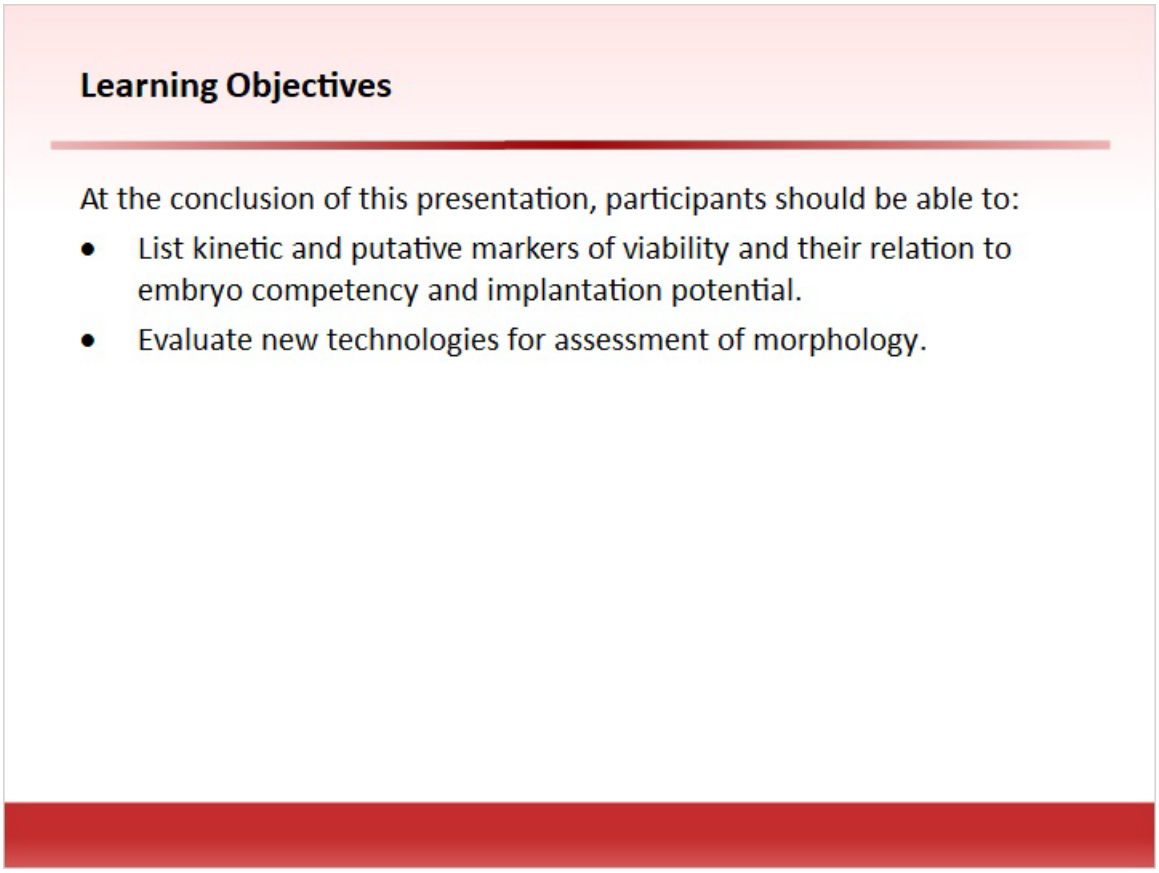
LABCC100 Lesson 27

1.1 Time-Lapse Imaging Morphometry (TLIM)

Notes:

Welcome to the American Society for Reproductive Medicine's eLearning modules. The subject of this presentation is Time-lapse Imaging Morphometry (TLIM).

1.2 Learning Objectives



Notes:

At the conclusion of this presentation, participants should be able to:
Summarize kinetic and putative markers of viability and their relation to embryo competency and implantation potential.
Evaluate new technologies for the assessment of embryo morphology.

1.3 Introduction to Embryo Culture

Introduction to Embryo Culture

- Embryo morphology and correlates with viability and implantation.
- Embryo selection to transfer or cryopreserve based primarily on developmental stage and morphological assessment.
- Success of embryo development, implantation, and healthy pregnancy relies on optimal culture conditions.
- Embryos are routinely cultured in conventional front-load incubators or top-load mini-incubators.
- Traditionally, embryo morphology evaluation occurs via daily sequential assessment and scoring of an embryo.

Notes:

Within the field of embryology, there is a correlation between embryo morphology and viability and implantation. Embryo morphology has been studied extensively, and it is customary to assess embryo development sequentially.
The decision as to what embryo to transfer or cryopreserve is based primarily on developmental stage and morphological assessment of the embryo, taking into account the number of blastomeres, absence or presence of cytoplasmic fragmentation, expansion of blastocyst cavity, and the number of cells that comprise the trophectoderm and inner cell mass.
The success of embryo development, implantation, and a healthy pregnancy relies heavily on optimal culture conditions and a controlled culture environment.
The standard practice for routine embryo culture has been to grow embryos in conventional front-load incubators or top-load mini-incubators. These incubators provide a suitable environment for embryo growth.
However, culture within these incubators requires that embryo morphology evaluation occurs by daily assessment of embryo growth along with embryo grading.

1.4 Control of Embryo Microenvironment

Control of Embryo Microenvironment

- Conventional incubators provide a stable environment under constant operation.
- Microenvironment is compromised by door openings.
- Recovery of microenvironment (temperature and gas concentrations) may take minutes to hours and varies among incubator types.

Comparison of microenvironment recovery

	Top-Load Mini-incubator	Front-Load Conventional Incubator
Temperature	4.9 ± 0.5 min*	31.5 ± 2.9 min
O_2	3.0 ± 0 min*	7.8 ± 0.9min

Note: time lapse for recovery after opening the door for 5 sec.
*P<.01 (mini incubator vs. conventional incubator)

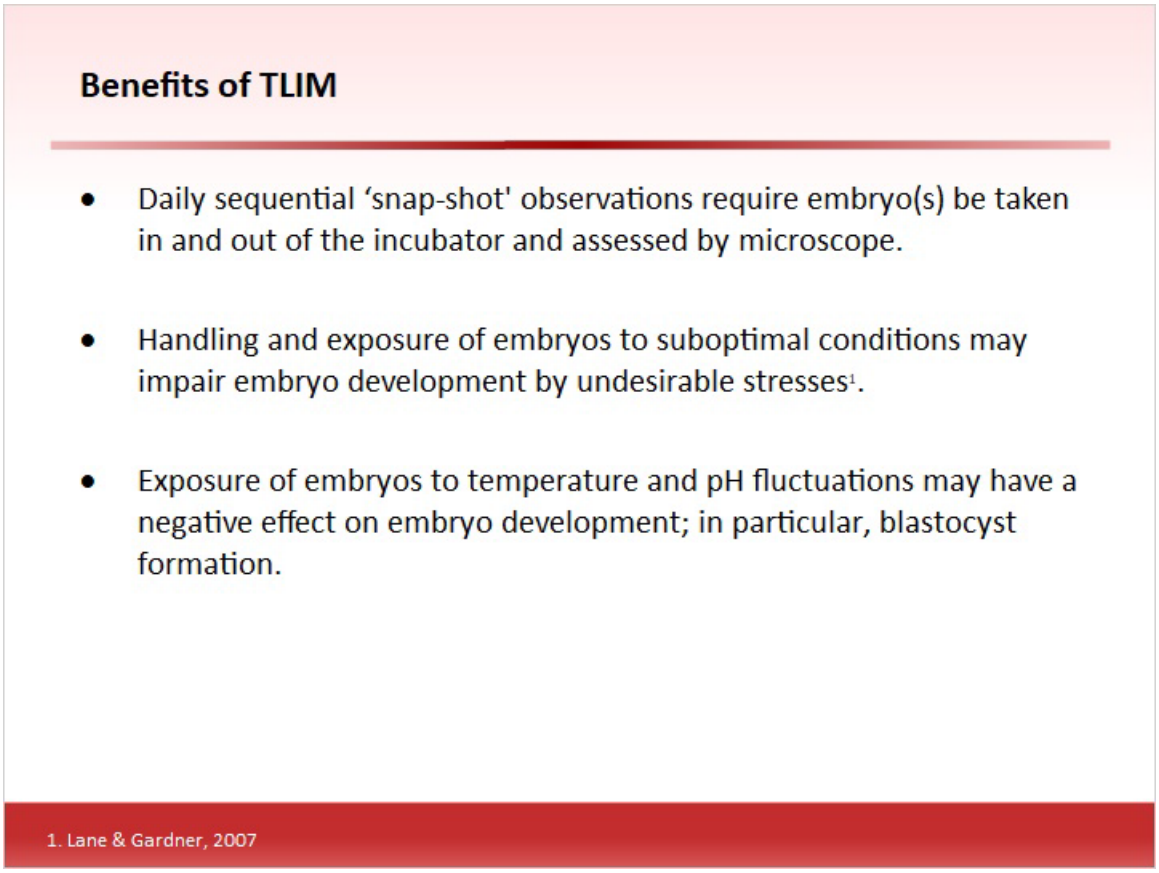
Adapted from Fujiwara et al., 2007; Swain, 2014

Notes:

Although conventional incubators provide a stable environment under constant operation, the microenvironment is disturbed by opening the incubator door. The recovery of the microenvironment (that includes temperature and gas concentrations) to achieve optimal growth conditions may take minutes to hours to stabilize. The recovery time of such conditions varies among incubator types.

One study that compared microenvironment recovery in top-load mini-incubators with conventional front-load incubators, shows that a top-load mini-incubator has a significantly faster recovery time of temperature and oxygen concentration compared with front-load conventional incubators. This comparison was done following only a door opening that lasted 5 seconds.

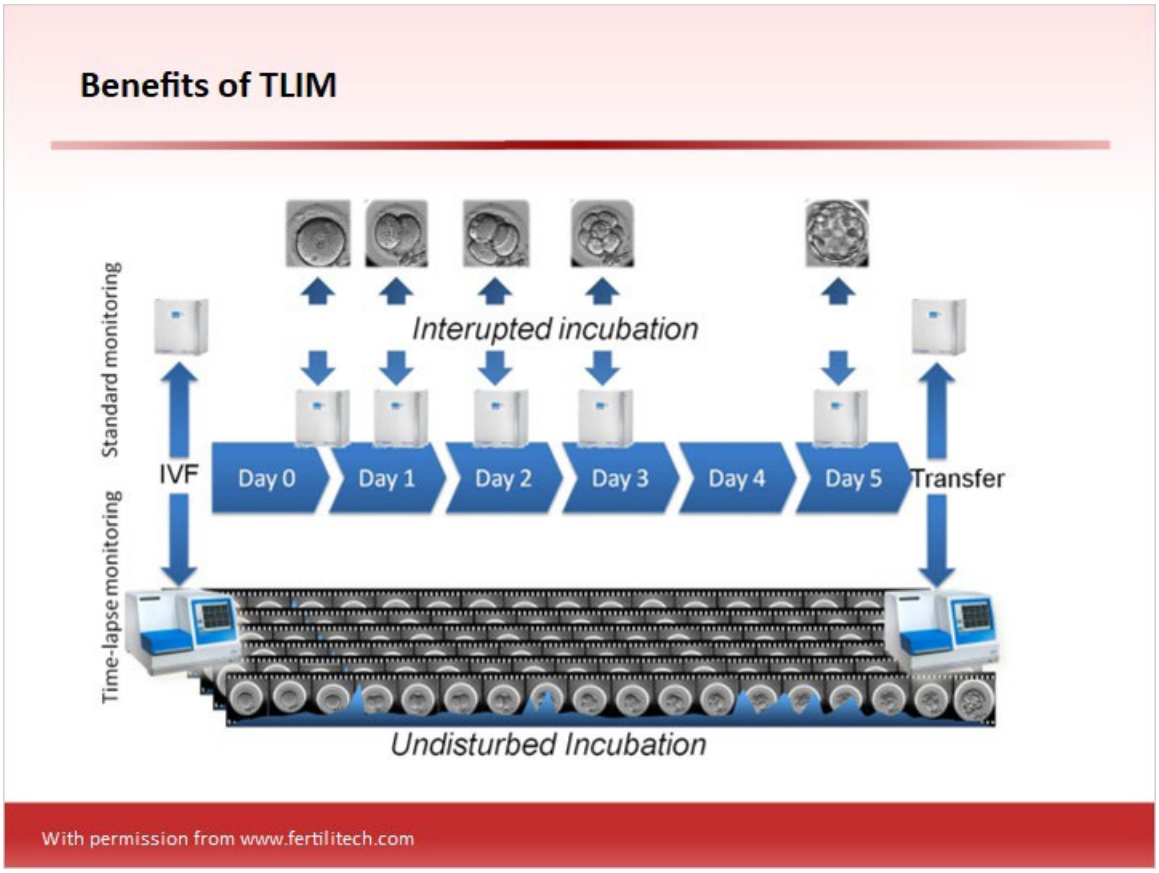
1.5 Benefits of TLIM



Notes:

In order to assess embryo development, 'snap-shot' observations require embryos be removed from the controlled microenvironment of the incubator and assessed by microscope.
Each observation, usually daily or multiple times during a day, involves the handling and exposure of embryos to suboptimal conditions that may impair embryo development by undesirable stresses.
These snap-shot observations expose embryos to temperature and pH fluctuations that may have a negative effect on embryo development; in particular, blastocyst formation.

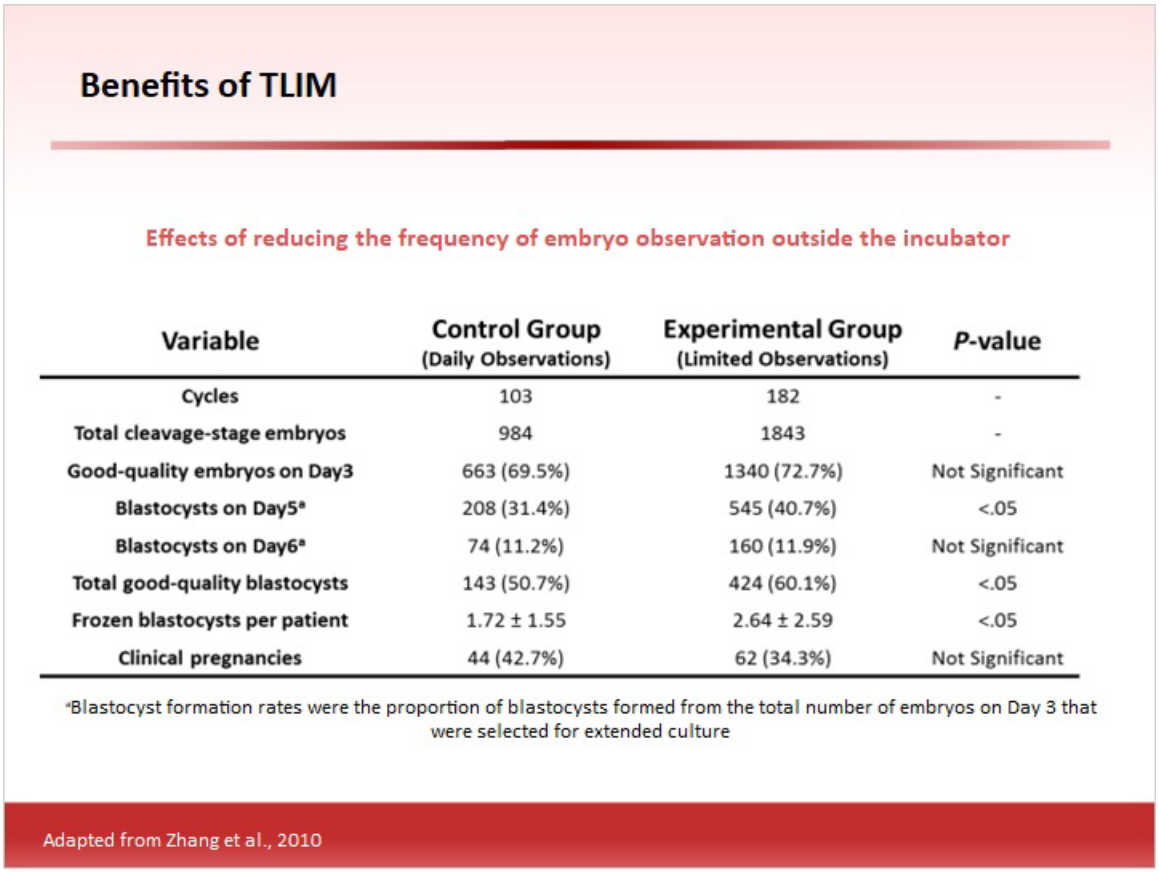
1.6 Benefits of TLIM



Notes:

This diagram represents the interrupted incubation that occurs during standard monitoring and daily assessment of embryos that are removed from the incubator and assessed with a microscope. Time-lapse technology provides the added benefit to allow for undisturbed incubation by using time-lapse monitoring. This type of monitoring significantly reduces any potentially harmful disruption to the incubation microenvironment. Although embryos do not have to be removed for daily morphological assessments, the time-lapse system will only be a completely undisturbed culture system if a single-step culture medium is used. Otherwise, the time-lapse monitoring will need to be paused, and the embryos removed, to allow for media refreshing or exchange as commonly performed in a sequential media culture protocol.

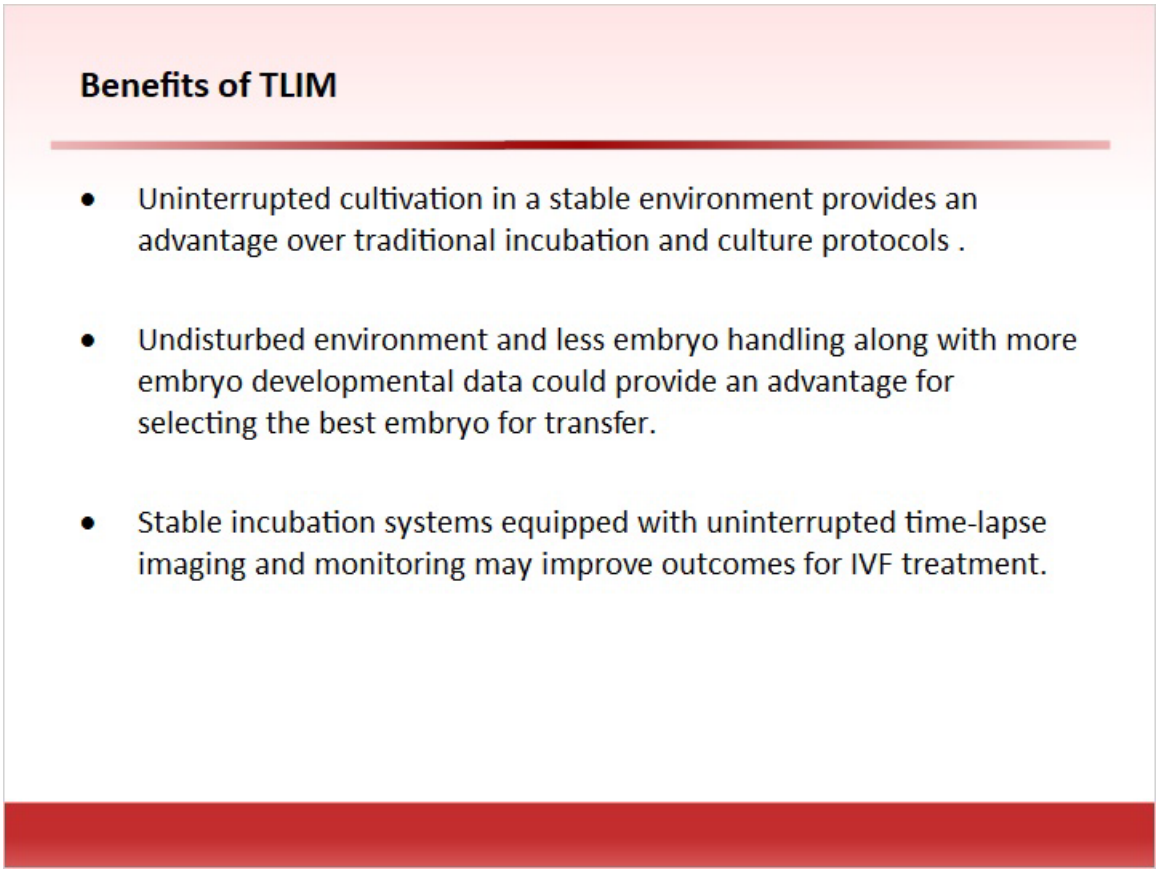
1.7 Benefits of TLIM



Notes:

A recent study described the effects of reducing the frequency of embryo observations outside the incubator. The control group consisted of IVF cycles where embryos were observed once every day for 6 days after insemination, or 6 times in total. The experimental group consisted of embryos that were removed from the incubator on Day 1, 3, 5, and 6 of embryo culture, or 4 times in total. Results showed a significant increase in blastocyst development on day 5 along with a significant increase in good quality blastocysts and blastocysts available for freezing. Therefore, by simply reducing embryo observation from 6 times to 4 times, embryo blastocyst development was significantly better. It is also important to note that incubator management, or the patient-to-incubator ratio, should be taken into consideration. If only one patient's embryo cohort are to be cultured per incubator, the frequency and negative effects of breaching the incubator environment will be greatly reduced than if multiple patients' embryos were cultured in the same incubator.

1.8 Benefits of TLIM



Notes:

TLIM provides an advantage over traditional incubation and culture protocols because of uninterrupted cultivation in a stable environment that does not need to be compromised for daily embryo assessments. Also, the undisturbed environment combined with less embryo handling and more embryo developmental data could prove to be crucial to the success of selecting the best embryo for transfer. By minimizing disturbances, stable incubation systems equipped with uninterrupted time-lapse imaging and monitoring may improve outcomes for IVF treatment.

1.9 Benefits of TLIM

Benefits of TLIM

- Culturing embryos with TLIM improves the relative probability of clinical pregnancy when compared with standard incubators.

			Logistic regression model			
Factor	**Comparison**	**Crude estimate**	**Estimate**	**Lower CL**	**Upper CL**	***P*value**
Odds ratio for clinical pregnancy based on 7,305 cycles with						
Incubation	TMS vs. SI	1.190	**1.201 20.1%**	1.059	1.363	.0043
Day of transfer	Day 5 vs. day 3	1.272	1.169	1.039	1.312	.0092
Donation cycle	Donation vs. autologous	1.786	1.921	1.674	2.205	.0000
Per year of age	Per year less in autologous	1.057	1.100	1.080	1.121	.0000
	Per year less in donation cycles	0.971	1.019	1.003	1.035	.0194
No. of oocytes	Per oocyte less in autologous cycles	0.951	0.974	0.959	0.989	.0005
	Per oocyte less in donation cycles	0.914	0.946	0.925	0.966	.0000
Odds ratio for clinical pregnancy based on 6,961 cycles with embryo transfer						
Incubation	TMS vs. SI	1.141	**1.157 15.7%**	1.018	1.315	.0254
Day of transfer	Day 5 vs. day 3	1.598	1.612	1.419	1.832	.0000
Donation cycle	Donation vs. autologous	1.653	1.709	1.483	1.969	.0000
Per year of age	Per year less in autologous	1.035	1.078	1.057	1.100	.0000
	Per year less in donation cycles	0.979	1.013	0.997	1.03	.1227
No. of oocytes	Per oocyte less in autologous cycles	0.958	0.992	0.976	1.007	.283
	Per oocyte less in donation cycles	0.923	0.967	0.946	0.989	.0033
No. transferred	3 embryos transferred vs. 1	1.448	1.714	1.199	2.451	.0031
	3 embryos transferred vs. 2	0.768	0.899	0.640	1.262	*.5376*

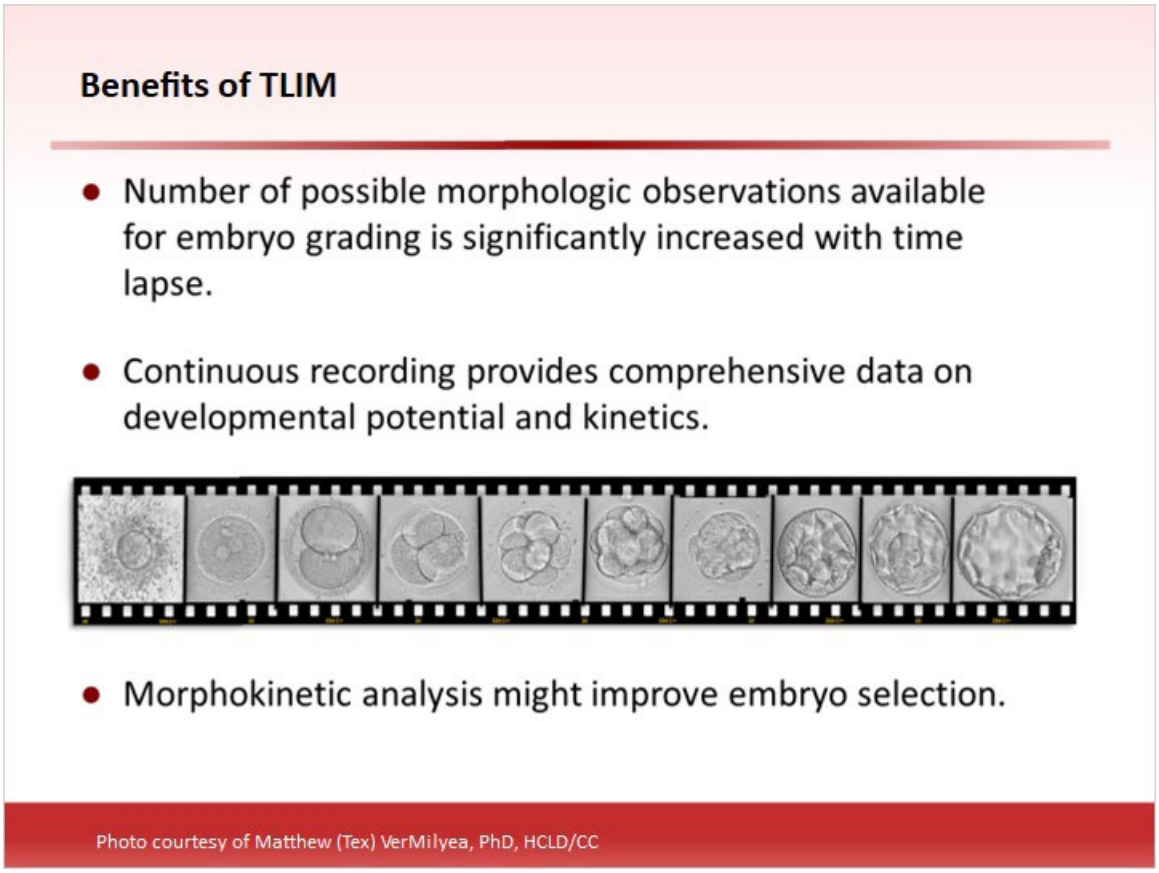
Logistic analysis of outcome, taking into account all significant confounding factors.

Adapted from Meseguer et al., 2012

Notes:

A logistic regression analysis that included all confounding factors has been used to evaluate the effect of culturing and selecting embryos with the use of TLIM. A retrospective analysis suggested that culturing and selecting embryos with TLIM significantly improves the relative probability of a clinical pregnancy when compared with standard incubators. The estimated increase of 20.1% per oocyte retrieval and 15.7% per embryo transfer is a significant improvement. Incubation in the undisturbed microenvironment in combination with improved embryo selection was associated with an increase in clinical pregnancy success.

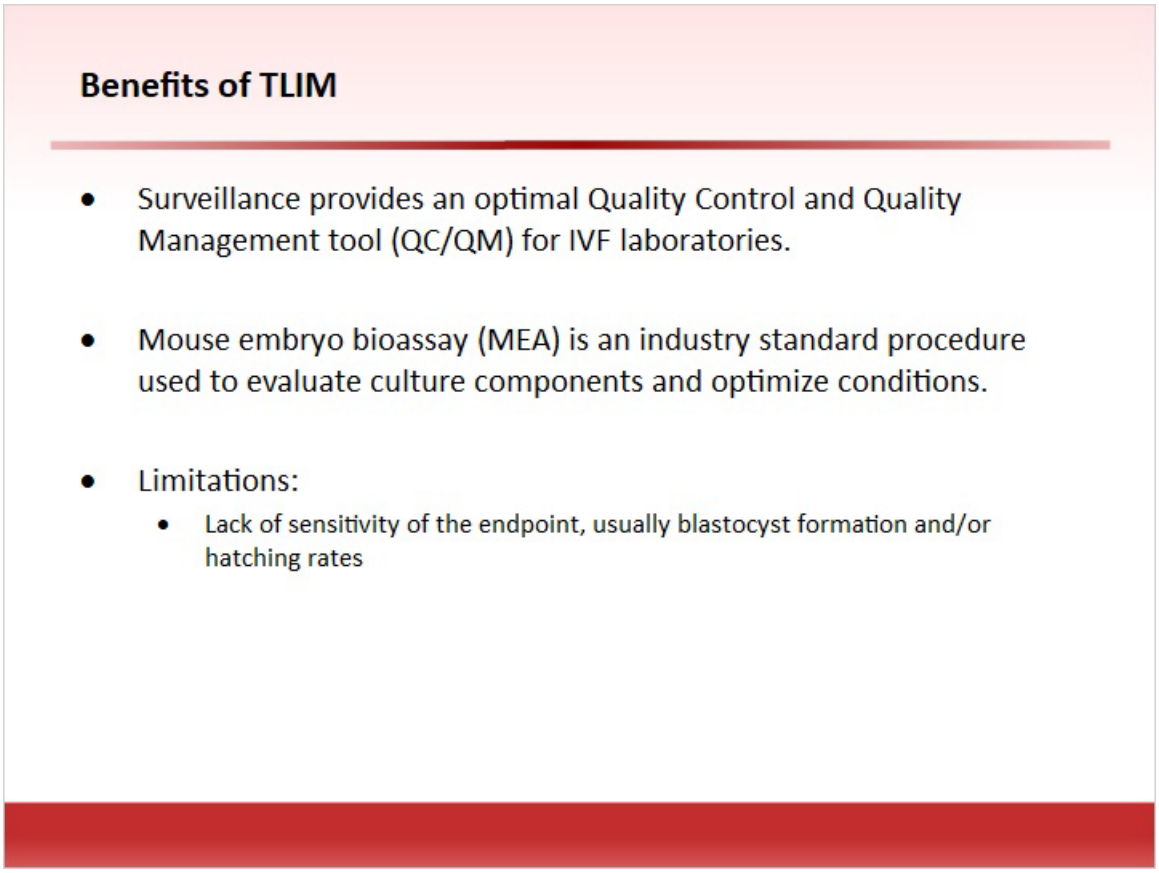
1.10 Benefits of TLIM



Notes:

Time-lapse imaging greatly increases the number of morphologic observations available for embryo grading and scoring. This continuous surveillance recording provides comprehensive data on embryo developmental potential and valuable insight into the intricacies of embryo kinetics. However, repeated quantitative measurements of key events such as pronuclei formation and disappearance, cytoplasmic fragmentation, blastomere division, compaction, cavitation, and cell lineage specification have not yet been demonstrated to be predictive of successful outcomes. The vast amount of data produced by manual annotation and automated assessment of embryo development through TLIM needs to be validated further in order to truly understand the potential benefits.

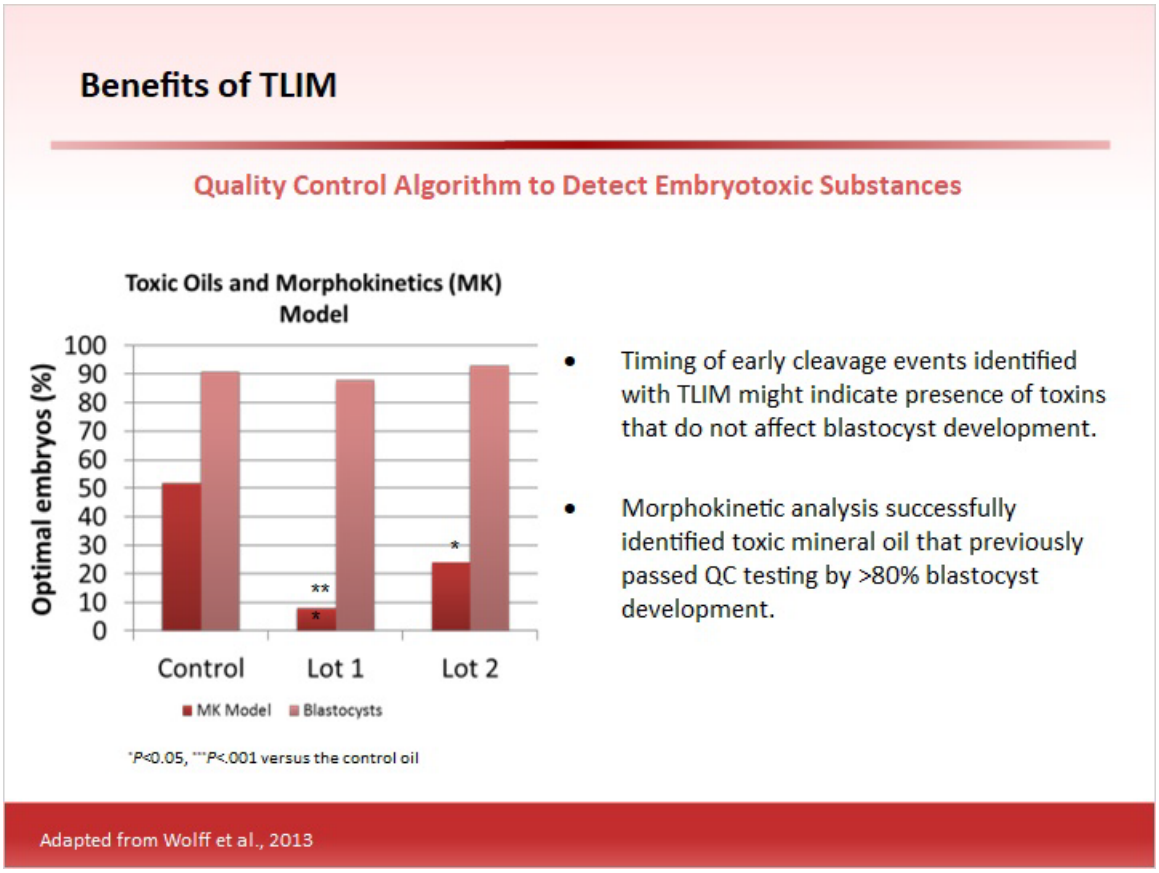
1.11 Benefits of TLIM



Notes:

Time-lapse technologies can also be applied to the maintenance of a rigorous quality assurance program in IVF laboratories. The continuous recorded surveillance provided by time-lapse technology presents an optimal Quality Control and Quality Management tool. The mouse embryo bioassay or MEA has been instrumental as a reliable diagnostic tool and used as an industry standard procedure to evaluate culture components and optimize conditions. However, it is important to note that limitations of such bioassays include the lack of sensitivity of the endpoint, usually blastocyst formation and/or hatching rates.

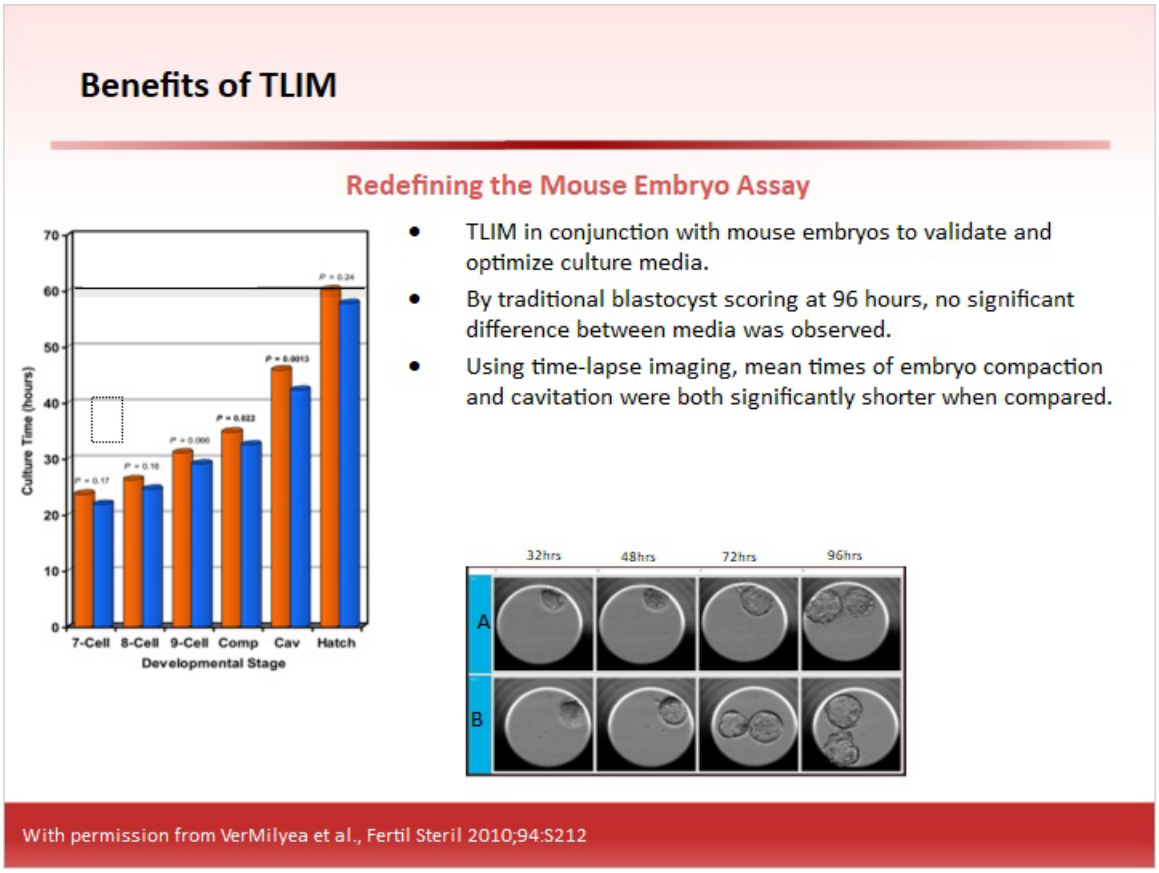
1.12 Benefits of TLIM



Notes:

As previously mentioned, time-lapse imaging might be useful for screening products for potential embryotoxic substances. In one study, two different lots of recalled pharmaceutical-grade mineral oil used in embryo culture were compared. Both lots of oil had previously passed the manufacturer's quality control testing by standard mouse embryo assay. The results showed that toxins that do not affect blastocyst formation could be detected by morphokinetic analysis of specific cell division events identified by the use of time-lapse imaging. Similar to the results of the manufacturer's quality control program, neither lot affected blastocyst development as rates were greater than 80%. Morphokinetic modeling showed a significant difference in the adverse effects on embryo development by both lots of oil.

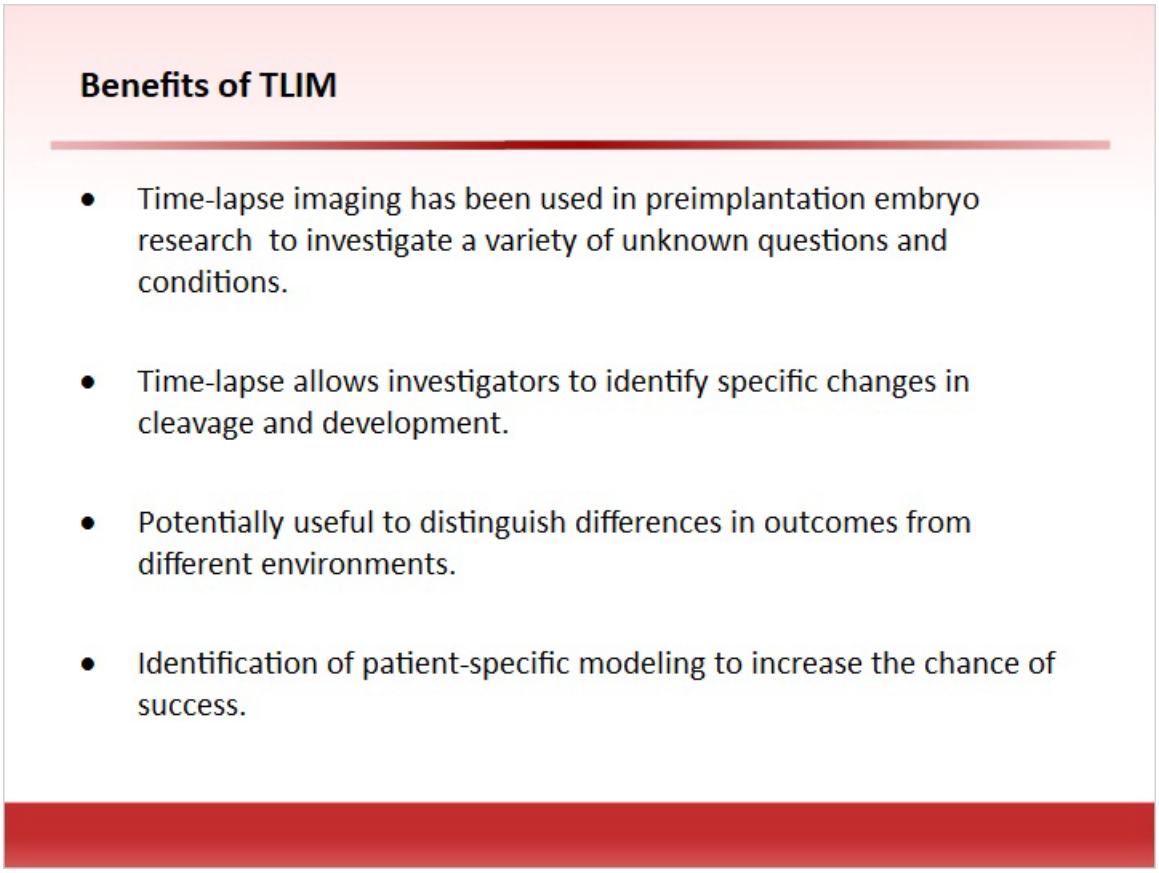
1.13 Benefits of TLIM



Notes:

By using time-lapse imaging in conjunction with the mouse embryo assay, a more detailed embryo developmental trajectory was observed compared with traditional static scoring of embryo development. In one such study, no significant difference between media was observed by using traditional developmental endpoints of blastocyst development following 96 hours of culture. However, the mean developmental times of embryo compaction and cavitation in differing culture media were both significantly shorter when compared using time-lapse observations.

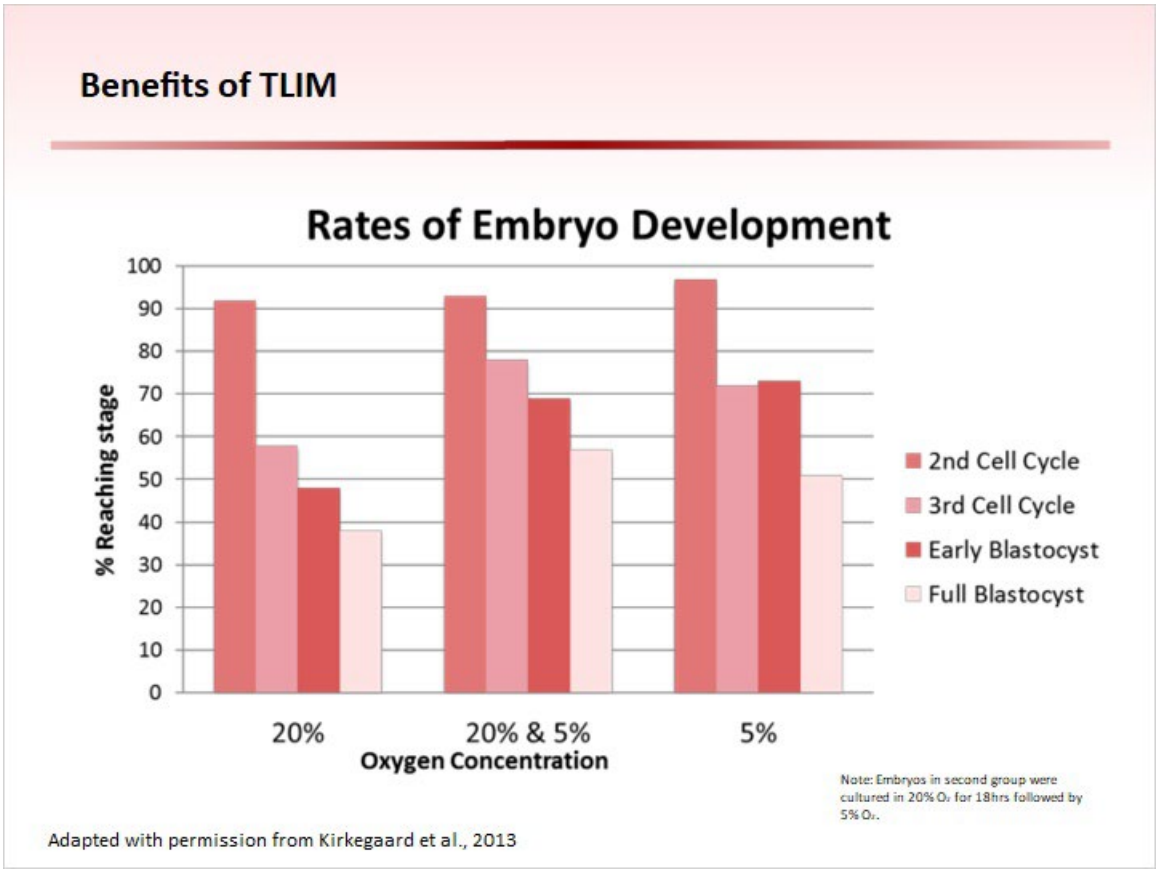
1.14 Benefits of TLIM



Notes:

Because of its unique characteristics as a noninvasive continuous assessment technology, time-lapse imaging allows investigators to identify specific changes in cleavage events and subsequent embryo development. Of particular interest is the use of TLIM to distinguish differences in outcomes from individual laboratory environments including gas concentrations and culture protocols. Ultimately, patient-specific modeling can be identified to increase the chances of success for infertile individuals.

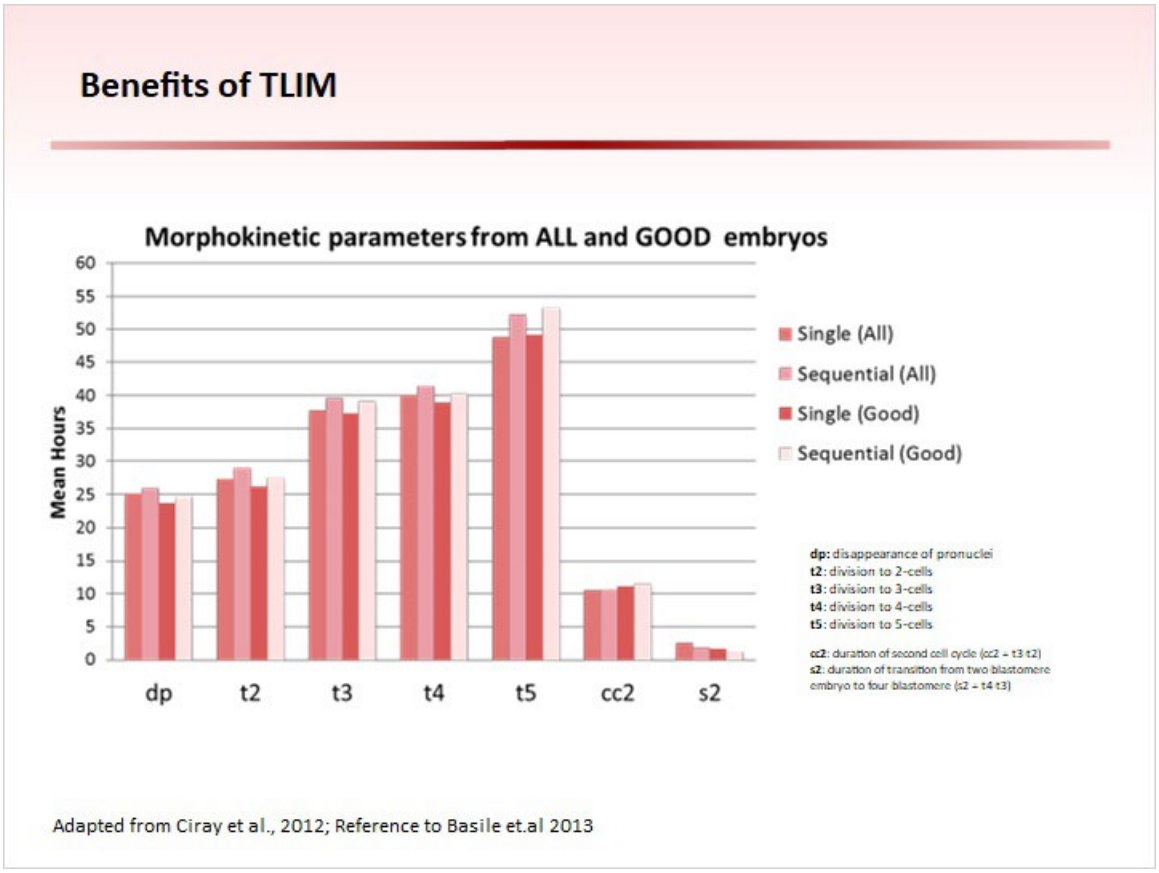
1.15 Benefits of TLIM



Notes:

TLIM has been used to study how oxygen concentration influences embryo development. In this study, embryos observed by time lapse and cultured in 20% oxygen had a reduction in cumulative development at the 8-cell and early and full blastocyst stages. Culture in 20% oxygen reduced developmental rates and delayed completion of the third cell cycle.

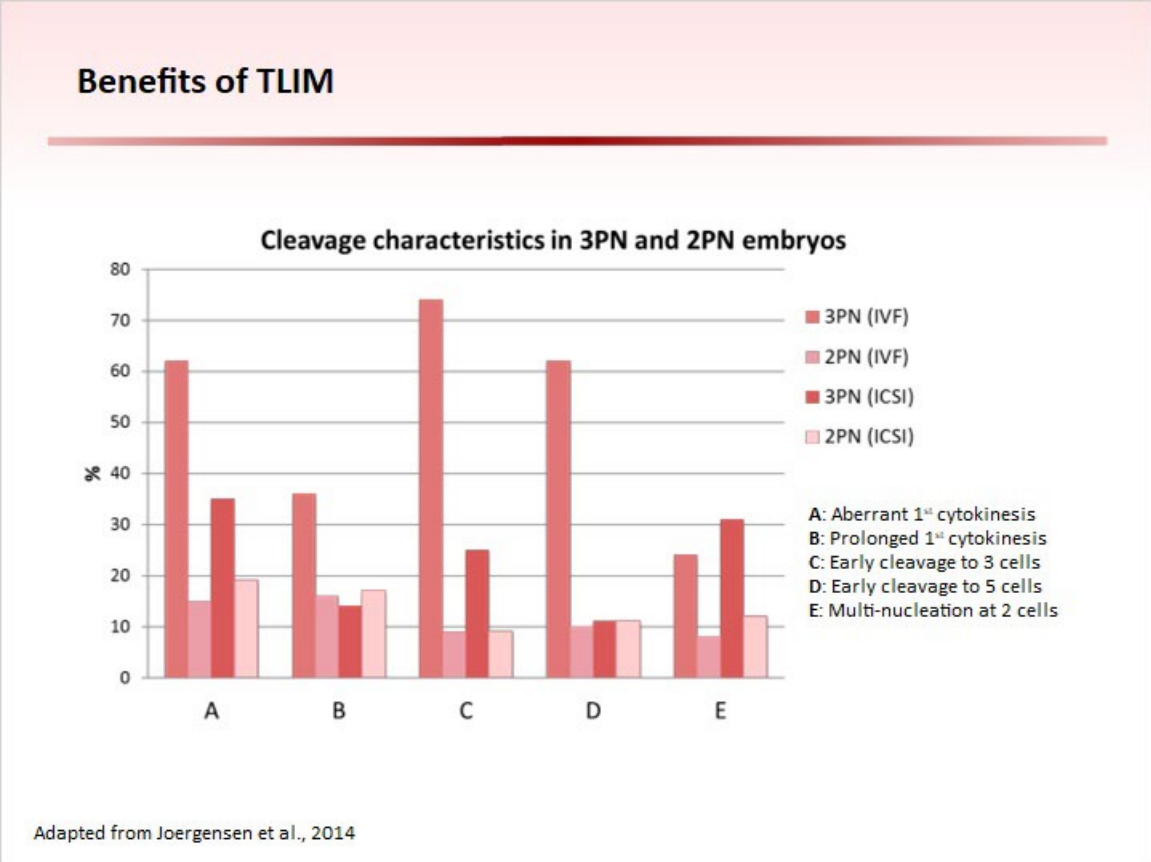
1.16 Benefits of TLIM



Notes:

TLIM has been used to study embryo culture media and culture protocols. Morphokinetic data from this study shows that the first cell cycle of embryos in a single medium was accelerated and embryos reached 2- to 5-cell stages earlier compared with those in sequential media. Subsequent cleavage events (cc2 and s2) remained similar. However, a recent, prospective study using sibling oocytes applied a morphokinetic algorithm to a comparison of different culture media. Results showed that embryo development in the different culture media was remarkably similar. This suggests that cell kinetics may not be culture media-dependent; however, investigations with other types of culture media need to be undertaken.

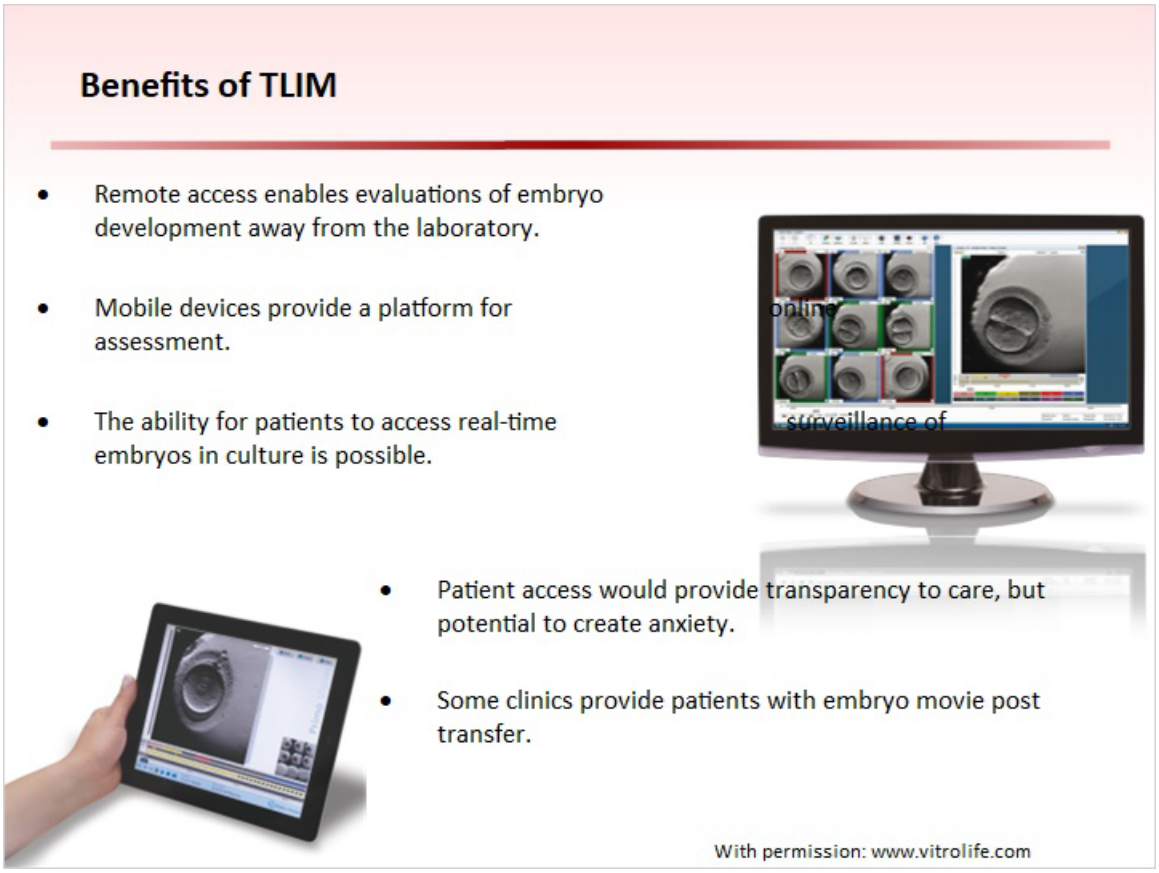
1.17 Benefits of TLIM



Notes:

An additional benefit of time-lapse imaging is the ability to observe pronuclei formation and fusion. Most recently, TLIM has been used to study differences in cleavage between triploidy IVF and ICSI embryos. Compared with 2PN, more 3PN embryos produced by conventional IVF displayed early cleavage into 3 cells. 3PN, more often than 2PN, ICSI embryos displayed early cleavage into 3 cells, and more 3PN IVF embryos than ICSI embryos displayed early cleavage into 3 cells.

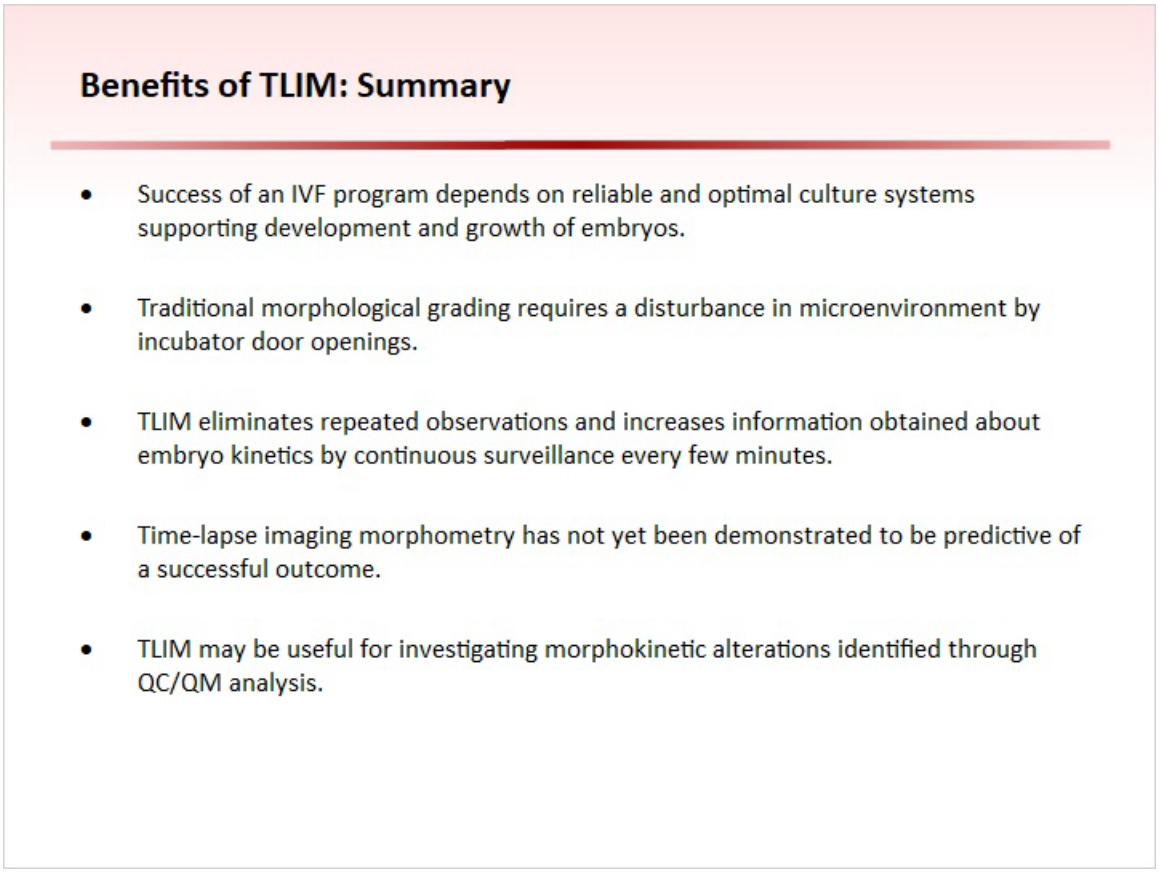
1.18 Benefits of TLIM



Notes:

Some time-lapse devices allow for remote access enabling evaluations of embryo development to be performed outside the laboratory. Remote access via mobile devices provides a platform for online assessment by other colleagues and professionals in the field. Although not currently available, the ability for patients to access real-time surveillance of embryos in culture is a near possibility. Such patient access would provide greater transparency to individualized patient care, but may also create anxiety for some patients. Currently, some clinics provide patients with a copy of their embryo's movie following transfer.

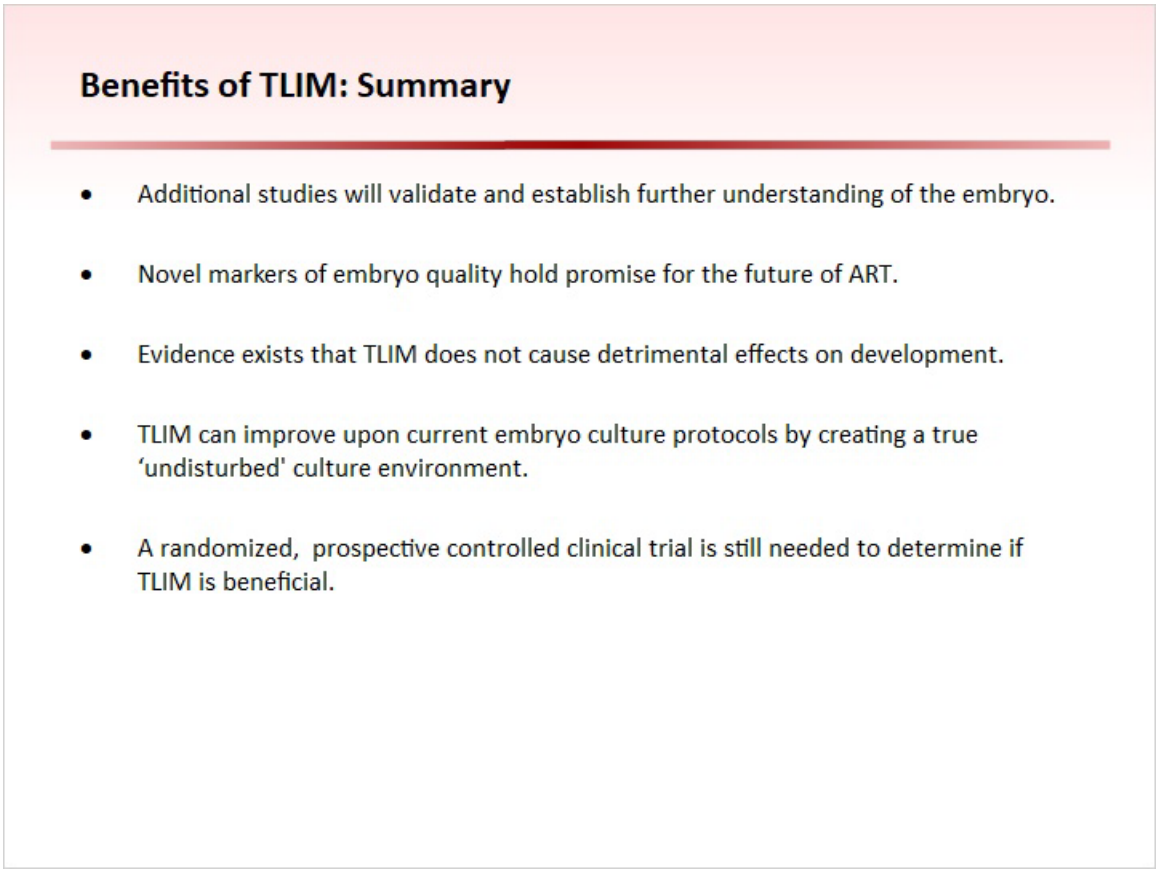
1.19 Benefits of TLIM: Summary



Notes:

The success of an IVF program depends heavily on a reliable and good culture system that supports development and growth of healthy embryos. Traditional morphological grading of embryos requires repeated breaching of the incubator microenvironment following incubator door openings that has shown to be harmful to embryo development and may have an impact on overall success. Time-lapse imaging provides a platform that eliminates repeated perturbations of the culture environment and greatly increases the information obtained regarding embryo developmental kinetics due to the continuous recorded surveillance feature. Time-lapse imaging morphometric analysis has not yet been demonstrated to be predictive of a successful outcome, but it might serve as a useful tool for investigating precise morphokinetic alterations that can be identified through quality control analysis.

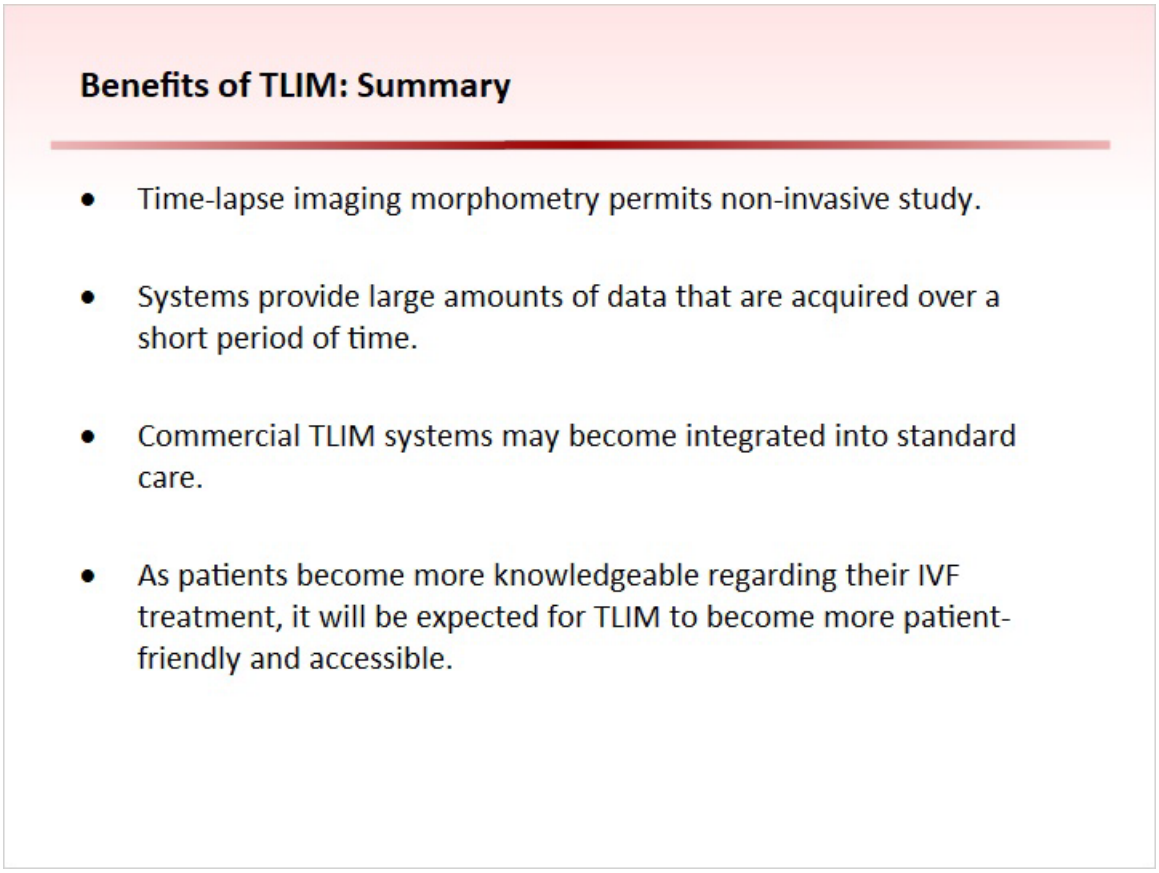
1.20 Benefits of TLIM: Summary



Notes:

In current and future research applications, additional time-lapse studies will validate and establish further understanding of the dynamic developing embryo. Novel non-invasive prediction markers of embryo quality that may improve identification of the best embryo for transfer are promising for the future of assisted reproductive technologies. Collectively, many studies have provided evidence that TLIM does not cause any observable detrimental effect in human embryo development, however, a randomized, prospective controlled clinical trial is still needed to determine if TLIM is beneficial.

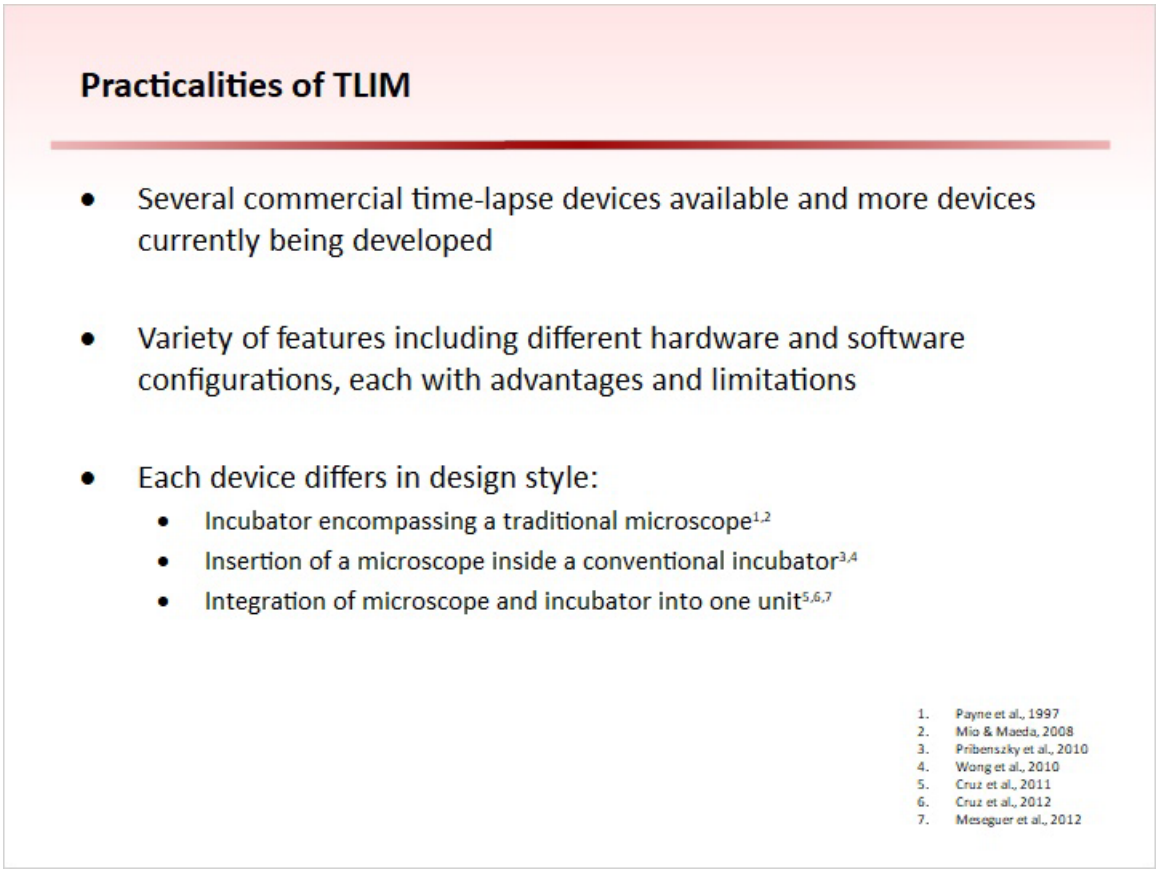
1.21 Benefits of TLIM: Summary



Notes:

Time-lapse technology provides a great tool for non-invasive studies of embryo development.
Due to the continuous surveillance capabilities these systems provide, large amounts of data can be acquired over a short period of time.
Commercially available imaging systems that are suitable for clinical use may soon become integrated into the standard care for infertility treatment worldwide.
As patients become more knowledgeable regarding their IVF treatment, it will be expected for TLIM to become more patient-friendly and accessible to patients. The potential for patients to view their embryo development on their hand-held device in the convenience of their own home is not too far off from reality.

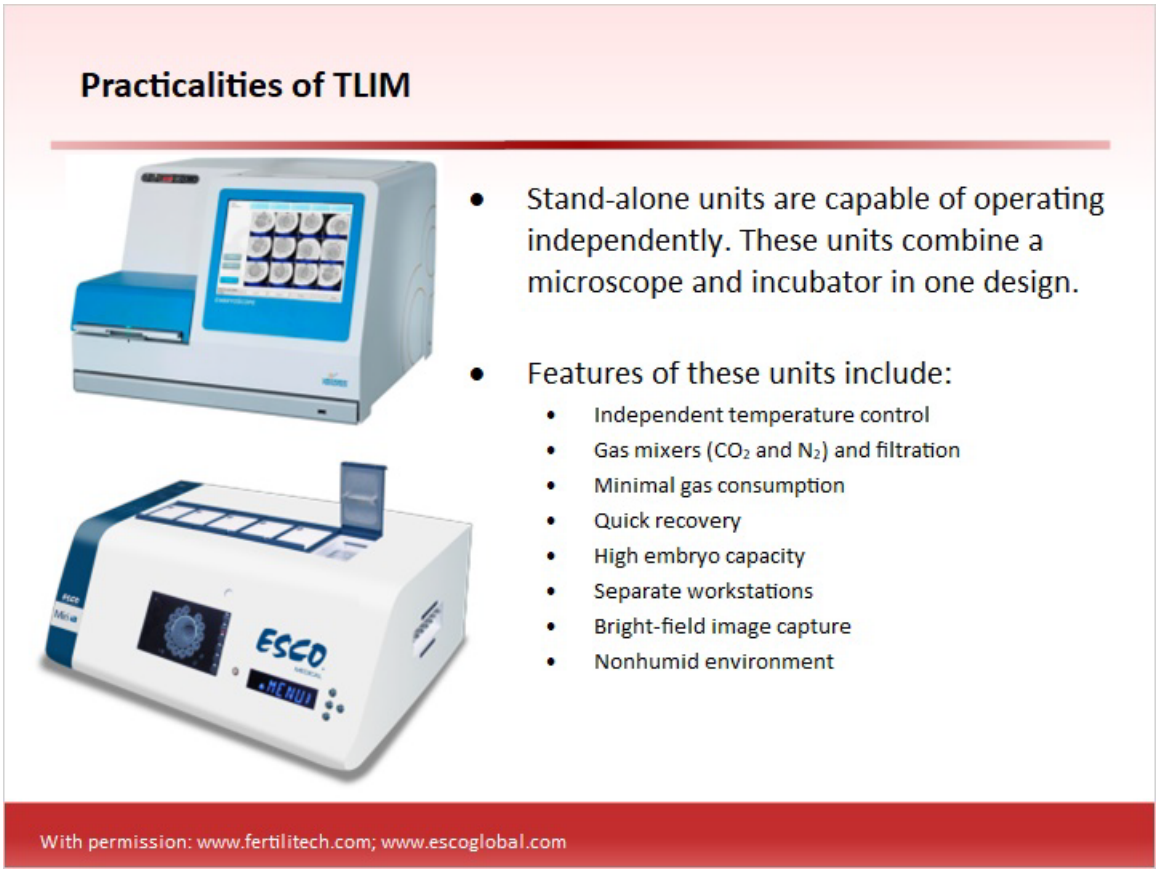
1.22 Practicalities of TLIM



Notes:

In recent years there has been an increase in commercially available time-lapse devices on the market with more being developed. All such devices offer a variety of features including various hardware and software configurations. Each device differs in design style; some include a traditional microscope enclosed by an incubator, the ability to insert a time-lapse microscope inside a conventional front-load incubator, and the integration of a time-lapse microscope and incubator into one unit. Each unit has its own advantages and limitations, but this all depends on the application with which time-lapse imaging is to be used along with the user's interest for the device.

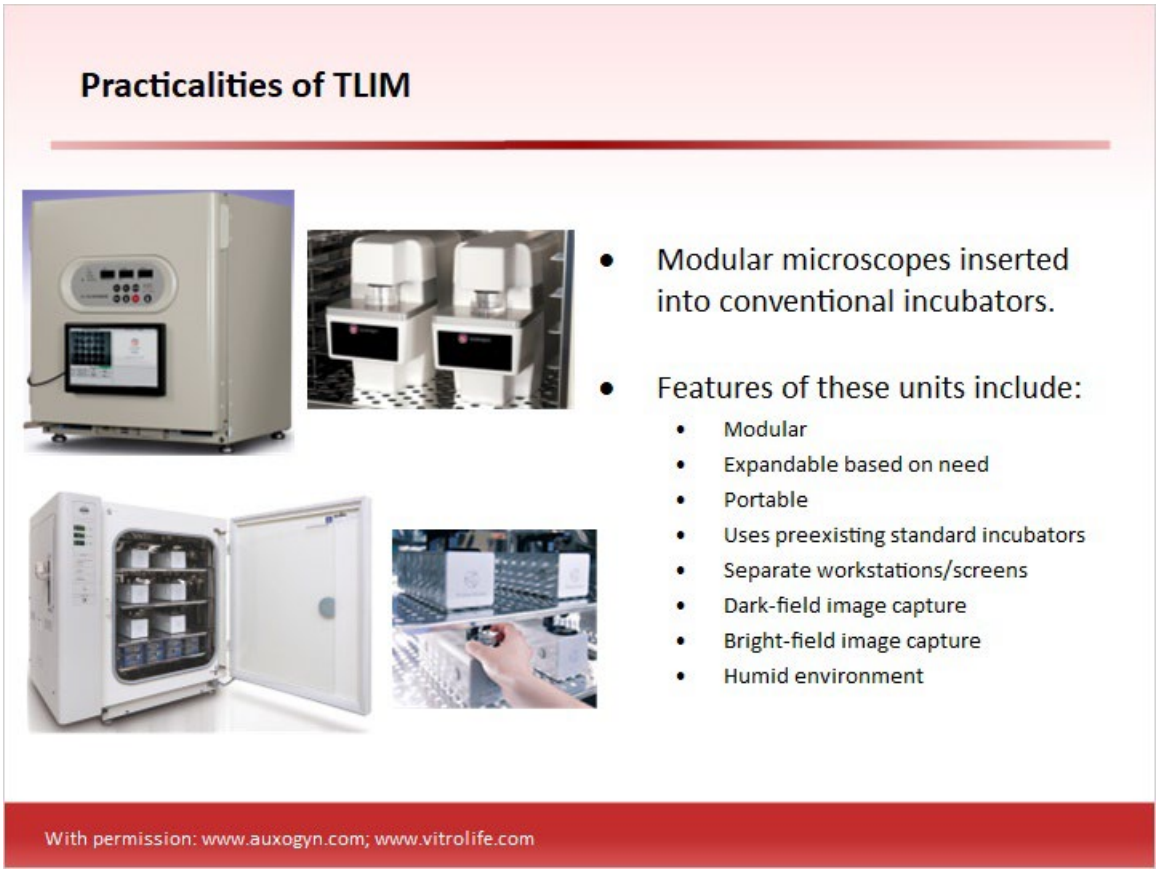
1.23 Practicalities of TLIM



Notes:

Some time-lapse devices are stand-alone units that are capable of operating independently of any other device. These units combine a microscope and incubator in one design and have a relatively large footprint. Therefore, space availability in the laboratory is a consideration for such stand-alone units.
Features of these units include: independent temperature control, gas mixers that combine carbon dioxide and nitrogen to provide specific gas concentrations, built-in gas filtration systems, minimal gas consumption, quick recovery rates, high embryo culture capacities, separate workstations for annotations and embryo viewing, bright-field image capture, and a nonhumidified environment.

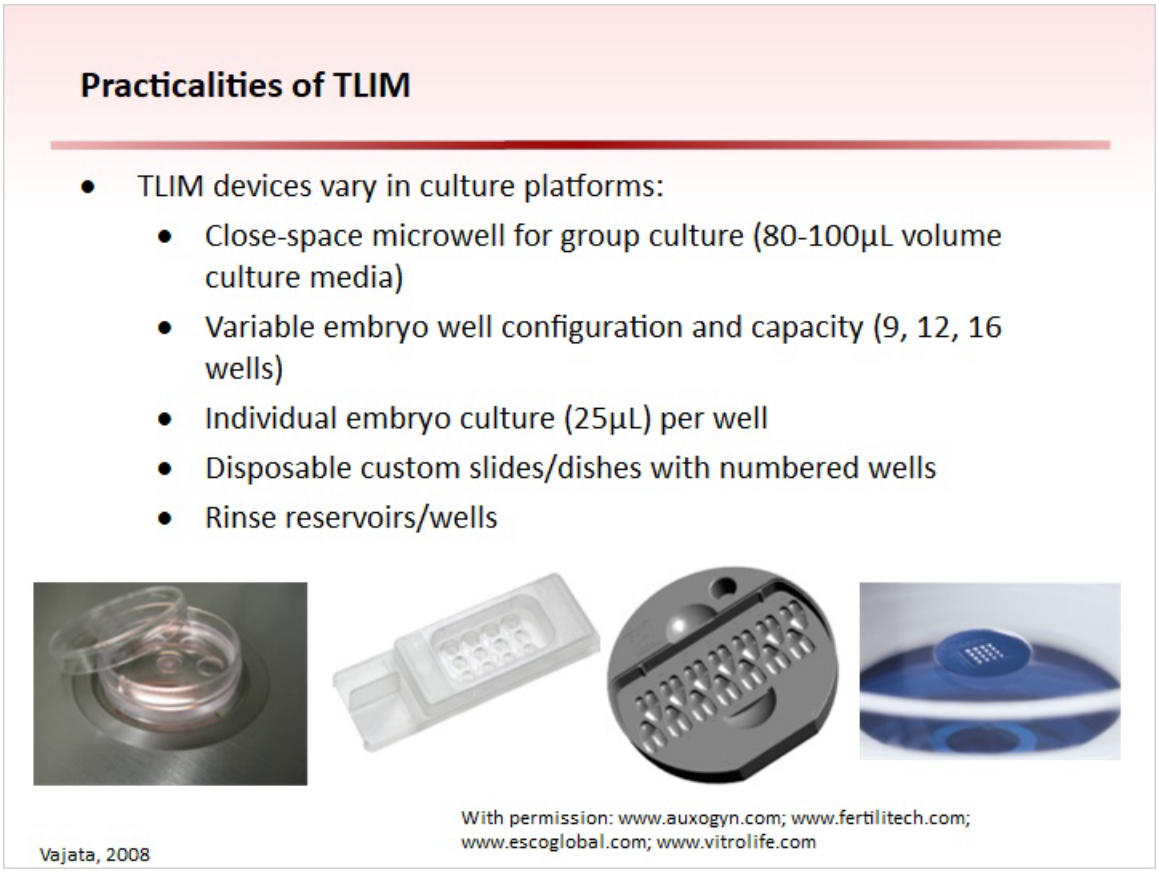
1.24 Practicalities of TLIM



Notes:

Other time-lapse devices are modular microscopes that are inserted into conventional incubators. These units utilize the culture environment provided by front-load conventional incubators. Features of these units include: modulation of devices, the ability to expand the number of units based on need, portability of the time-lapse microscope hardware, utilizing preexisting standard incubators, separate workstations/screens, dark-field image capture, bright-field image capture, and a humid environment.

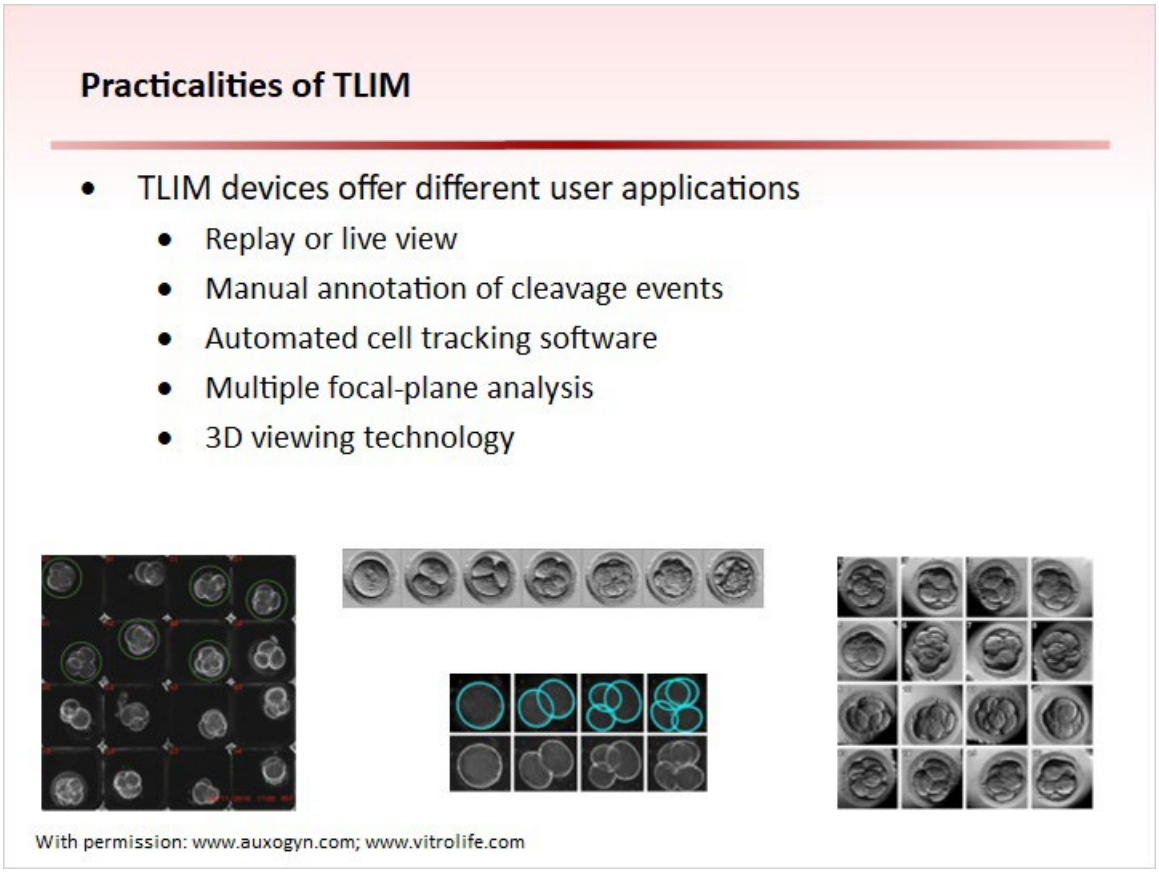
1.25 Practicalities of TLIM



Notes:

TLIM devices also vary in size and capacity of culture platforms. Each device uses a custom culture dish, usually made of high-optic materials. Some platforms provide a close-space microwell intended for group culture. These well-of-the-well systems have been shown to improve embryo development in previous studies. The volumes of these platforms usually hold 80-100 µL of culture media. Other culture platforms have variable embryo well configuration and the capacity to hold 9, 12, and 16 embryos. The ability to culture some embryos individually in 25 µL per well is also available. Each platform is disposable and custom made with numbered wells for each embryo. Some dishes or slides contain rinse reservoirs or holding wells allowing an embryologist to remove an embryo from the microwell and place in a holding well for embryo transfer. These custom culture platforms are usually quite expensive and are not interchangeable across devices.

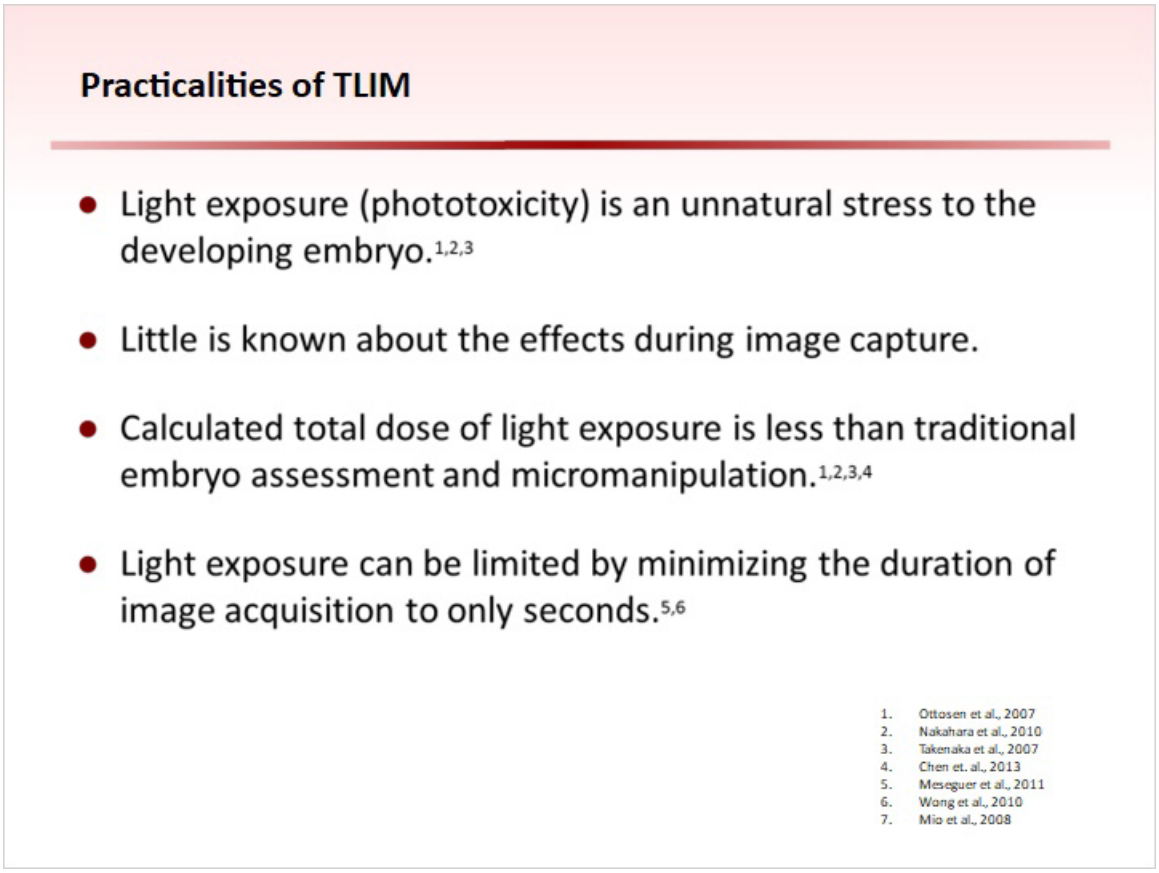
1.26 Practicalities of TLIM



Notes:

TLIM devices also offer different user applications. Special consideration should be taken when deciding on what features are most important to the user. Some application features include the ability to replay embryo videos frame-by-frame and the ability to view the embryo in real-time. Some applications require manual annotation of cleavage events, while others utilize automated cell tracking software that automatically recognizes cleavage events to a certain stage of development. The use of multiple focal-plane analysis and 3D-viewing technologies allow for more depth and detailed analysis of embryo quality.

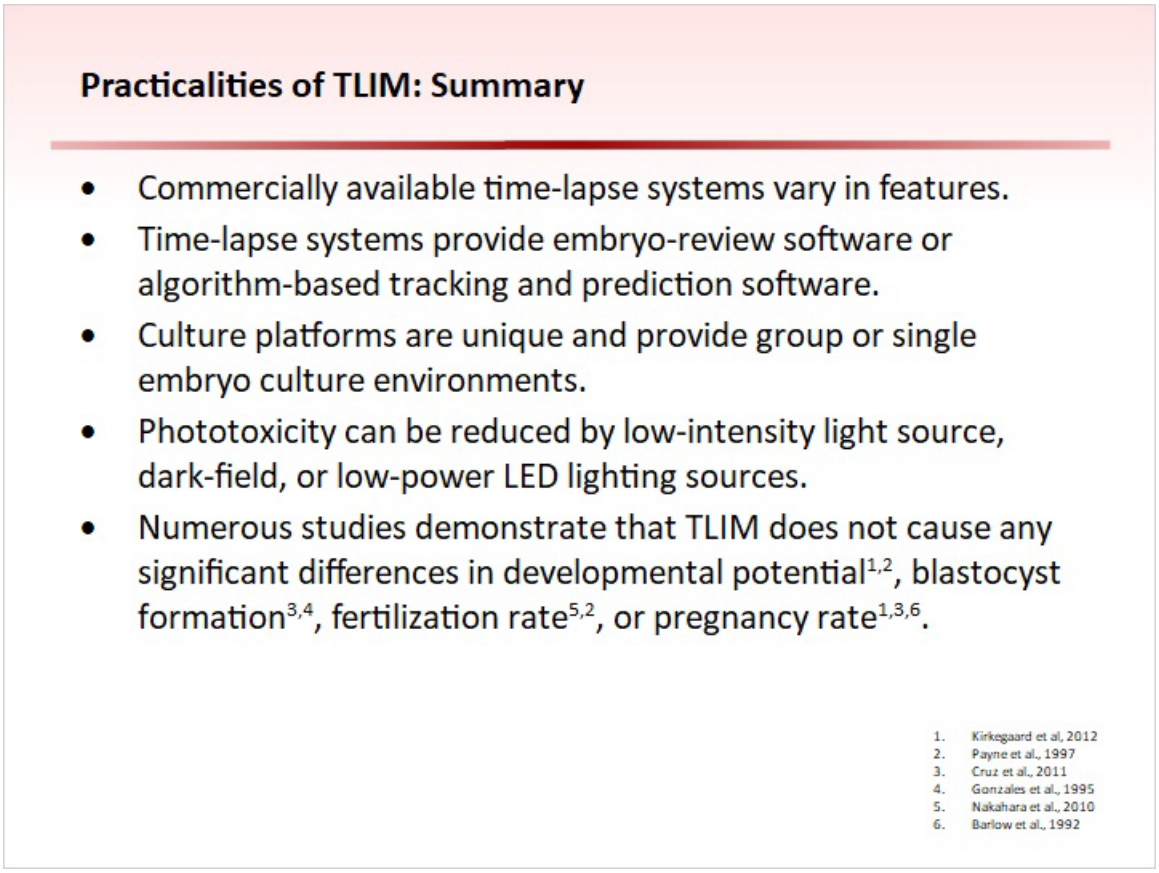
1.27 Practicalities of TLIM



Notes:

While little is known about the effects of light exposure during image capture in time-lapse devices, it is known that light exhibits an unnatural stress to the developing embryo. The calculated total dose of light exposure to embryos in time-lapse devices is less than what is commonly used in traditional embryo assessment and micromanipulation. Some devices allow the user to limit the amount of light to which embryos are exposed by minimizing the duration of image acquisition to only seconds. Although embryos cultured in time-lapse devices are periodically exposed to light when digital images are obtained, reports suggest that embryo development and the clinical pregnancy outcome after time-lapse imaging were not significantly different from pregnancies resulting from nonimaging observation.

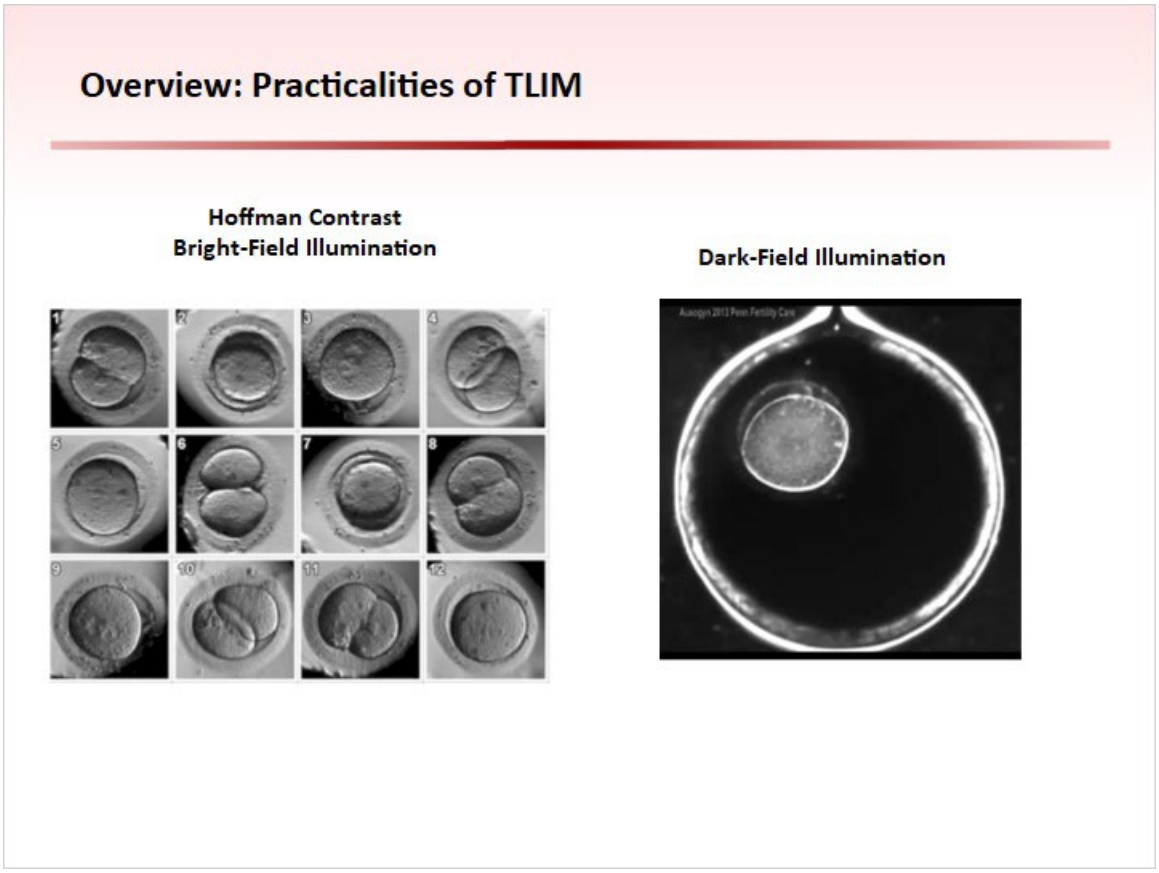
1.28 Practicalities of TLIM: Summary



Notes:

It is important to know that commercially available time-lapse systems vary in hardware and software features. Depending on the application of the device, time-lapse systems may provide adequate embryo-review software to more complex algorithm-based tracking and prediction software. Embryo culture platforms are also unique to individual time-lapse devices and provide either a group- or single-embryo culture microenvironment. Possible embryo phototoxicity can be reduced by visible light capabilities such as a low-intensity light source, dark-field, or low-power LED lighting sources. Numerous time-lapse studies have shown that TLIM does not cause any significant differences in developmental potential, blastocyst formation, fertilization rate, or pregnancy rate.

1.29 Overview: Practicalities of TLIM



Notes:

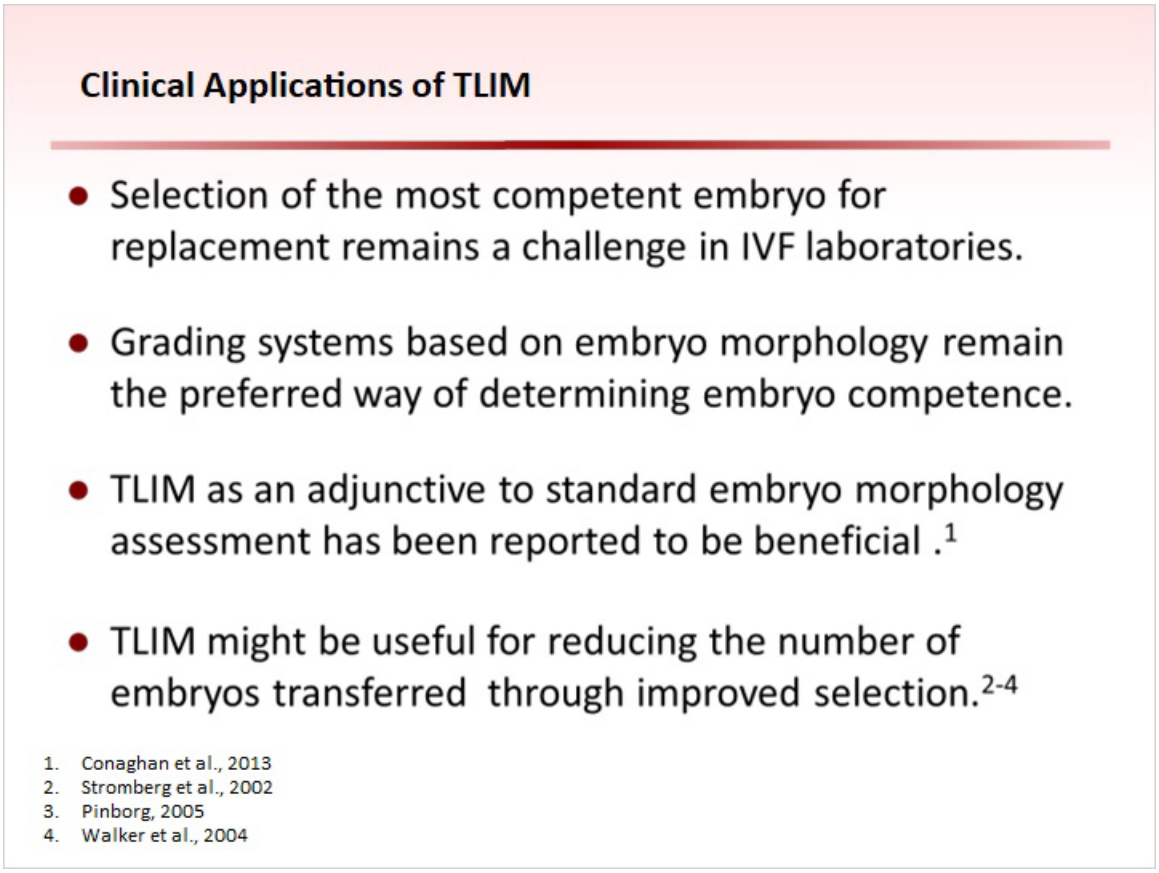
The next slide will show videos of bright and dark field illumination. In bright-field illumination, the embryos appear darker on a bright background. Bright-field microscopy is the standard technique for retaining the natural colors of the embryo and the contrast comes from the absorbance of light by the embryo. In bright-field microscopy, embryo features are visible where a shadow is cast on the surface or when part of the surface is less reflective, as in the case of pronuclei formation. With dark-field illumination, the embryo appears bright because it reflects the light from the microscope into the objective. The interpretation of dark-field images must be done with care as common dark features from bright-field images may be invisible and vice-versa. Some features of embryos that are unable to cast shadows will not appear in bright-field images, but the light that reflects off the sides of the feature will be visible by dark field. This is necessary for embryo cell-tracking software used in conjunction with dark-field microscopy, but has its limitations when wanting to observe pronuclei formation and syngamy.

1.30 Videos

Notes:

Videos

1.31 Clinical Applications of TLIM



Notes:

Next, the clinical applications of TLIM will be discussed.
The selection of the most competent embryo for replacement into the uterus remains a challenge in IVF laboratories. Advances in such technologies, including time-lapse may help to minimize these challenges and provide greater insight in embryo selection. Traditionally, grading systems based on embryo morphology remain the preferred way of determining embryo competence. However, the use of TLIM adjunctively with embryo morphology may provide improved and objective selection of embryos that are more suitable for transfer, thereby reducing the number of embryos transferred. Ultimately, this may lower the risks associated with multiple pregnancies.

1.32 Clinical Applications of TLIM

Clinical Applications of TLIM

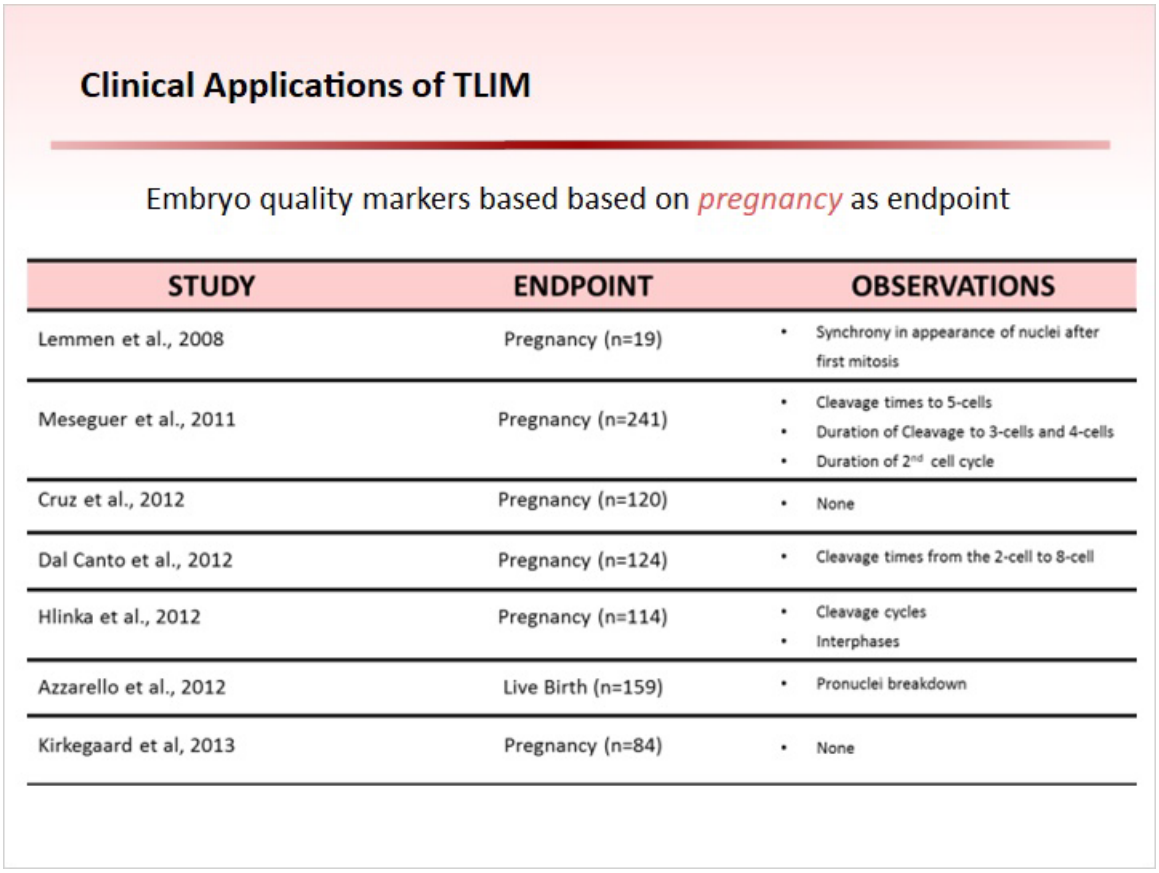
Embryo quality markers based based on *development* as endpoint

STUDY	ENDPOINT	OBSERVATIONS
Wong et al., 2010	Blastocyst development Y/N (n=100)	• Duration of 1st cytokinesis • Time between 1st and 2nd mitosis • Synchronicity of 2nd and 3rd mitosis
Hashimoto et al., 2012	Blastocyst score (n=80)	• Duration of 2nd and 3rd mitotic divisions • Pronuclear disappearance
Cruz et al., 2012	Blastocyst score (n=834)	• Cleavage times to 4-cell • Duration of 3-cell stage • Direct cleavage of 1-cell to 3-cell • Unevenness at 2-cell stage
Dal Canto et al., 2012	Expanded blastocyst (n=459)	• Cleavage times from the 2-cell to 8-cell • Duration of all cleavage stages
Hlinka et al., 2012	Blastocyst development (n=180)	• Cleavage cycles • Interphases
Conaghan et al., 2013	Blastocyst quality (n=1233)	• Duration of 2- and 3-cell stage
Kirkegaard et al, 2013	High quality blastocysts (n=571)	• Duration of 1st cytokinesis • Duration of 3-cell stage • Direct cleavage from 1-3cells

Notes:

The studies shown here identify embryo markers by time-lapse that correspond to embryo development. Endpoints include the ability of an embryo to develop to the blastocyst stage, the quality of the blastocysts, and the overall score or grade of the blastocyst. The duration, frequency, and synchronicity of early cytokinesis events were commonly observed.

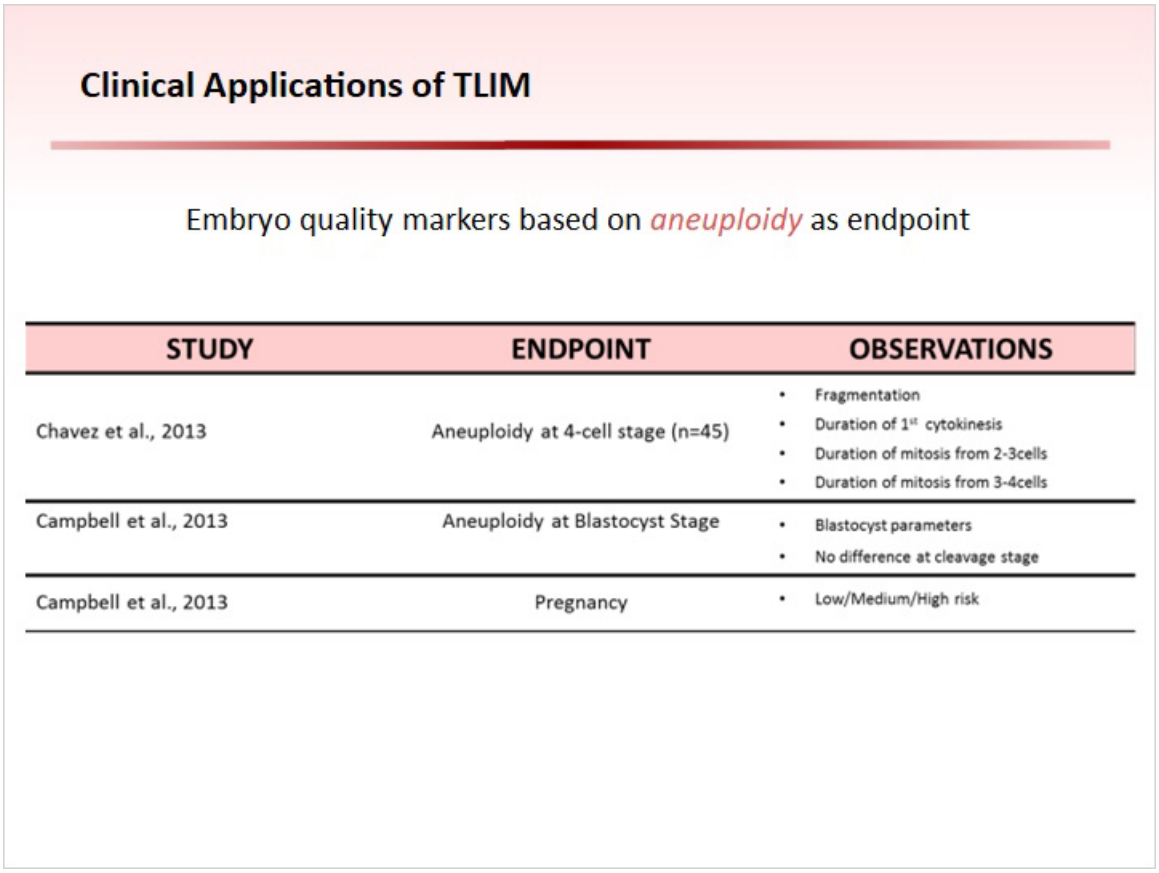
1.33 Clinical Applications of TLIM



Notes:

The studies shown here identify embryo markers by time-lapse that correspond to pregnancy. Pregnancy success and live-birth rates were used as endpoints for these studies. Various observations of embryo development, including cleavage times and duration, pronuclei breakdown, and cellular interphase events were used to identify endpoints.

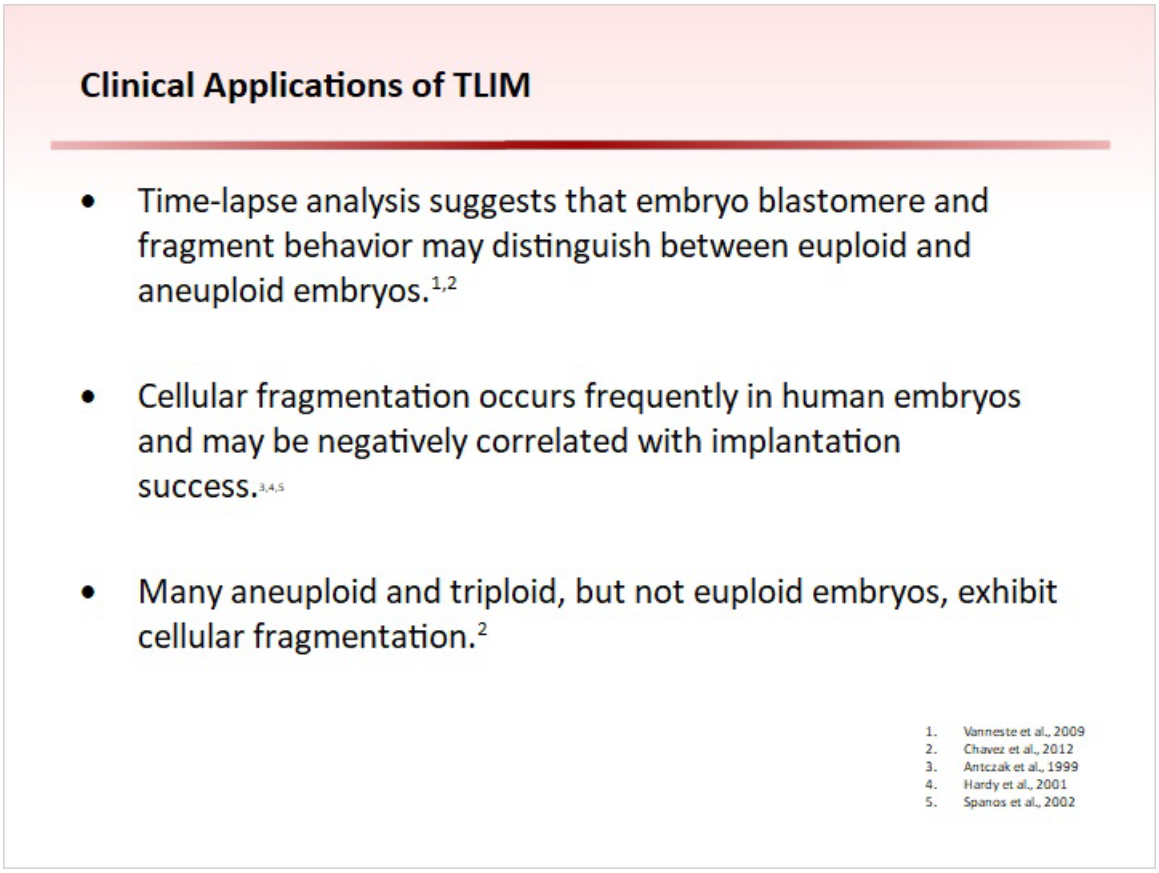
1.34 Clinical Applications of TLIM



Notes:

The studies shown here describe the use of time-lapse imaging and morphokinetic analysis to identify the risk of embryos for having a single or multiple-aneuploid chromosome constitution. The identification of normal or abnormal embryos by time-lapse imaging, in conjunction with genetic screening of embryos, provided the endpoints for such studies. Observations of embryo development, including cellular fragmentation, duration of mitosis, and blastocyst development were used to predict that embryos were more likely to be aneuploid. Although it is not clear if time-lapse imaging could replace the invasive methodology of embryo biopsy and preimplantation genetic testing, results from such studies are encouraging.

1.35 Clinical Applications of TLIM



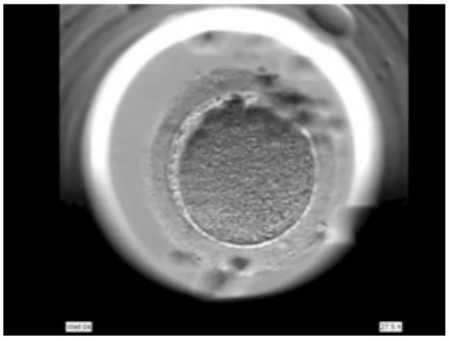
Notes:

Considering that the rate of human embryo aneuploidy is 50%-80%, additional time-lapse analysis suggests that embryo blastomere and fragment behavior may distinguish between euploid and aneuploid embryos. Cellular fragmentation, or generation of cytoplasmic fragments, occurs frequently in human embryos and may be negatively correlated with implantation success.
Observations by TLIM have shown that many aneuploid and triploid, but not euploid embryos, exhibit cellular fragmentation.

1.36 Video: Clinical Applications of TLIM

Video: Clinical Applications of TLIM

- Fragments may remain as units that contain chromosomal DNA or may be reabsorbed by blastomeres.[1,2,3]



Click on Video to View

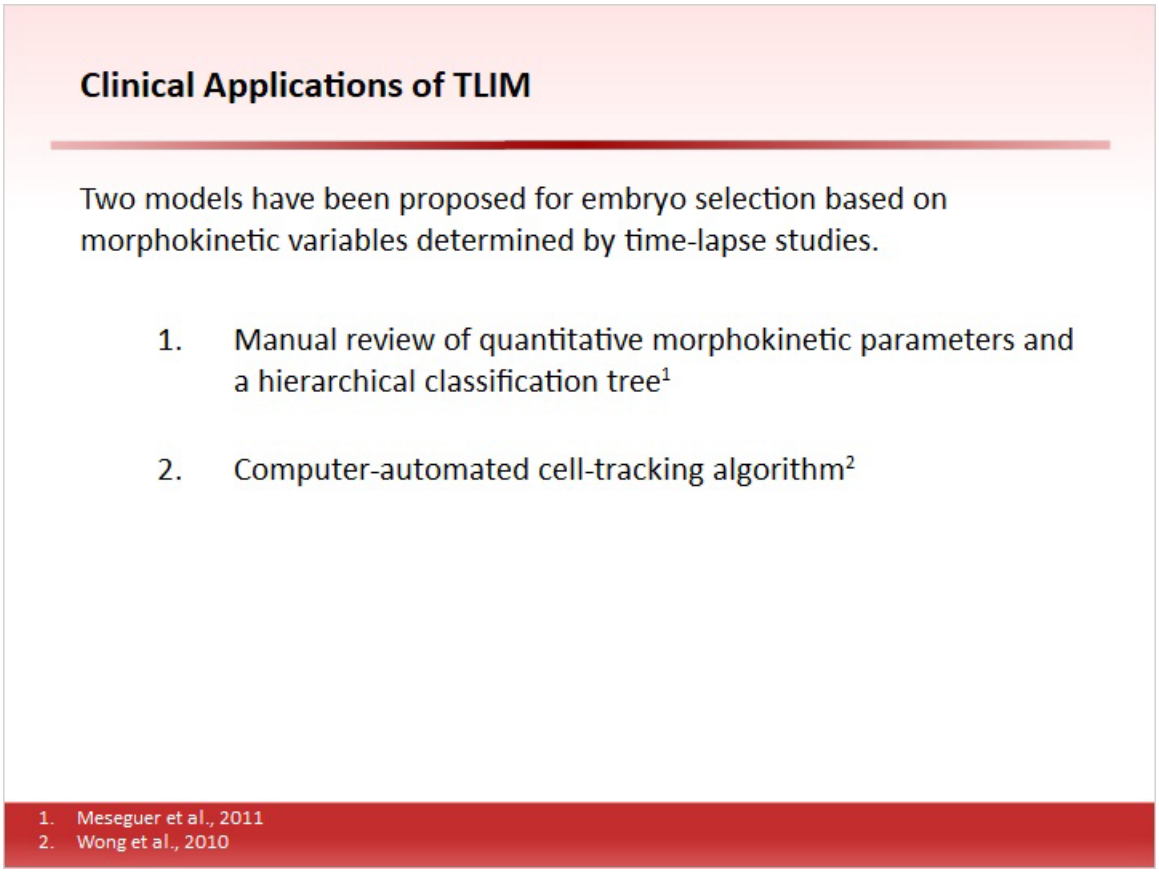
- If fragments fuse with a blastomere from that it originated, it could potentially restore embryonic euploidy.[4,5]
- Fusion of a fragment with a segregated chromosome to a neighboring blastomere may result in complex genotypes.[6,7]

1. Van Blerkom et al., 2001
2. Hardarson et al., 2002
3. Lemmen et al., 2008
4. Barbash-Hazan et al., 2009
5. Munne et al, 2004
6. Vanneste et al., 2009
7. Johnson et al., 2010

Notes:

Videos: Noninvasive analysis suggests that fragments may remain as a separate entity that contains chromosomal DNA and cytoplasm, or fragments may be reabsorbed by blastomeres. If a fragment containing DNA fuses with a blastomere from which it was created, it could potentially restore embryonic euploidy enabling chromosomal correction during embryo development.
Equally likely is the fusion of a fragment with a segregated chromosome to a neighboring blastomere resulting in complex genotypes.
These fascinating studies would not have been possible without the continuous surveillance of embryos provided by TLIM.

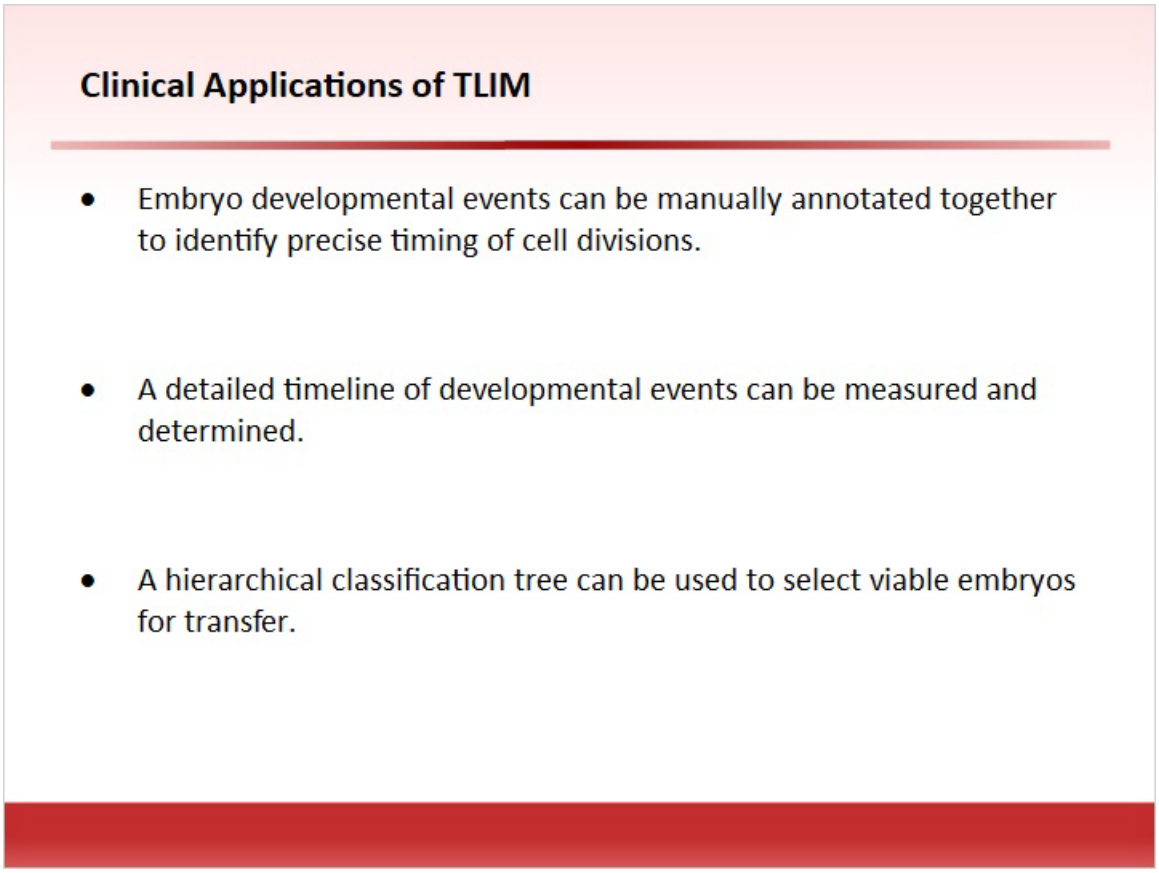
1.37 Clinical Applications of TLIM



Notes:

To date, two models have been proposed for embryo selection based on morphokinetic variables determined by time-lapse studies. One model involves the manual review of quantitative morphokinetic parameters and the use of a hierarchical classification tree to identify good embryos. The other model involves a computer-automated cell-tracking algorithm that predicts that embryos are most likely to become blastocysts.

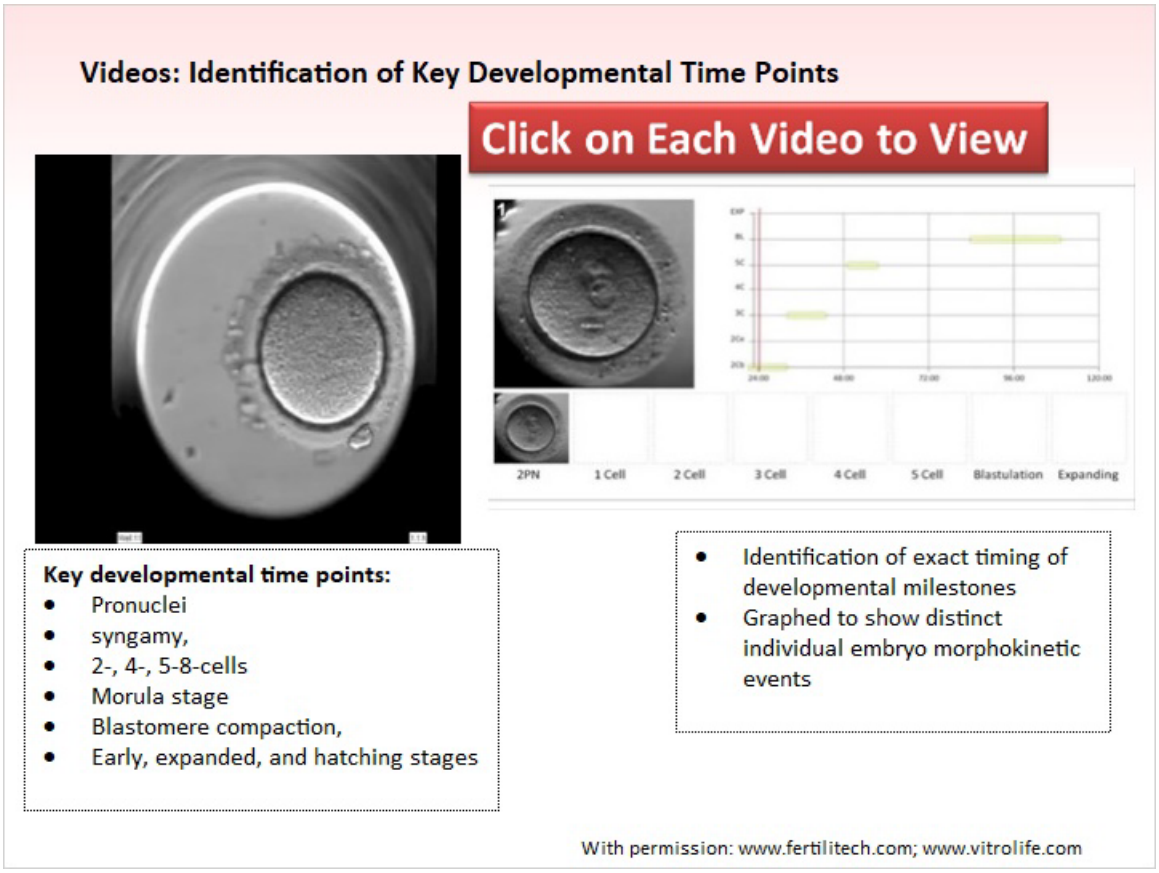
1.38 Clinical Applications of TLIM



Notes:

Using image analysis software, embryo developmental events can be manually annotated together and used to identify precise timing of cell divisions. By identifying the time of cell cleavage events as the first visual point when a newly formed blastomere is completely separated by individual cell membranes, a detailed timeline of developmental events can be measured and determined. Also, by observing correlations between morphokinetic parameters and embryo implantation, a proposed hierarchical classification tree can be used to select viable embryos for transfer.

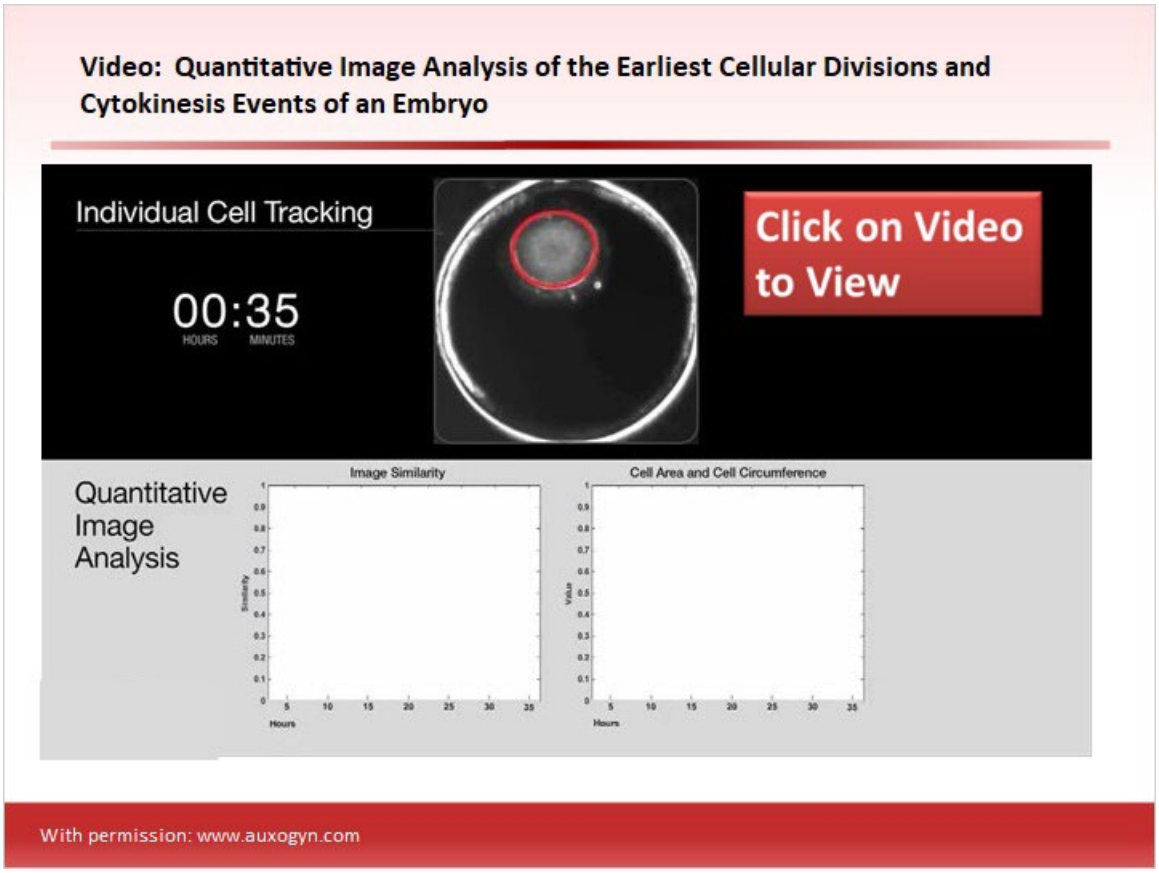
1.39 Videos: Identification of Key Developmental Time Points



Notes:

In the video to the left, key developmental time points can be identified by time-lapse including the appearance of pronuclei, syngamy, first-cell division to create 2 cells, second-cell division cycle to create 4 cells, the third-cell division cycle to create 5 to 8 cells, morula stage followed by blastomere compaction, and subsequent stages of blastocyst development including early, expanded, and hatching stages. In the video to the right, the exact timing of such developmental milestones can be identified and graphed to show distinct individual embryo morphokinetic events. These annotations can then be compared and applied to a classification tree to help better identify embryos with the greatest chance of success.

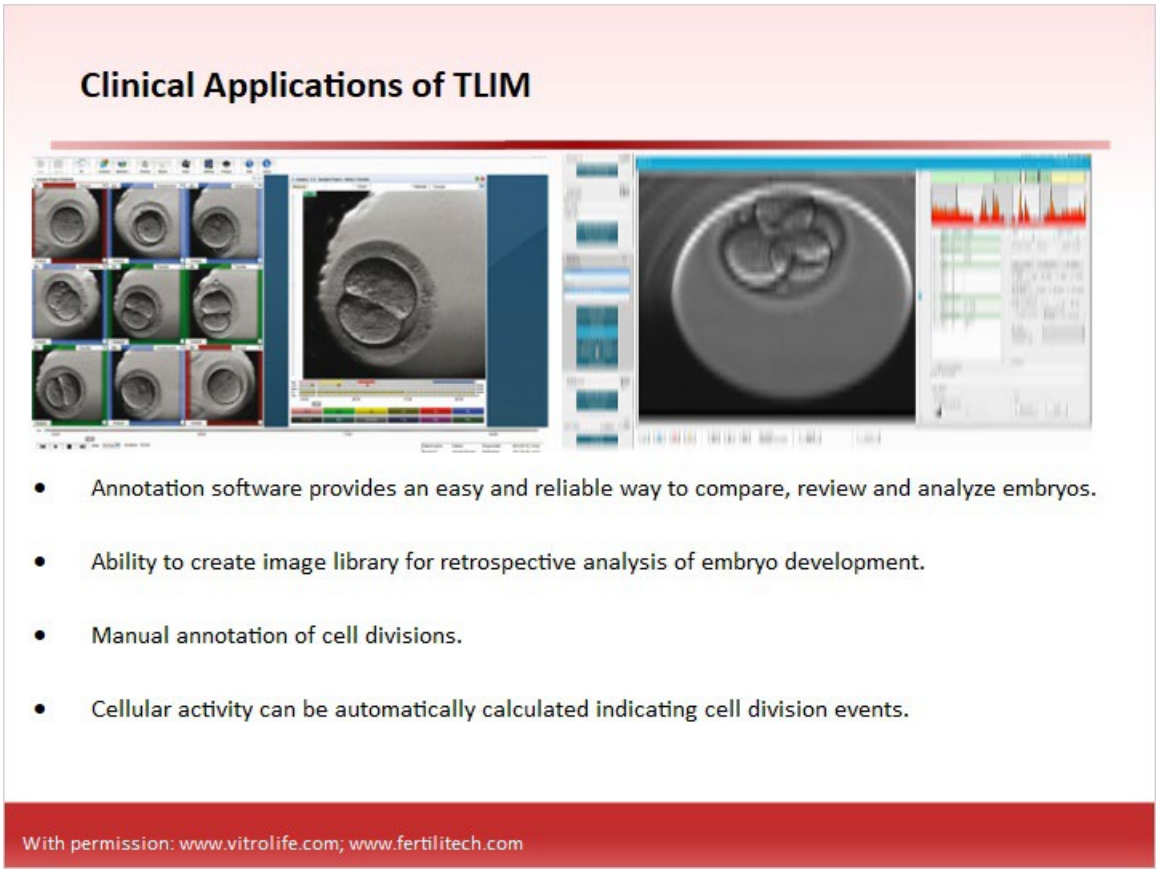
1.40 Video: Quantitative Image Analysis of the Earliest Cellular Divisions and Cytokinesis Events of an Embryo



Notes:

The automated tracking of individual cells that is provided by some time-lapse software allows for quantitative image analysis of the earliest cellular divisions and cytokinesis events of an embryo. Such software, as shown here, provides quantitative data on each embryo's developmental potential by analyzing embryo development against validated cell division timings.

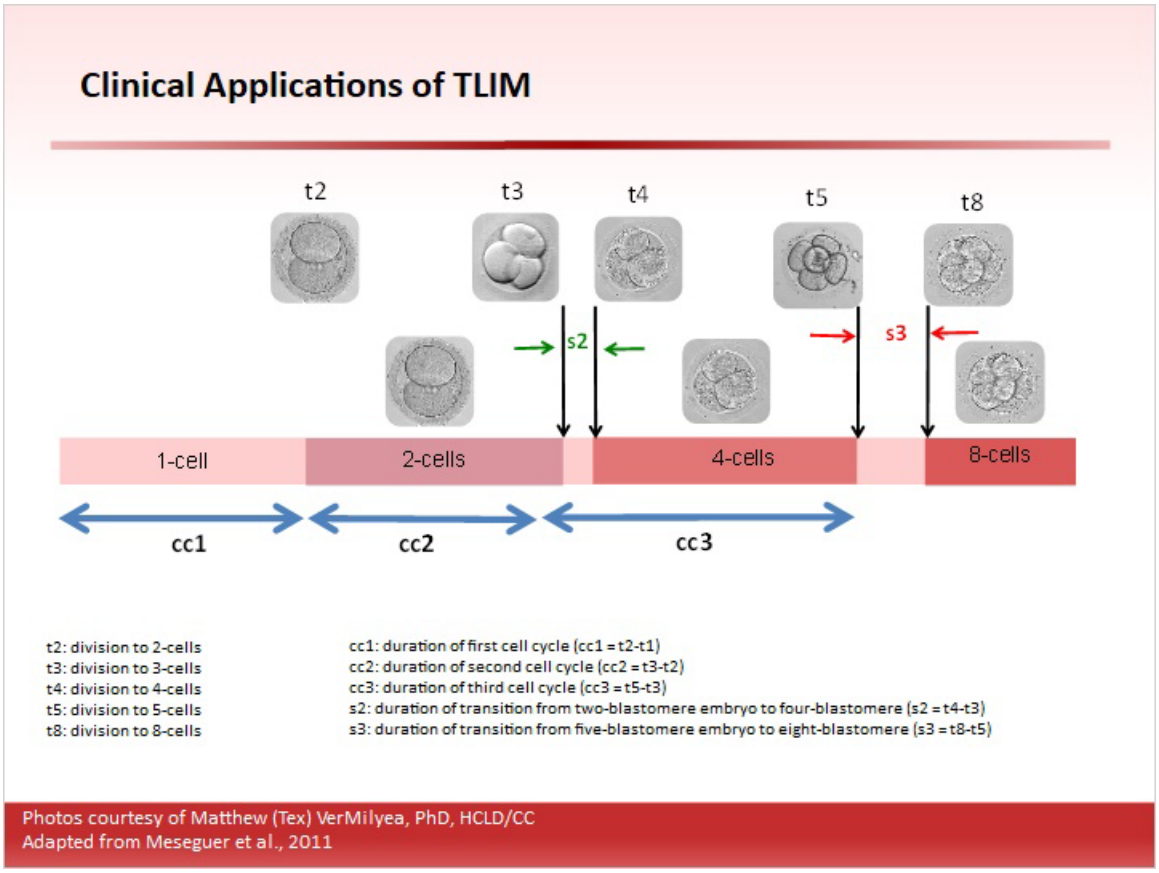
1.41 Clinical Applications of TLIM



Notes:

Time-lapse annotation software provides the embryologist with an easy and reliable way to compare, review, and analyze the development of selected embryos. There is also the ability to create an image library for retrospective analysis of embryo development. Cellular divisions can be manually annotated and displayed in tables and graphs for ease of visual comparison among embryos in the same cohort. Further analysis of cellular activity can be automatically calculated indicating cell division events.

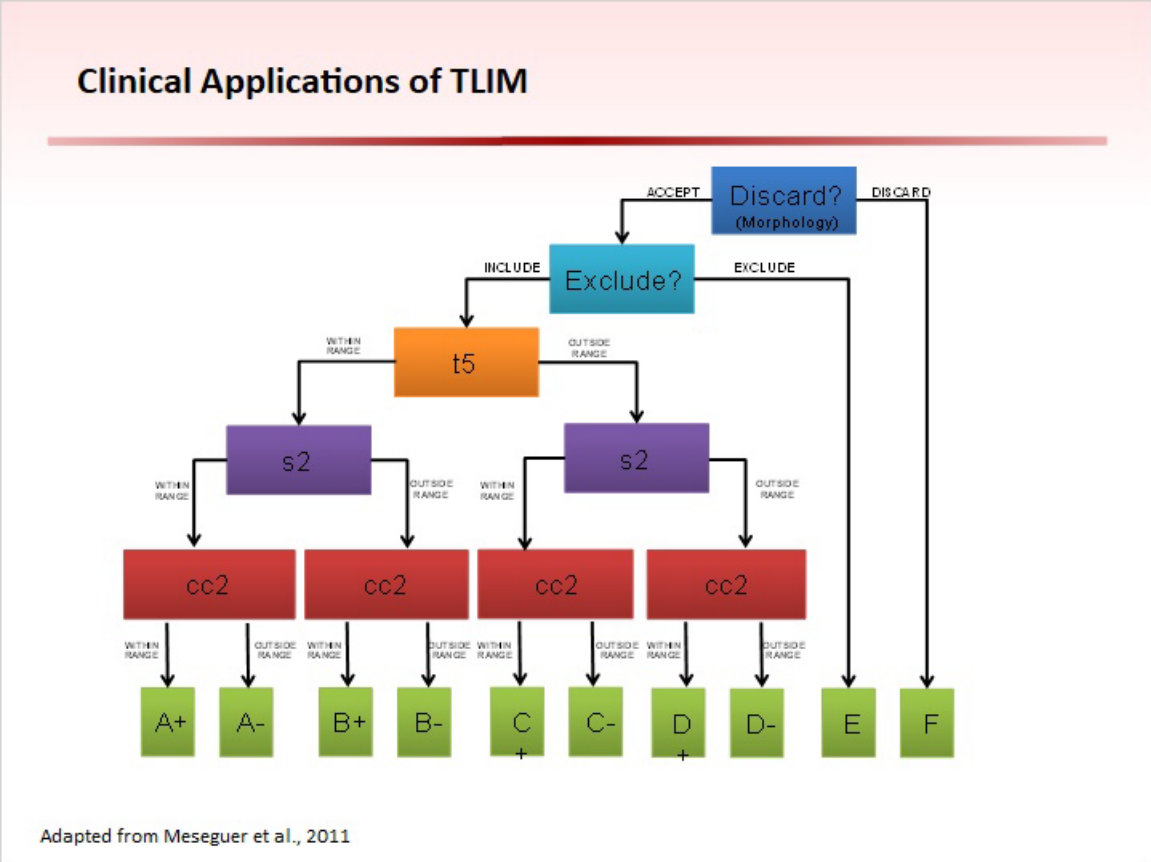
1.42 Clinical Applications of TLIM



Notes:

The retrospective analysis of time-lapse images has provided the annotation and identification of cleavage events in the early embryo. The precise timing of the cell divisions can be identified as the following: the first cell division to two cells is t2 followed by the second division or 2 to 3 cells as t3; the third division to 4 cells or t4; and the division to 5 cells or t5. For this study, all events were expressed as hours post-intracytoplasmic sperm injection (ICSI). The duration of the second cell cycle, or cc2, is the time from division of a 2-blastomere embryo until division to a 3-blastomere embryo. Therefore cc2 equals t3 minus t2. The second synchrony, or s2, is the duration of the transition from a 2-blastomere embryo to a 4-blastomere embryo. Therefore, s2 equals t4 minus t3. This corresponds to the duration of the period as a 3-blastomere embryo.

1.43 Clinical Applications of TLIM

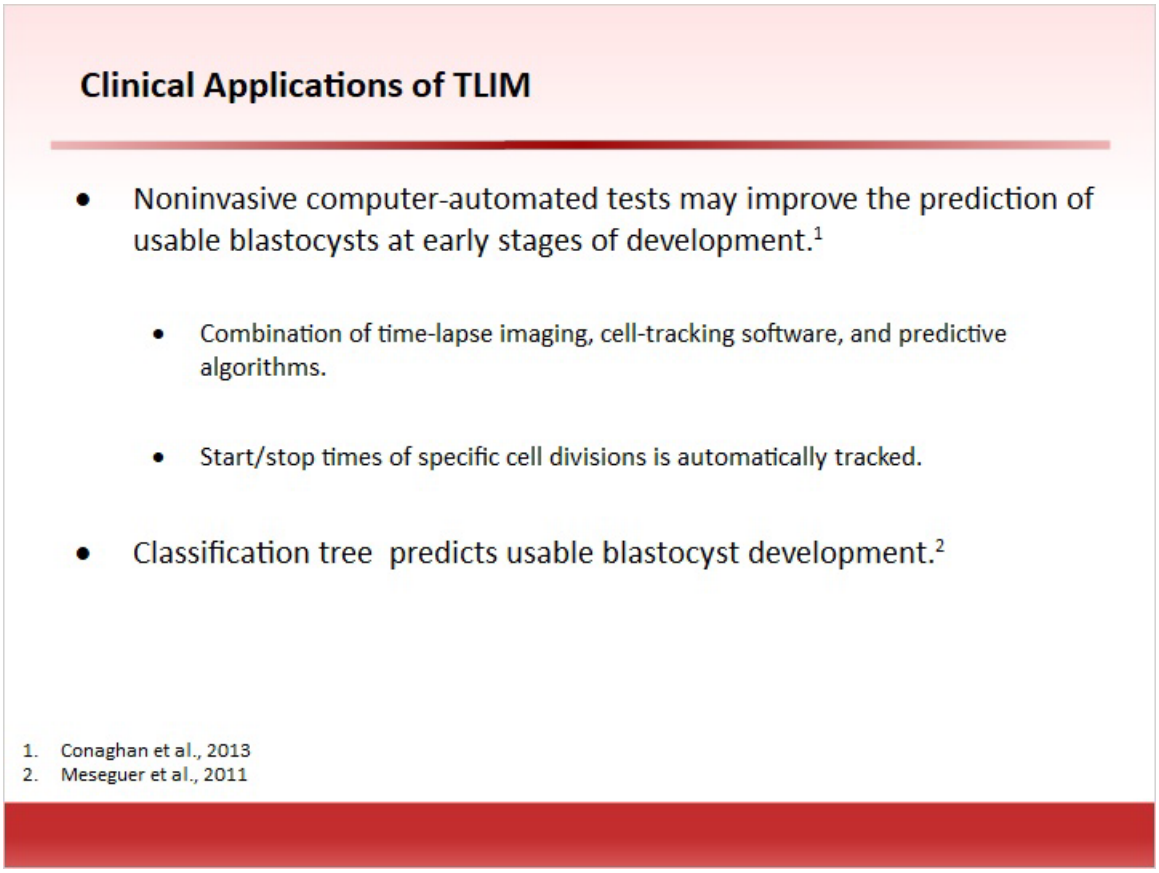


Notes:

Utilizing data from traditional morphological evaluation and identified selection criteria, a hierarchical classification tree model subdivides embryos into 6 categories from A to F. This classification is based on morphological screening, absence of exclusion criteria, timing of cell division to 5 cells, synchrony of divisions from 2-cell to 4-cell stage, and the duration of the second-cell cycle cc2. Categories A through D are further subdivided into subgroups as positive or negative, giving a total of 10 categories. The hierarchical classification tree begins with a morphological screening of all embryos in a cohort that allows for the elimination of any atretic or abnormal or arrested embryos. These nonviable embryos are discarded and not considered for transfer and fall into category F. The next branch of the classification tree is to exclude embryos that fulfill any of the 3 exclusion criteria. These criteria include A) uneven blastomere size at the 2-cell stage; B) rapid cleavage from 1 to 3 or more cells; or C) multinucleation at 4-cell stage. Any embryos that fulfill such criteria are not considered for transfer and fall into category E. The successive branches in the classification tree follow a strict hierarchy based on the timing variables t5, s2, and cc2 that were described previously. If the value of t5 falls in the established optimal range (48.8-56.6 hours) the embryo is categorized as either A or B. If this value is not within the t5 range, the embryo then falls into category C or D. If

the value of s2 falls inside the optimal range of less than or equal to 0.76 hours) the embryo is categorized as A or C, depending on t5. Similarly, if the value falls outside of the optimal range for s2, the embryo falls into categories B or D, depending on t5. Lastly, the embryo is categorized with a positive sign if the value of cc2 is inside the optimal range of less or equal to 11.9 hours. Alternatively, if the value is outside the optimal range for cc2, the embryo is labeled with a minus.

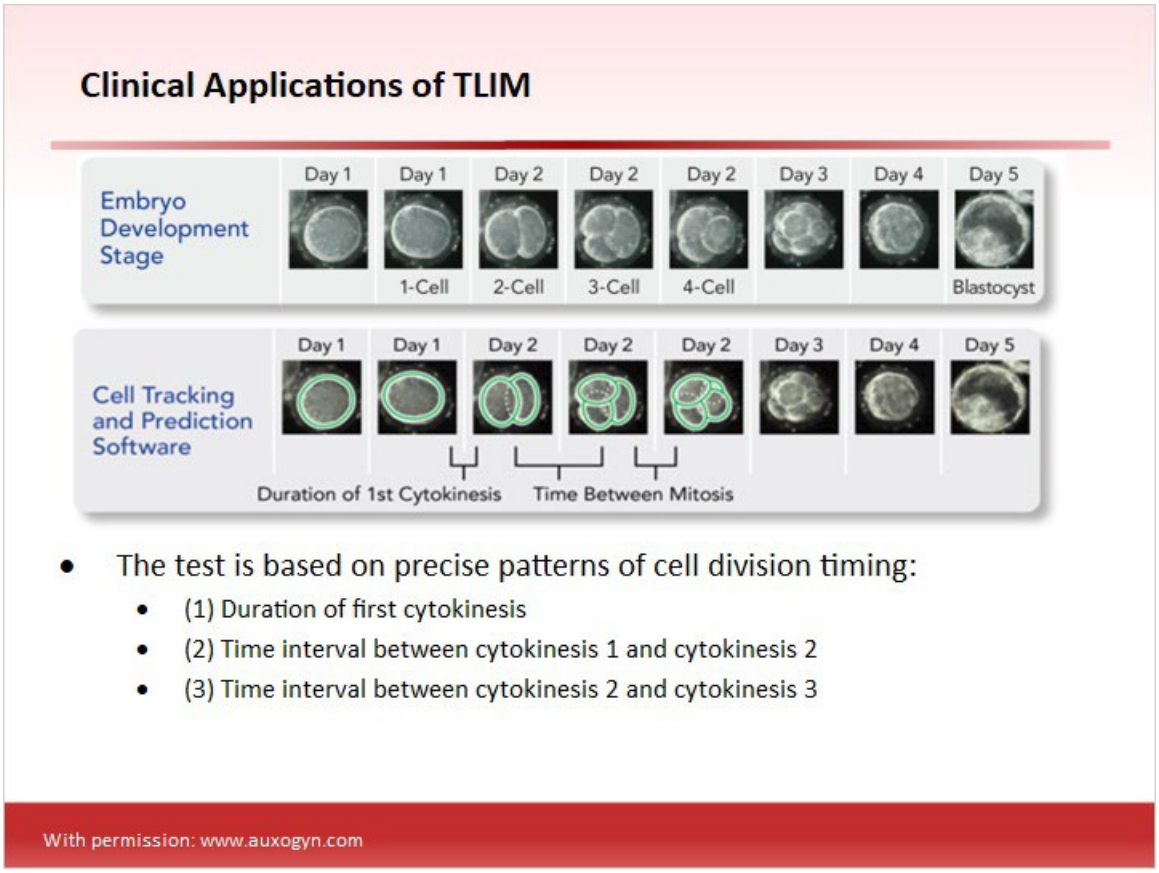
1.44 Clinical Applications of TLIM



Notes:

The application of a noninvasive computer-automated test has also been shown to improve the prediction of usable blastocysts formation by day 3 of embryo culture. This technology combines time-lapse imaging, cell-tracking software, and predictive algorithms. The start/stop times of specific cell division time intervals from the 1- to 4-cell stage are automatically followed and parameter measurements are then applied through a classification tree that predicts usable blastocyst formation on day 3.

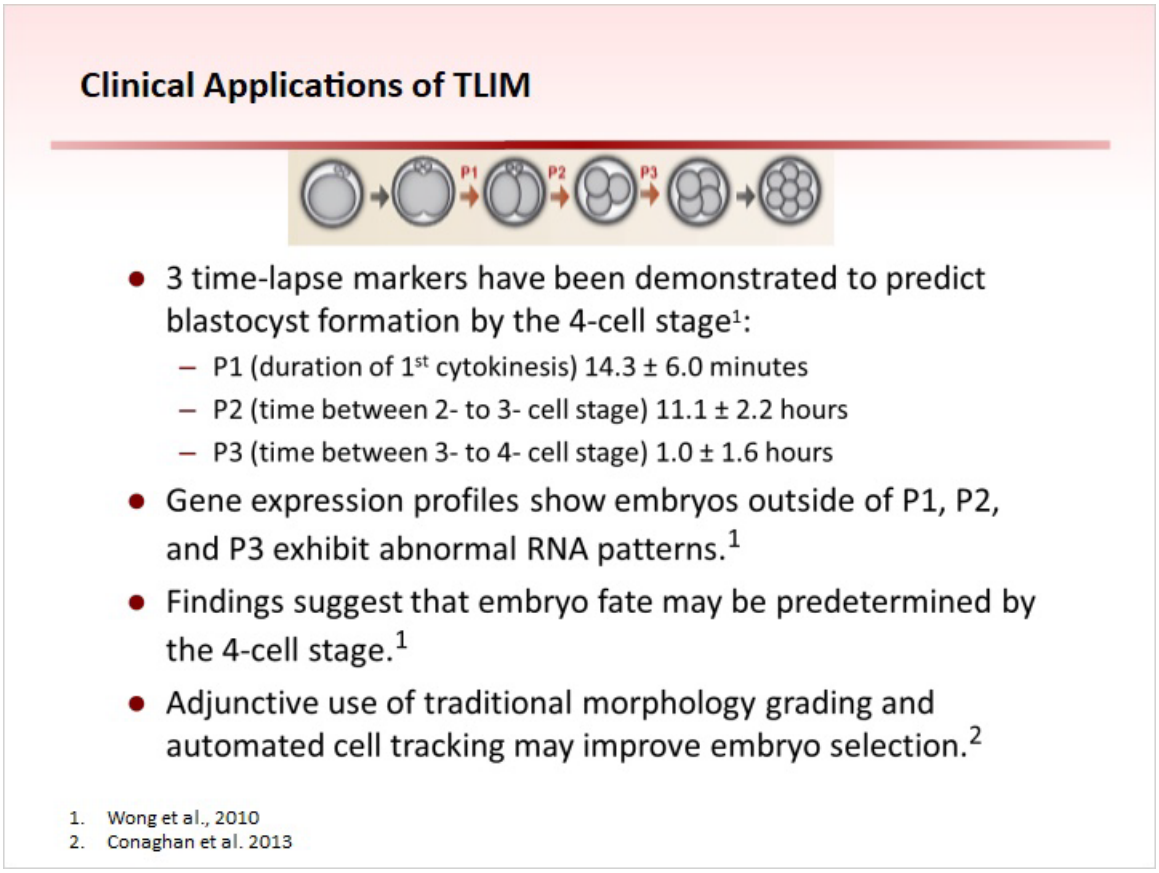
1.45 Clinical Applications of TLIM



Notes:

One such test automatically tracks cell divisions that occur on day 1 and day 2. A prediction of an embryo's ability to develop to a blastocyst is based on the duration of the first cytokinesis and the time interval between cytokinesis 1 and 2 followed by 2 and 3. In other words, how long an embryo takes to go from one cell to two cells and the time from two to three cells and three to four cells.

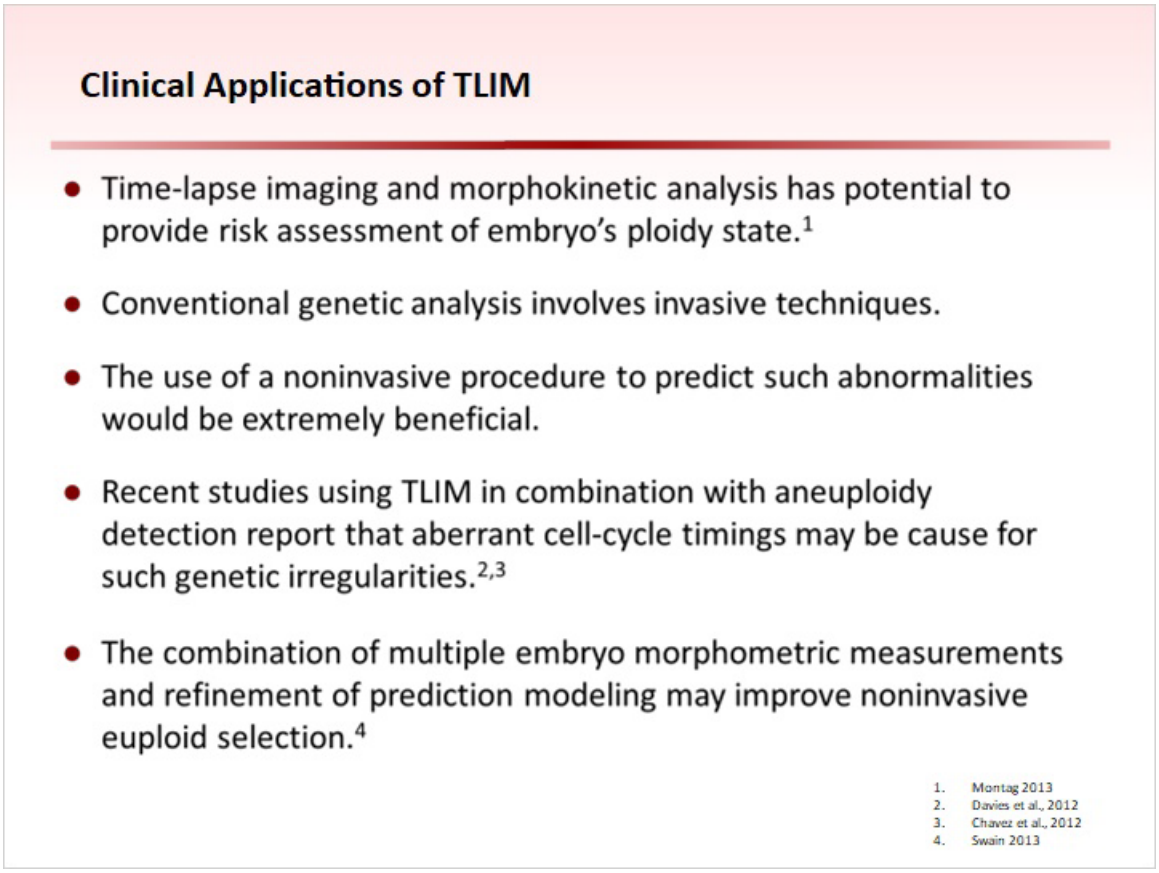
1.46 Clinical Applications of TLIM



Notes:

In this test, 3 time-lapse markers have been demonstrated to predict blastocyst formation by the 4-cell stage. These markers are identified as P1, P2, and P3 and the optimal time ranges are shown here.
Single embryo gene expression profiles also show that embryos outside of P1, P2, and P3 exhibit abnormal RNA patterns for embryo cytokinesis, microRNA production and maternal mRNA reserves. These findings suggest that embryo fate may be predetermined very early in embryo development, specifically by the 4-cell stage.
Key morphokinetic markers of embryo development which can be identified through automation and algorithms may provide embryologists with improved embryo selection when used in combination with traditional embryo grading.

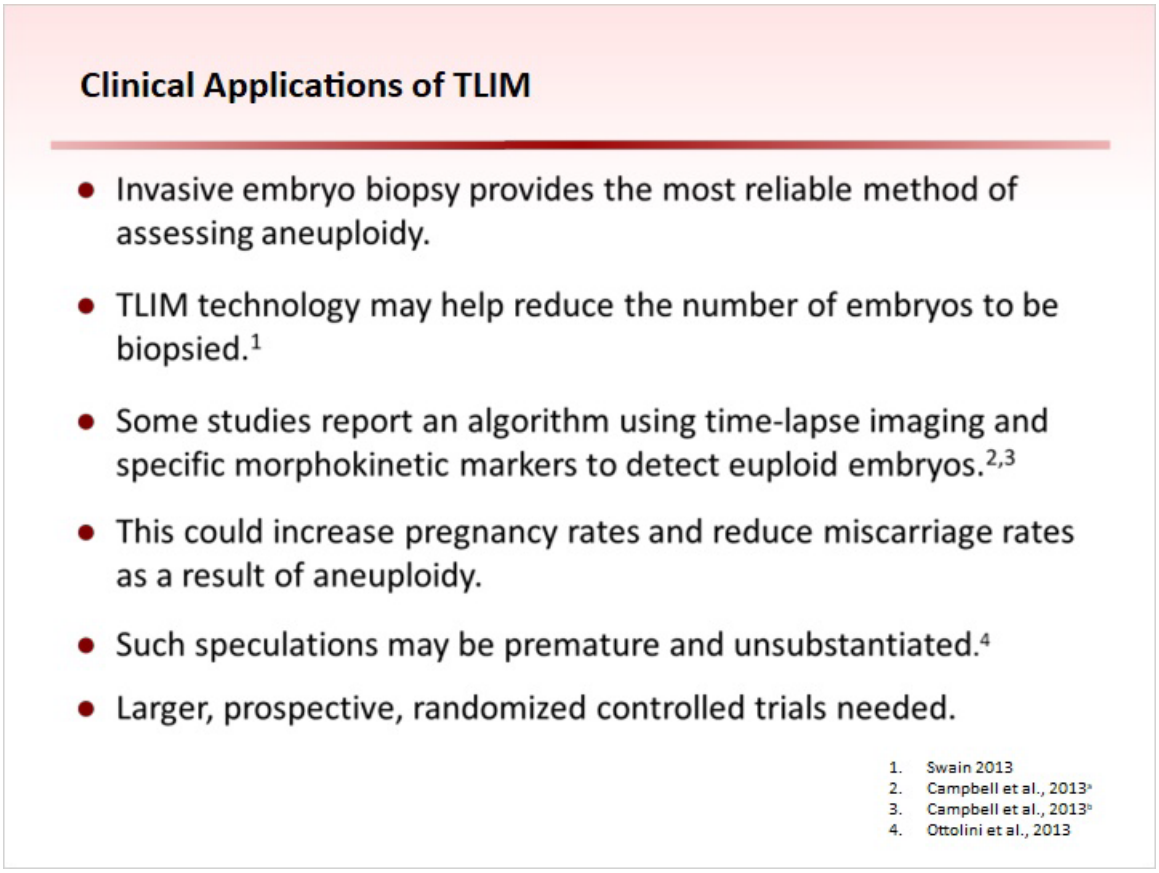
1.47 Clinical Applications of TLIM



Notes:

The use of time-lapse imaging and morphokinetic analysis to identify the risk of embryos for having an aneuploidy chromosome configuration is of great interest. Traditionally, such genetic analysis involves either a blastomere or trophectoderm biopsy. These are considered invasive techniques that require the removal of cells from an embryo for screening. The availability of a noninvasive procedure to predict such abnormalities would allow widespread use of this application for those with TLIM in their laboratories. Recent studies using TLIM in combination with aneuploidy detection report that aberrant cell-cycle timings may be cause for such genetic irregularities. The ability to integrate multiple developmental parameters and endpoints may someday provide a reliable method of assessing embryo chromosomal complement using TLIM.

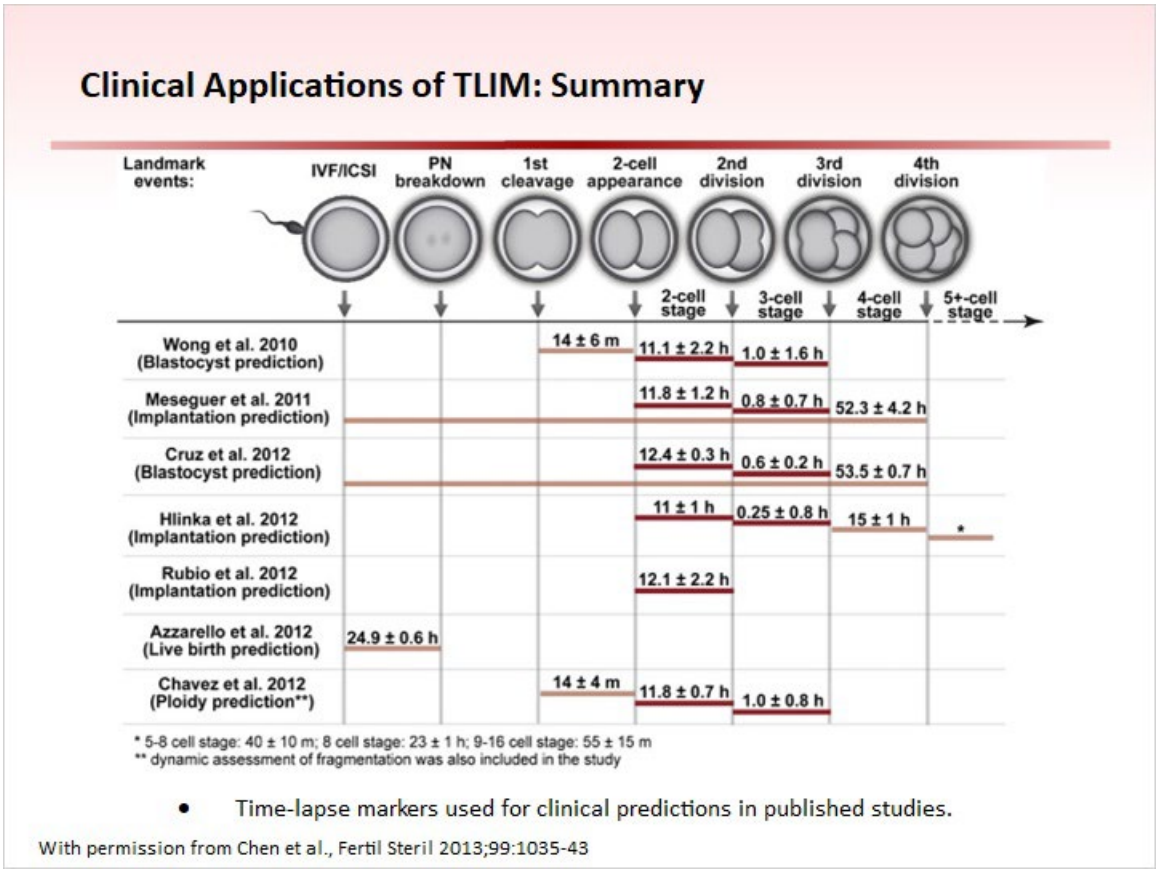
1.48 Clinical Applications of TLIM



Notes:

Although visual and automated observations of embryo morphokinetics by TLIM may help in the selection of euploid embryos, the accuracy is yet to be determined. TLIM may be used to help identify embryos that are more at risk of being chromosomally abnormal and therefore reduce the number of embryos to biopsy. Ideally, an algorithm using time-lapse imaging and specific morphokinetic markers to detect euploid embryos with great accuracy will be further refined. Such an algorithm could increase pregnancy rates and reduce miscarriage rates as a result of aneuploidy; however, others debate that such speculations are premature and unsubstantiated by the current published data. Data from a prospective, randomized, controlled trial is needed to confirm such findings.

1.49 Clinical Applications of TLIM: Summary



Notes:

In recent years, embryo cleavage events have been captured by time-lapse imaging technology and charted to correspond with embryo development and potential outcome. Recent publications have used time-lapse markers to predict various endpoints including development to the blastocyst stage, implantation potential, live-birth prediction and ploidy prediction. By using either manual annotation and traditional morphological evaluation in conjunction with a classification tree or computerized tracking and predictive algorithms, time-lapse technologies hold promise for helping embryologists and clinicians select the most suitable embryos for transfer.

1.50 Thank you!

Notes:

Thank you for participating in this educational activity.