimplantation genetic screening: a prospective study with sibling oocytes. BMC Med Genomics. 2014;22;7:38.*
2. *Campbell A, Fishel S, Bowman N et al. Modelling a risk classification of aneuploidy in human embryos using non-invasive morphokinetics. Reprod Biomed Online. 2013;26(5):477-485.*
3. *Campbell A, Fishel S, Bowman N et al. Retrospective analysis of outcomes after IVF using an aneuploidy risk model derived from time-lapse imaging without PGS. Reprod Biomed Online. 2013;27(2):140-146.*
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6. *Zhang J, Tao W, Liu H et al. Morphokinetic parameters from a time-lapse monitoring system cannot accurately predict the ploidy of embryos. J Assist Reprod Genet. 2017;34(9):1173-1178.*
7. *Zaninovic N, Irani M, Meseguer M. Assessment of embryo morphology and developmental dynamics by time-lapse microscopy: is there a relation to implantation and ploidy? Fertil Steril. 2017;108(5):722-729.*

8.Kramer YG, Kofinas JD, Melzer K et al. Assessing morphokinetic parameters via time lapse microscopy (TLM) to predict euploidy: are aneuploidy risk classification models universal? J Assist Reprod Genet. 2014;31(9):1231-1242.

Despite the advancement of technology and different techniques available, researchers have not arrived at any consensus to determine the competency of human embryos derived from IVF or to select the most competent embryos for transfer.

The results of the comparative studies of Time-lapse system vs. PGD/PGS for selection of good quality embryos have shown mixed results.

Few studies have shown that Time-lapse system alone or in combination with PGS vs. PGD/PGS alone is a useful technique to identify and select euploid embryos.

In a prospective study by Yang et al., there was a comparison of the effects of the time-lapse system and the conventional incubator on embryo ploidy and implantation potential in PGS patients using a sibling oocyte model. The researchers retrieved a total of 1163 metaphase II (MII) oocytes from 138 PGS patients and cultured them in the time-lapse system (n = 582; group A) and in the conventional incubator (n = 581; group B).
Euploid blastocysts within the most predictive morphokinetic parameters (Group A) or with the best morphological grade available (Group B) were selected for transfer to individual patients on day 6. Ongoing pregnancy and implantation rates were compared.
According to the results, there was euploidy in 105 (39.6%), aneuploidy in 156 (58.9%) and no results in 4 (1.5%) of the biopsied blastocysts.
Although euploid embryos developing to the blastocyst stage in the time-lapse system group compared to the conventional incubator group showed a non-significant trend, there were more euploid embryos in time-lapse system (46.0% vs. 39.6%, respectively, p >0.05; NS; Figure 1).

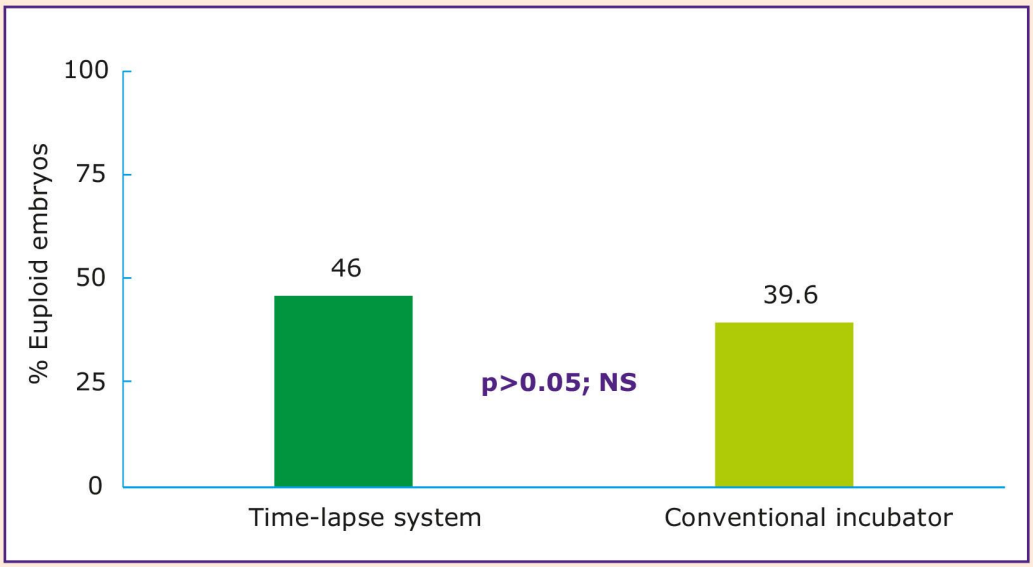

Figure 1. Euploid embryos in Time-lapse system and conventional incubator

There were significant differences observed in clinical pregnancy rates, implantation rate, ongoing pregnancy rate and miscarriage rate between the time-lapse system and the conventional incubator (71.1% vs. 45.9%, respectively, p=0.037), (66.2% vs. 42.4%, respectively, p=0.011), (68.9% vs. 40.5%, respectively, p=0.019), (3.1% vs. 11.8% respectively, p=0.273).

Campbell et al., in a study evaluated whether morphokinetic variables could be used as a potential aid to select euploid embryos for transfer. Time-lapse images from 98 blastocysts were collected and analyzed blinded to ploidy. In this study, the couples selected were recommended PGS due to different factors.
The results showed that time of initiation of blastulation (t_{SB}) was significantly delayed (p=0.004 and 0.006 respectively) more than 6 h for both single aneuploid embryos and multiple aneuploid embryos compared with euploid embryos (Table 1).

Table 1. Timing of divisions for euploid, single aneuploid and multiple aneuploid embryos

	Euploid				Single aneuploid					Multiple aneuploid				
	25th percentile (hpi)	Median (hpi)	75th percentile (hpi)	n	25th percentile (hpi)	Median (hpi)	75th percentile (hpi)	n	p-value	25th percentile (hpi)	Median (hpi)	75th percentile (hpi)	n	p-value
t_{SB}	91.7	95.1	101.5	38	96.4	103.4	110.2	30	0.004*	97.0	101.9	107.3	30	0.006**

Mann–Whitney–Wilcoxon test, p-values of the two types of aneuploidy against the euploid: *p < 0.05; **p < 0.01. hpi = hours post insemination; n = number of embryos.

Table adapted from Reprod Biomed Online. 2013;26(5):477-485.

However, there were no significant differences observed between aneuploid and euploid embryos in the length of the first or second cell cycle, synchrony of the second or third cell cycle, duration of blastulation and multinucleation at the 2-cell stage and irregular division pattern.
This study indicated that time-lapse monitoring of embryo development to blastocyst could be effectively used to avoid high risk aneuploid embryos and preferentially select embryos with a greatly reduced risk of aneuploidy based on morphokinetic timing.
The researchers opined that this non-invasive approach may be offered to patients as an alternative to PGS or, indeed, as a complementary system. It may also be used for patients electing against invasive genetic screening technology or for clinics without the skills or access to PGS.

Campbell et al., in a retrospective cohort **study evaluated the effectiveness and potential impact of time-lapse imaging for unselected IVF patients without biopsy and PGS.**
The outcomes of the study were fetal heart beat (FHB), live birth (LB) or failed implantation in the 88 transferred blastocysts. Aneuploidy risk classification was used (low, medium or high) to classify the risk of aneuploidy for each transferred embryo with a KID (known implantation data) value.
The study results showed a significant difference for FHB (p < 0.0001) and LB (p < 0.01) rates among the low and medium risk embryos.

The incidence of positive FHB in the low-risk class was 72.7% and the incidence of positive LB in the low-risk class was 61.1%, which was correspondingly 74% and 56% relative increase compared to overall rate across all class of embryos (42% and 39.1% respectively; Table 2).

Table 2. Known implantation data rates for fetal heart beat and live birth for each aneuploidy risk class

Risk class	FHB KID		LB KID	
	No. of embryos	FHB KID rate	No. of embryos	LB KID rate
Low	33	72.7[a]	18	61.1[b]
Medium	51	25.5[a]	26	19.2[b]

LB KID data were calculated only from treatments where the information could have been obtained (over 10 months from time of embryo transfer).
FHB = fetal heart beat; KID = known implantation data; LB = live birth.
[a]$p < 0.0001$.
[b]$p < 0.01$.

Table adapted from Reprod Biomed Online. 2013;27(2):140-146.

This study demonstrated for the first time that time-lapse imaging can be used to classify human preimplantation embryos according to their risk of aneuploidy without biopsy and PGS correlating well to a clinical outcome.

In a unicentric retrospective study, Rocafort et al., investigated if automated TLI selection could diagnose euploidy and/or other parameters of embryo quality in order to identify the most competent embryo.
About 244 patients undergoing PGS cycles with autologous oocytes or oocyte donation (OD) with single euploid embryo transferred were evaluated. PGS+TLI combination significantly delivered better implantation, clinical and ongoing pregnancy rates compared with the PGS-only group **when euploid embryos with high implantation potential as predicted by TLI were transferred.** This improvement was observed when only transfers of good morphological quality embryos were compared.

On the contrary, several studies have also discussed whether time-lapse parameters can predict ploidy.

Reigner et al., in a systematic review that included 13 studies with heterogeneous design, patients, day of embryo biopsy, statistical approach and outcome measures demonstrated that no single or combined morphokinetic parameter was consistently identified as predictive of embryo ploidy status. The researchers opined that morphokinetic parameters should not be used as a surrogate for PGS to determine embryo ploidy *in vitro.*

Zhang et al., analyzed 256 blastocysts from 75 patients with TLI+PGS technique and showed that morphokinetic parameters failed to improve the chance of selecting euploid embryos.

Zaninovic et al., opined that despite morphokinetic parameters of Time-lapse system are a useful

aid in differentiating between euploid and aneuploid embryos, they are not sufficiently accurate to replace preimplantation genetic testing for aneuploidy.

Further Kramer et al., also showed that the use of non-invasive morphokinetics is unlikely to discriminate aneuploid from the euploid embryos.

Despite these mixed opinions, researchers state that morphokinetics may be useful as an adjunct to PGS in selecting those PGS-screened euploid embryos with the best chances of implantation and live birth.
The use of time-lapse system to screen out embryos with the highest risk of aneuploidy could result in a paradigm shift in the IVF clinical practice and improve the incidence of live birth for most patients.

Time-lapse vs. conventional incubator - what are the scientific evidences?

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3. *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.*
4. *Kahraman S, Çetinkaya M, Pirkevi C et al. Comparison of Blastocyst Development and Cycle Outcome in Patients with eSET Using Either Conventional or Time Lapse Incubators. A Prospective Study of Good Prognosis Patients. Journal of Reproductive and Stem Cell Biotechnology. 2012;3(2):55-61.*
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8. *Wu L, Han W, Wang J etal. Embryo culture using a time-lapse monitoring system improves live birth rates compared with a conventional culture system: a prospective cohort study. Hum Fertil (Camb). 2018;21(4):255-262.*
9. *Mascarenhas M, Fox SJ, Thompson K et al. Cumulative live birth rates and perinatal outcomes with the use of time-lapse imaging incubators for embryo culture: a retrospective cohort study of 1882 ART cycles. BJOG 2019;126:280–286.*
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12. *Pribenszky C, Nilselid AM, Montag M. Time-lapse culture with morphokinetic embryo selection improves pregnancy and live birth chances and reduces early pregnancy loss: a meta-analysis. Reprod Biomed Online. 2017;35(5):511-520.*
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Traditionally morphological evaluation of the embryo is the most accepted method to assess and select embryos as embryo quality is the most predominant factor for the success of IVF.
However, the limitations of the static morphological evaluation of embryos like embryo exposure to suboptimal culture conditions, limited predictive value for ploidy status etc. makes the

technology to take a relook into the more advanced options for the embryo selection.
The advent of the Time-lapse technology with continuous monitoring of the embryo development and precise timing of the different kinetic events portrays it as a new option in the routine embryology laboratory.

Several clinical studies are available that effectively portray the advantages of the use of Time-lapse system over conventional incubator methods for embryo selection, pregnancy rates and improved perinatal outcomes.
While some studies have shown that there is no difference between the use of the Time-lapse system and conventional incubator methods, some studies have clearly demonstrated the advantages of the Time-lapse system compared to the use of conventional incubators.

In a prospective cohort study, Cruz et al., evaluated whether incubation conditions with a time-lapse incubator (TLI) was comparable to standard laboratory incubation (SI) in 478 embryos from 60 couples by comparing embryo quality, development and ongoing pregnancy rates.
The study results showed that time-lapse monitoring (TLM) did not affect the embryo quality grading, blastocyst development or viability compared to standard incubator. TLI was useful for monitoring the timing of early cleavages (Table 1).

Table 1. Blastocyst rate, proportion of frozen and transferred embryos incubated in the TLI vs. the standard incubator

	Blastocyst	Frozen	Transferred
TLI (n=238)	54.8 (n=130)	7.6 (n=18)	21.0 (n=50)
Standard incubator (n=240)	50.6 (n=121)	10.9 (n=26)	24.1 (n=58)
p	ns	ns	ns

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

A randomized study by Kahraman et al., evaluated the embryo development until the blastocyst stage in either conventional (CI) or time-lapse incubators in good prognosis patients. About 64 patients (n=33 TLI and n=31 for CI) were included in the study and the primary outcome was the proportion of good and top quality blastocysts on day 5.
The study results showed that blastocyst development rate was 66.6% for TLI and 64.3% for CI, which was not statistically significant (p=0.86). The good and top quality blastocysts (GQB) were similar for both groups, although a 3% increase of GQB in TLI group (32.97 vs. 29.96, p=0.43).
A comparable rate of implantation and pregnancy were achieved in both types of incubators.

In a two center, randomized, controlled clinical trial, 676 oocytes from 59 patients in their second or third treatment cycle were cultured in the TLI or in a CI. The primary endpoint was proportion of 4-cell embryos on day 2 (Table 2).
According to the study results, there was no significant difference between the TLI and CI in the primary outcome. There were also no differences in clinical pregnancy rate or implantation rate.

Table 2. Primary endpoint data

	ITT/PP	TLI	CI	$RR_{TLI/CI}$	$RD_{TLI/CI}$	*p*-value*
Number of randomized oocytes	ITT	n=338	n=338	–	–	1.0
	PP	n=297	n=303	–	–	0.73
Number of four-cells day 2	ITT	n=92	n=113	0.81 (0.65;1.02)	−6.2 % (−13.1;0.7)	0.08
	PP	n=84	n=107	0.80 (0.63;1.01)	−7.0 % (−14.4;4.0)	0.07

RR Risk Ratio; RD Risk Difference
TLI Time-lapse incubator; CI Conventional incubator
ITT Assessed by intention-to-treat; PP Assessed per protocol
*Time-lapse incubator compared with conventional incubator

Table adapted from J Assist Reprod Genet. 2012;29(6):565–572.

The researchers showed that culture in the TLI supported embryonic development equally to a CI but TLI could be used as a potential tool for more refined and flexible embryo evaluation.

Comparing the TLI environment to the SI environment, Sciorio and colleagues demonstrated that TLI supports embryo development equally to a benchtop incubator. They confirmed the safety of the TLI as it provides a suitable culture environment that does not affect embryo quality at cleavage stage and blastocyst development.
About 581 sibling human zygotes from 47 patients were compared after culture in either TLI or SI. The primary endpoints were proportion of 4 cell embryos on day 2, proportion of top-quality embryos on day 3 (6-10 cells, less than 10% fragmentation), and proportion of blastocysts on day 5/6.
A statistically significant higher proportion of good quality embryos was seen in the TLI group compared to the SI on day 2 (66.8 vs. 50.5%, p=0.014) and on day 3 (75.1 vs. 56.0%, p=0.006; Figure 1).

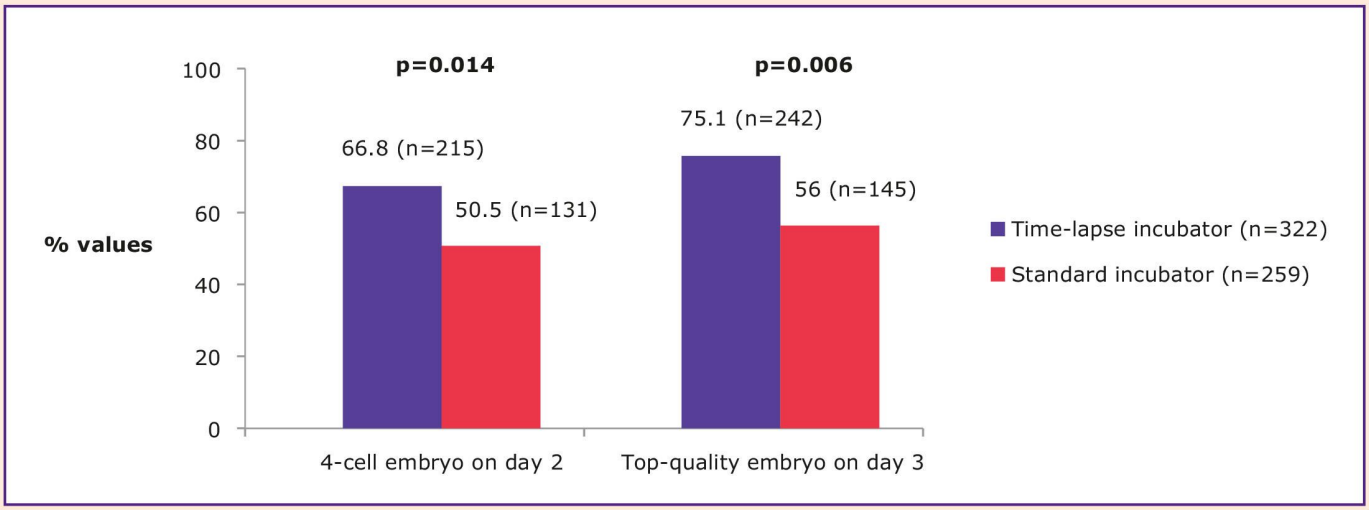

Figure 1. Embryo development in TLI environment vs. SI environment

In the TLI, a non-significant higher proportion of embryos developed to good quality blastocysts compared to the benchtop (49.4 vs. 42.0%, p = 0.24, NS).
There were no significant differences in the proportion of blastocysts on day 5/6 in the two incubators.

Mesegeur et al., in a largest retrospective, observational cohort study compared the reproductive outcome of culturing and selecting embryos using a novel TLI system with a standard incubator (SI).
Donation and autologous ICSI cycles cultured in TLI (n=1,390) or in SI (n=5,915) were evaluated. **Clinical pregnancy was the main outcome measure.**
The results, presented as a logistical regression analysis showed a substantially relative clinical pregnancy improvement of +20.1% compared to SI (SI-44.9% and TLI-53.9%; Figure 2) per oocyte retrieval and +15.7% per embryo transfer for treatments using TLI over those using SI.
The increased clinical pregnancy rates were attributed to controlled and stable incubation conditions, minimal handling of embryos and the use of quantitative morphokinetic parameters for selecting viable embryos.

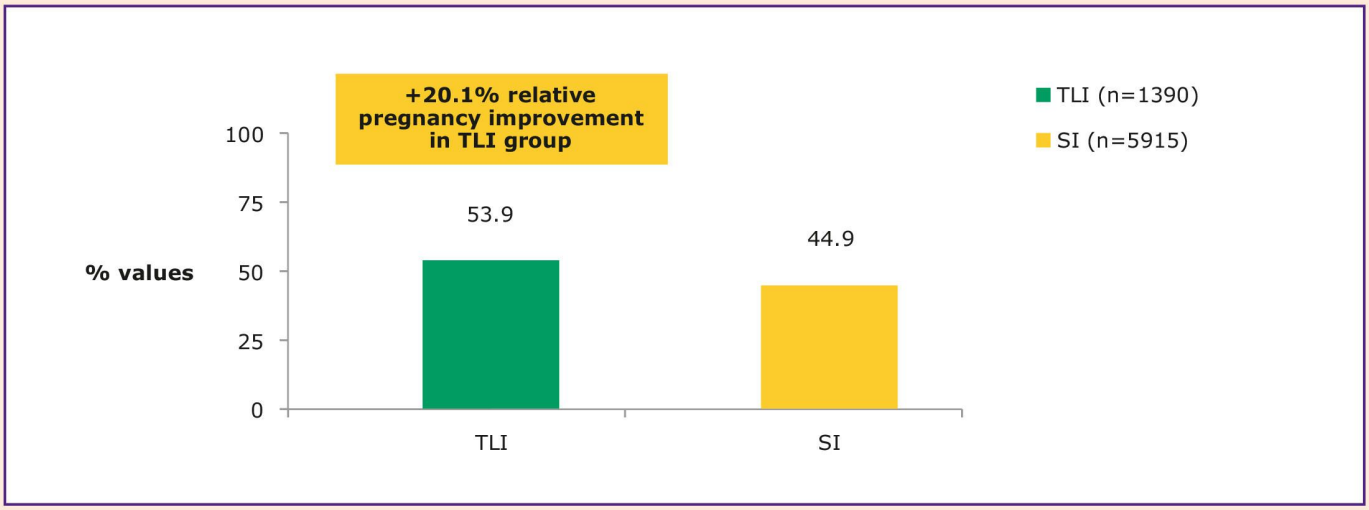

Figure 2. Improved clinical pregnancy in TLI system vs SI

In a prospective, cohort study of 608 patients undergoing IVF by Wu et al., the effects of a TLI (n=304) on embryo quality and clinical pregnancy outcomes were compared to that of an SI (n=304).
The TLI group showed a significantly higher transferable embryo ratio at Day 3, a higher number of transferable embryos and number of good-quality embryos cryopreserved at Day 3. While there was no significant difference in the implantation and clinical pregnancy rates, the TLI group had a higher ongoing pregnancy rate and live birth rate compared with the SI group (Table 3).
According to the researchers, a stable environment of the TLI might have been the reason for the beneficial effects.
The observed beneficial effects could be the result of a more stable environment provided by the TLM system.

In one of the largest studies comparing cumulative live birth rates and perinatal outcome data

Table 3. Comparative evaluation of outcomes with TLI and SI			
Outcomes	TLI group (n=304)	SI group (n=304)	p value
Transferable embryo ratio at Day 3	61.65%	52.87%	< 0.0010
Number of transferable embryos	4.71 ± 2.38	4.09 ± 2.35	0.0053
Good-quality embryos cryopreserved at Day 3	2.72 ± 2.35	2.11 ± 2.33	0.0056
Ongoing pregnancy rate	67.32%	57.22%	0.0410
Live birth rate	65.37%	55%	0.0380

between TLI and SC incubators, Mascarenhas et al., demonstrated improved perinatal outcomes after fresh and frozen embryo transfer.

The retrospective cohort study compared 1,064 IVF cycles using TLI (TLI cycles) and 818 IVF cycles using SC (Standard Culture cycles). Cumulative live birth rate per oocyte retrieval and perinatal outcomes were the main outcome measures.

TLI cycles resulted in higher live birth rate from fresh embryo transfer. After both fresh and frozen embryo transfers, the mean birthweight was higher in the TLI group.

A lower risk of early preterm birth (PTB) and very low birthweight (LBW) in the TLI group was seen after a fresh embryo transfer and the frozen embryo transfers resulted in lower risk of early PTB and LBW in the TLI group (Tables 4 and 5).

Table 4. Comparison of perinatal outcomes after fresh embryo transfer between TLI and SC cycles			
	TLI	SC	Odds ratio/mean difference (95% CI)
Early PTB, n (%)	2 (0.6)	11 (4.4)	0.11 (0.02–0.51)*
Mean birthweight in g, mean (SD)	3311.71 (603.15)	3153.05 (717.48)	174.78 (64.80–284.77)*
Very LBW, n (%)	2 (0.6)	11 (4.4)	0.11 (0.02–0.52)*

*Adjusted odds ratio.
**Adjusted mean difference (adjusted for age, number of oocytes retrieved and number and stage of embryos transferred)

Table 5. Comparison of perinatal outcomes after frozen embryo transfer between TLI and SC cycles			
	TLI	SC	Odds ratio/mean difference (95% CI)
Early PTB, n (%)	0	4 (3.0)	0.46 (0.40–0.52)
Mean birthweight in g, mean (SD)	3341.23 (563.49)	3155.74 (749.93)	175.91 (16.98–334.84)**
LBW, n (%)	11 (7.3)	21 (15.9)	0.39 (0.18–0.85)*

*Adjusted odds ratio.
**Adjusted mean difference (adjusted for age, number of oocytes retrieved and number and stage of embryos transferred)

Tables 4 and 5 adapted from BJOG 2019;126:280–286.

In a prospective, randomized, double-blinded, controlled study, Rubio et al., clinically validated the embryo culture and selection in a TLI system and compared the same with a SI system based exclusively on morphology.
The main outcome measures were rates of embryo implantation, pregnancy, ongoing pregnancy (OPR), and early pregnancy loss.
The ongoing pregnancy rate statistically significantly increased in the TLI group compared with the SI group. A statistically significantly decreased early pregnancy loss and increased implantation rate was seen in the TLI group versus SI group respectively (Table 6).

Table 6. Outcome results per intention to treat, per cycle, per transfers and per embryo transferred

Outcome	TMS group	Control group	RR	*P* value
Ongoing pregnancy (% of all treated cycles)	51.4 (46.7–56.0)	41.7 (37.0–46.6)	1.23 (1.06–1.43)	0.005
Ongoing pregnancy (% of all transfers)	54.5 (49.6–59.2)	45.3 (40.3–50.4)	1.20 (1.04–1.39)	0.01
All pregnant cycles	271	228		
Early pregnancy loss (% of all pregnancies)	16.6 (12.6–21.4)	25.8 (20.6–31.9)	0.64 (0.45–0.91)	0.01
All transferred embryos	775	699		
Implantation rate (% of all transferred embryos)	44.9 (41.4–48.4)	37.1 (33.6–40.7)	1.43 (1.05–1.39)	0.02

Note: Results shown as proportion with 95% confidence limits in brackets, relative risk (RR) with 95% confidence limits in brackets and the corresponding p value (Fisher's exact test).
Total number of cycles are also presented in brackets.

Table adapted from Fertil Steril. 2014;102(5):1287-1294.

In a recently published one-year pseudo-randomized prospective study, Kalleas and colleagues compared the clinical outcomes from large cohort of medium-high prognosis patient cycles using undisturbed embryo culture at low oxygen in TLI (n = 243) to those with a high quality bench top incubator, CI (n = 203).
There was higher overall live birth rate in the TLI group compared to the CI (43.2% vs. 34.5%; OR = 1.43) with significantly reduced early pregnancy loss (5.8% vs. 13.8%; OR = 0.37; Figure 3) respectively.
There was a higher proportion of 4-cell embryos on day 2 and 8-cell embryos on day 3 in the TLI, compared to the CI.
A TL system with undisturbed embryo culture and low oxygen delivered higher live birth rate and was an appropriate use in ART.

A meta-analysis of five studies with 1637 patients by Prizbensky et al., evaluated whether time-lapse monitoring (TLM) intervention could improve overall clinical outcome (pregnancy, early pregnancy loss, stillbirth and live birth rate) compared with embryo selection based on single time-point morphology in IVF cycles.
The results showed that TLM resulted in a significantly higher ongoing clinical pregnancy rate (51.0% versus 39.9%, p<0.001), significantly lower early pregnancy loss (15.3% versus 21.3%; OR: 0.662; p=0.019) and a significantly increased live birth rate (44.2% versus 31.3%; OR 1.668; p=0.009; Figure 4).

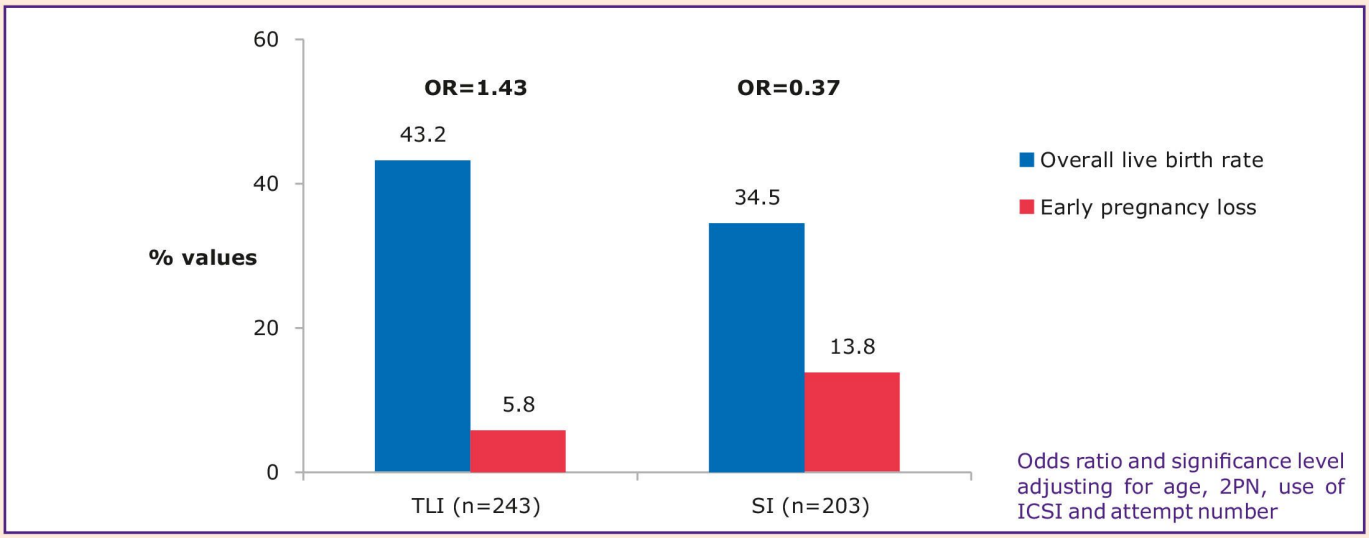

Figure 3. Live birth rate and pregnancy loss in undisturbed embryo culture at low oxygen in a time-lapse incubator compared to a high-quality benchtop incubator

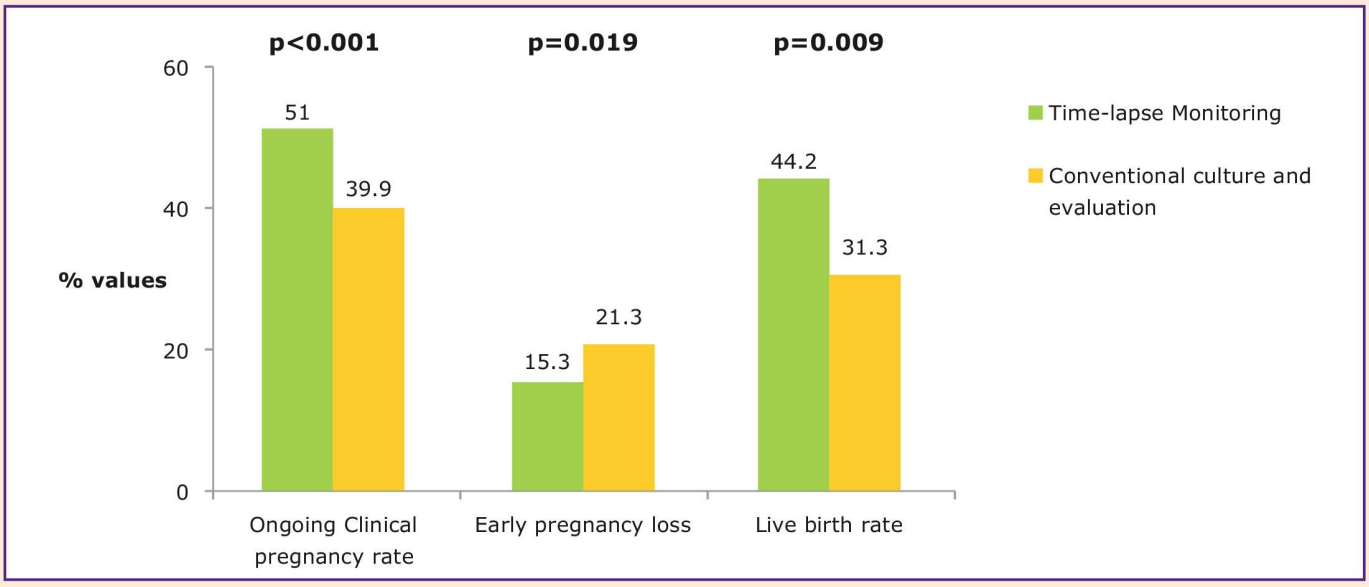

Figure 4. Analysis of the Time-lapse monitoring effect on clinical outcome

The meta-analysis supported the growing evidence for the clinical benefit of using imaging systems in human IVF. The researchers also warranted high quality evidence owing to inconsistencies across the studies.

Despite these positive evidences, a meta-analysis and systematic review of ten randomized controlled trials by Chen et al., showed that clinical TLI may have the potential to improve IVF outcomes but the evidence was insufficient to support regular TLI use when considering ongoing pregnancy rates and blastocyst formation rates.

videos – what do they say?

video 1: asymmetric embryo

1.Levin M. Left-right asymmetry in embryonic development: a comprehensive review. Mech Dev. 2005 Jan;122(1):3-25.
2.Toledo-Jacobo L, Henson JH, Shuster CB. Cytoskeletal polarization and cytokinetic signaling drives polar lobe formation in spiralian embryos. Dev Biol. 2019;456(2):201-211.
3.Feil R. Epigenetic asymmetry in the zygote and mammalian development. Int J Dev Biol. 2009;53(2-3):191-201.

Symmetry is a prominent aspect of developmental morphology during embryogenesis.
Asymmetric cell division is a hallmark of many embryonic and stem cell divisions. During early embryogenesis, asymmetric cell fates depend on the differential segregation of developmental determinants amongst the daughter blastomeres. Embryos may cleave asymmetrically by positioning the mitotic spindle towards one pole of the cell, or by forming asters of unequal sizes.
In mammals, there is a requirement of both a maternal and a paternal genome for embryonic and fetal development. Perturbation of the differential organization of the maternally and paternally derived genomes, before fertilization, or in the early embryo, can give rise to aberrant growth and developmental disorders in humans.

video 2: fragmented embryo

1.Kim, Seok-Gi et al. "Early fragment removal on in vitro fertilization day 2 significantly improves the subsequent development and clinical outcomes of fragmented human embryos." Clinical and experimental reproductive medicine vol. 45,3 (2018): 122-128.

Fragmentation of embryos is often observed during embryo culture in IVF-embryo transfer (ET) cycles. Factors responsible for Embryo fragmentation include inadequate culture conditions, poor quality of the ovum and spermatozoon, increased maternal age, chromosomal abnormalities, abnormal cell cycle, apoptosis, and oxidative stress in embryos.
The presence of fragmentation limits the subsequent development of embryos due to the loss of cytoplasmic mitochondria, mRNA, and regulatory proteins, which are essential for cell division, as well as physical interruption of the gap junctions in blastomeres, which interferes with the cell-cell interactions required for cleavage and compaction.

video 3: Multiple pronuclei

1.Li M, Zhao W, Xue X et al. Three pro-nuclei (3PN) incidence factors and clinical outcomes: a retrospective study from the fresh embryo transfer of in vitro fertilization with donor sperm (IVF-D). Int J Clin Exp Med. 2015 Aug 15;8(8):13997-4003.

Three pro-nuclei (3PN) prevalence among all pregnancies has been estimated to be approximately 1% to 3%.
Two circumstances can lead to 3PN formation: the combination of one maternal and two paternal sets or the combination of one paternal and two maternal sets. 3PN results either from polyspermic fertilization or from oocyte-derived meiotic failure.
The reasons for causing 3PN fertilization are complicated. Some investigators have suggested that the incidence of 3PN fertilization after IVF is a result of advanced maternal age or severe sperm abnormalities and other investigators have suggested that the propensity toward 3PN is a function of ovarian stimulation, as indicated by high peak E2 levels, large oocyte yields, high gonadotropin doses, and lengthy stimulations.

video 4: single pronuclei

1.Hondo S, Arichi A, Muramatsu H et al. Clinical outcomes of transfer of frozen and thawed single blastocysts derived from nonpronuclear and monopronuclear zygotes. Reprod Med Biol. 2019;18(3):278-283.
2.Gras L, Trounson AO. Pregnancy and birth resulting from transfer of a blastocyst observed to have one pronucleus at the time of examination for fertilization: Case report, Human Reproduction.1999;14(7):1869–1871.

In ART, fertilization is confirmed by regularly observing the presence of zygotes with two pronuclei (2PN), 18-18 hours after insemination. However, sometimes, zygotes with one pronucleus (1PN) is found, with reported frequency of 1.6%-7.7%. Such embryos are considered to be unfertilized or abnormally fertilized ova, and therefore, they are not suitable for clinical use and are discarded. The absence of a second PN may be explained as oocyte parthenogenetic activation, irregular pronuclear formation resulting from asynchrony of pronuclear appearance, or possibly male and female pronuclear fusion.

video 5: unfertilized oocyte

1.Liu Y, Han XJ, Liu MH et al. Three-day-old human unfertilized oocytes after in vitro fertilization/intracytoplasmic sperm injection can be activated by calcium ionophore a23187 or strontium chloride and develop to blastocysts. Cell Reprogram. 2014 Aug;16(4):276-80.
2.Kuczyński W. Dhont M, Grygoruk C et al. Rescue ICSI of unfertilized oocytes after IVF. Human Reproduction 2002;17(9):2423–2427.

During IVF procedures, unfertilized human oocytes are routinely discarded.
Various reasons for failed fertilization after IVF include sperm defects, disturbances in sperm-oocyte interaction and oocyte abnormality.
However, these unfertilized oocytes may provide rich sources of oocytes for parthenogenesis as well as for embryonic stem cell research.

The videos shared are a property of Dr G.A. Rama Raju and Krishna IVF. We also sincerely thank the contribution and efforts put by Dr. Siva Narayana for preparing and editing these videos and enabling their digital incorporation in the issue.

Link to open the videos:

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4) Click on the link to open the videos https://hcp.merckgroup.com/in-en/fertility/resources/alive-7-videos.html

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Future of Time-lapse Imaging technology

1. *Bhide P, Srikantharajah A, Lanz D et al. TILT: Time-Lapse Imaging Trial-a pragmatic, multi-centre, three-arm randomised controlled trial to assess the clinical effectiveness and safety of time-lapse imaging in in vitro fertilisation treatment. Trials. 2020;21(1):600.*
2. *ESHRE Working group on Time-lapse technology, Apter S, Ebner T, Freour T et al. Good practice recommendations for the use of time-lapse technology. Hum Reprod Open. 2020 Mar 19;2020(2):hoaa008. doi: 10.1093/hropen/hoaa008.*
3. *Lundin K and Park H. Time-lapse technology for embryo culture and selection. Ups J Med Sci. 2020;125(2): 77-84. doi: 10.1080/03009734.2020.1728444.*
4. *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 Apr 4;2:21.*
5. *Aparicio-Ruiz B, Romany L, Meseguer M. Selection of preimplantation embryos using time-lapse microscopy in in vitro fertilization: State of the technology and future directions. Birth Defects Res. 2018 May 1;110(8):648-653.*

As on date, due to the absence of high-quality evidence there is no current national guidance, recommendation or policy for the use of TLI technology. Moreover, the use of TLI is not consistently incorporated into standard IVF care.

It is only in the last decade that TLI technology has been introduced into human IVF as a routine procedure much later than in other fields of biosciences, and yet it has led to major changes in the way that embryos are observed and handled.

In addition, the construction of powerful algorithms for widespread use is hindered by large variations in culture conditions between individual IVF laboratories despite large data availability. Despite all these odds and also being expensive, TLI technology appears to be a very useable tool for the laboratory, with safe and stable culture conditions.

Artificial intelligence (AI), or machine learning, describes a nonbiased approach to multiparameter analysis. In the context of TLT, attempts are underway to use higher-powered computer-processing power to analyze large data sets of images to identify combinations of parameters that might link to embryo viability.

Khosravi et al., used AI approach based on deep neural networks (DNNs) and TLT to analyze more than 10,000 embryos and developed a model that was able to predict blastocyst quality with an AUC of >0.98. In this study, the chance of pregnancy based on individual embryos varied from 13.8% (age≥41 and poor-quality) to 66.3% (age<37 and good-quality) depending on automated blastocyst quality assessment and patient age. The AI-driven approach uncovered new, potentially personalized strategies to select embryos.

Also, TLI could be used in conjunction with PGS to choose which blastocysts to analyze within a cohort or to improve selection when more than one euploid blastocyst is available.

Consequently, every effort should be made to make the patients aware of the benefits and limits of TLT although it is difficult. Days are not far when TLI may evolve into a full-blown embryo selection modality, once combined with AI and non-invasive analytical approaches.
Albeit the prediction of future achievements of TLT is a difficult exercise, there is little doubt that this technology is here to stay. Mastering its use is therefore becoming imperative for embryologists and IVF laboratories.

feedback form

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Issue 7 | April 2021

Thank you for going through the contents of **Alive Newsletter Issue 7.** To ensure that future issues will be of interest to you, we would greatly appreciate your feedback on the format and content of this issue.

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Satisfaction Score for ALIVE Newsletter Time-lapse Imaging in ART Part 2: Issue 7; April 2021

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 7 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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Godrej One, 8th Floor, Pirojshanagar Nagar, Eastern Express Highway,
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