TIME-LAPSE EMBRYO MONITORING

TIs, Tms, Time-Lapse Microscopy, Continuous Embryo Imaging, Primo Vision, Embryoscope, Eeva, Early Embryonic Viability Assessment, Geri

A system for non-invasive embryo observation that allows detection of numerous developmental embryo anomalies.

About Time-lapse embryo monitoring

Time-lapse monitoring is a tool that has been evaluated as an aid to identify the embryo(s) with the highest implantation potential. It allows continuous, non-invasive embryo observation without the need to remove the embryo from optimal culturing conditions. The extra information on the cleavage pattern, morphologic changes and embryo development dynamics could help us identify embryos with a higher implantation potential. These technologic improvements enable us to objectively select the embryo(s) for transfer based on certain algorithms.

The most critical step during in vitro fertilization (IVF) is embryo selection for transfer. For the last two decades, the conventional method of embryo selection for transfer has been based on critical assessment of morphologic parameters during embryonic development. Currently, these morphological assessments are limited to once a day at set time points, since repeated removal of embryos from the incubator environment for observation may result in undesired temperature and pH shifts in the embryo culture dish.

Embryo development is a dynamic event and static observations of embryonic growth can therefore be limiting in their ability to discern differences between embryos at similar cell stages. The introduction of time-lapse imaging and monitoring systems in the clinical IVF laboratory has allowed more detailed observations on embryo developmental kinetics. The precise timing of specific key events of early embryo development are indicators of an embryo’s developmental potential. The ability to continuously monitor an embryo’s progression towards these benchmarks may therefore aid in selecting the best embryos for uterine transfer.

Time-lapse technology benefits

When using conventional incubators, the embryologists usually remove embryos from the incubator once per day to assess cleavage and morphology, but this type of monitoring only gives them a snapshot of a dynamic process. The embryos do not tolerate removal from optimal culturing conditions, which limits the number of observations that can be made. This problem is a significant one for the embryologists, and time-lapse technology offers a solution. With this technology, the embryos can be monitored without removing them from the incubator. A camera is built into the incubator and takes pictures of the embryos at preset intervals. With the help of the proper software, a video can be made that depicts their development. This type of monitoring allows for the collection of much more information on the timing of the cleavages and the dynamics of the morphologic changes.

Various time-lapse systems are currently used. Two of the most widely used technologies, the Primo Vision and Embryoscope systems, both use bright field technology, whereas the EEVA (Early Embryonic Viability Assessment) system uses dark field technology. All systems incorporate a digital inverted microscope that takes a picture of the embryos at 5-20 minute intervals. The images are processed by custom image acquisition and then displayed on a computer screen. The pictures taken at preset intervals are then connected into short films that can be rewound and fast forwarded for detailed analysis.

Dark field vs. Bright field technology
Dark field technology is used to evaluate solely embryo kinetics (cytokinesis). Bright field technology is used to evaluate both the embryo kinetics and the presence of the nuclei. Bright field type is more sensitive in recognizing poor quality and abnormal embryos with low birth potential than dark field technology.

Types of time-lapse monitoring systems

Embryoscope

The Embryoscope (Pic. 2) is an incubator with an integrated time-lapse system, where the embryos, cultured individually in microwells, are moved one by one into the field of view of the built-in microscope at each of the image acquisitions. In the Embryoscope system, embryos are cultured in special culture dishes. This multi-well dish allows the monitoring of up to 12 individually cultured embryos. The Embryoscope can follow 6 of these dishes (max. 72 embryos) simultaneously. It takes pictures every 12-20 minutes and can evaluate the embryos in 7 focal planes. It uses low intensity red LED illumination (635 nm) with <0.5 secundum per image light exposure.

In the Embryoscope, the tray holding the culture dishes is under constant movement to bring each embryo individually into the field of view. When the tray is fully loaded (72 embryos), it takes 20 minutes until the next image of a given embryo is taken. This interval does not allow the embryologist to detect rapid changes accurately (e.g., 51 which should last <30-35 min). The constant movement, electromagnetic effects, heat and volatile organic compounds released from the lubricants related to this technology carry the potential to exert adverse effects though no such negative effect has been directly confirmed yet. However, this technology enables the system to maximize resolution.

Primo Vision

Primo Vision is a compact digital inverted microscope system that is designed to be placed inside of existing small- to large-sized conventional incubators. Control of the system, patient database build-up, embryo development analysis and decision-making are performed outside of the incubator through a controlling unit. Embryos in the Primo Vision system are also cultured in multi-well dishes (Primo Vision embryo culture dish) that contain 9-16 wells. However, embryos in this system are covered by a single drop of culture medium. This system allows individual embryo observation while maintaining the benefits of group culture. The Primo system can monitor up to 16 embryos from the same patient. The units (maximum of 6) are connected with a controlling unit that is outside the incubator with a USB connection. The system uses low intensity green LED (550 nm) illumination and is also able to evaluate the embryos in up to 11 focal planes. Each controlling unit is able to follow a maximum of 96 embryos at the same time.

Each Primo Vision microscope is able to monitor up to 16 embryos at the same time without moving them. With this method, the embryos are cultured in a completely undisturbed environment. This system requires significantly less frequent image acquisitions (because all 16 embryos are observed at the same time); hence, the exposure to light, electricity and electromagnetic effects is even lower than that possible with the Embryoscope. This technology, however, does not provide us with the same image resolution. It needs to be emphasized that the light exposure compared to the current standard light microscope evaluation is significantly reduced with Primo Vision and Embryoscope.

EEVA system

Like the other systems, the EEVA system requires a special microscope to be placed in the incubator. This system uses dark field illumination to better outline the cell membranes. Embryos are cultured in the specially designed EEVA dish. Based on the timing of the early cleavage events up until the 4-cell stage, the software selects the embryos that are most likely to develop to the blastocyst stage (a thin-walled hollow structure in early embryonic development that contains a cluster of cells called the inner cell mass from which the embryo arises). The system analyzes early embryo development and provides quantitative data on each embryo’s developmental potential to the blastocyst stage by tracking the progress for 2 days post-fertilization.

The EEVA system uses dark field illumination, which allows more accurate observations of the blastomere membranes; therefore, divisions can be monitored accurately but the method gives far less information regarding intracellular morphology and has limited ability to follow embryos beyond day 2 with increasing number of cells. The automated system could confuse large fragments with blastomeres, which could therefore affect its precision. The dark filed technology, however, exposes the embryos to significantly higher light load compared to the Primo Vision and Embryoscope. The EEVA system comes with software that predicts which embryo is most likely to turn into a blastocyst based on observations of early markers by day 2 of development. The capacity of EEVA system is based on the capacity of the incubator. The use of the EEVA system has been shown to decrease inter-observer variability and increase the embryologist’s ability to correctly
identify the best embryos.

**Geri**

Geri is an incubator built from six chambers designed to have a single patient in single chamber, while each chamber is controlled independently by own camera. This eliminates the need to move embryos around the incubator, while at the same time minimising any movement of the camera. This compact benchtop incubator has minimal footprint to save lab space. Geri provides basic functions, but have additional software modules to increase the scope of time-lapse functionality.

**Miri**

Miri has six completely separated culture chambers, each hold 14 embryos with a total capacity of 84 embryos. A multi-room incubator has its unwavering advantage over the conventional boxed CO2 incubator. The Miri has twelve temperature sensors (2 for each chamber) to ensure constant temperature stability. Time-lapse system has 5 minute picture intervals from throughout the culture period.

**Success or failure factors**

Time-lapse embryo monitoring is recommended especially for those patients who have repeated failed IVF cycles, the egg donation case, advance maternal age or single embryo transfer.

Time-lapse technology is expected to **improve the embryologist's ability to select the embryo with the highest implantation potential** (even by day 3 or at the blastocyst stage), and this improvement should be translated into an improved clinical outcome. Automated systems that identify the embryo(s) to be transferred with the help of a software program also ease the embryologist’s work.

It has been previously reported, based on the standard morphologic assessment, that earlier cleaving embryos have a better chance to develop into blastocysts and implant. It was also noted that embryos that reach the blastocyst stage are less likely to be aneuploid (an abnormal number of chromosomes in a cell), and implantation rates are higher when blastocysts are transferred. Therefore, many centers use extended culture to the blastocyst stage and perform the transfers on day 5 after retrieval. This practice, however, adds to the work of the embryologist, increases the costs associated with embryology procedures and may be associated with adverse effects due to epigenetic changes, though the data are sparse to support such an effect. On the other hand, blastocyst stage transfer has been shown to result in about a 40% increase in pregnancy rates when compared to cleavage stage transfer. This improvement may be due to better embryo selection or improved embryo-endometrium synchrony. Therefore, despite the slightly higher cost of the cycle with blastocyst transfer, it may save money in the long-run by reducing the number of cycles that have to be performed.

The risk of multiple gestation and the associated maternal and neonatal morbidity/mortality (Plc. 3) has increased significantly over the past few decades. While stricter transfer policies have eliminated the majority of the high-order multiples, these changes have not yet had much of an impact on the incidence of twins. A twin pregnancy can be avoided by the transfer of a single embryo only. However, the traditionally used method of morphologic embryo selection is not predictive enough to allow routine single embryo transfer; therefore, time-lapse embryo monitoring allows identify embryos with a higher implantation potential.

**Complications**

It is important to establish the safety of any new technology. The periodic light exposure, electromagnetic effects, fumes from lubricants, and heat accumulation from the moving parts of the equipment are a potential cause for concern. Nevertheless, recent studies prove that those fears are unnecessary, because embryos are exposed to lower light intensity compared to static observation. Moreover, time-lapse monitoring systems use wave length that does not harm embryos such as white light.

In the Primo Vision system, embryos are exposed to less light compared to the Embryoscope, and because there are no moving parts in the Primo Vision system, one does not have to worry about potential negative effects related to this issue.
Prognosis

The adverse effects associated with using conventional incubators have limited the frequency of microscopic evaluation of embryos, as only limited information about growth and changes in embryonic morphology can be obtained at a few discrete time points. The recent development of time-lapse culture and monitoring has overcome this limitation by combining incubation and observation of embryos into one unique system.

Embryos cultured and selected by time-lapse technology have significantly improved the relative probability of clinical pregnancy (+20.1% per oocyte retrieval, +15.7% per embryo transfer). The elevated clinical pregnancy rate is attributed to a combination of stable culture conditions and the use of morphokinetic parameters for embryo selection.

It has been well documented that the main cause of embryo arrest, implantation failure and pregnancy loss is the presence of numerical chromosome abnormality or aneuploidy. Aneuploidy is the most common abnormality in in vitro fertilized embryos, and increases with maternal age. An interesting model for classifying the risk of aneuploidy has been proposed based on morphokinetics of human embryos that were cultured in the time-lapse system.

Sources


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