

## **NON-INVASIVE EMBRYO ASSESSMENT-BREAKTHROUGH TECHNOLOGIES**

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The success rate resulting from in vitro fertilization (IVF) treatment has improved over the years, e.g., overall 34% of cycles result in a live birth in US (CDC 2007). This increase is largely attributed to improvements in embryo culture conditions and optimization of ovarian stimulation.

Unfortunately, human reproduction is not an efficient process and only 15% of embryos transferred result in a pregnancy. The added financial, physical, and emotional burdens of the IVF process led to a desire for greater success within a given cycle. To achieve high pregnancy rates, multiple embryos are transferred in each cycle to overcome the low implantation rate. As a result, the multiple-pregnancy-rate is disconcertingly high-- nearly 1 of every 3 live births associated with IVF resulted in a multiple gestation, which is 20-time higher than the spontaneous multiple pregnancy rate in the general population.

Twin pregnancies and high-order multiple pregnancies significantly compromise both maternal and perinatal health. The optimal goal of IVF should be the delivery of a healthy singleton. In the future we may be compelled via legal, financial, and moral obligations to reduce multiple pregnancies. The clinical dilemma, therefore, is to restrict the number of embryos for transfer while maintaining or improving IVF success rates. Selecting a viable embryo becomes the key solution.

Over the past decade many technologies were developed to improve embryo selection, in particular, efforts are focused on non-invasive methodologies. This presentation will review the progress in embryo morphology evaluation and the new technologies that enable dynamic embryo development evaluation.

Blastocyst culture was developed to improve embryo selection. It has been broadly reported that embryo implantation rates and live birth rates were significantly improved with blastocyst transfer, as compared to cleavage stage transfer. It has been adopted by many clinics, especially ones that perform elective single embryo transfer. However, the risks associated with a longer culture time have been gradually recognized and are considered significant. Not all embryos can survive the stress of in vitro culture and some embryos arrest when they otherwise would have generated a viable pregnancy if transferred back to their natural environment sooner. Growing evidence suggested longer in vitro culture time may be associated with epigenetic disorders. It was reported that blastocyst transfer was associated with elevated monozygotic twinning rates. Recently studies have shown that poor neonatal outcomes resulted from blastocyst transfers (such as an increased risk of preterm delivery, low birth weight, low APGAR score, and respiratory diagnoses) as compared to cleavage transfers.

Given the above risks related to blastocyst culture, many IVF programs continue to perform cleavage embryo transfer as the standard procedure. Embryos are selected based on traditional morphology scores. Numerous studies have indicated a relationship between embryo morphology and implantation, for example a 2-pronuclear score, early cleavage observation, day 2 and/or day 3 cleaved embryo assessments, and day 5 or day 6 blastocyst grading. The combination of scores at different development stages has also been investigated. The graduated embryo score accounts for the embryo morphology evaluations performed 16-18 hours, 25-27 hours and 64-67 hours following fertilization. It has been suggested that sequential embryo scoring may be predictive of aneuploidy in poor-prognosis patients.

Despite the extensive efforts by embryologists to rate the embryos using a regular light microscope, it is clear that traditional morphological grading only captures static images at a few time points, and is of very limited predictive value. Studies showed that morphological criteria assessed on day 3 did not accurately predict blastocyst formation. Fundamentally, traditional morphology grading is of limited predictive value due to the lack of knowledge of the underlying biochemical basis for embryo morphology.

Preimplantation embryos undergo major changes in their gene expression patterns throughout in vitro culture. The regulation of early embryo development and the mechanism of implantation remains poorly understood. Embryonic development is controlled predominantly by factors stored in the oocyte during the early cleavage stages and the embryonic genome turns on at the 4-cell to 8-cell stage.

Recently, it was reported that time-lapse image analysis correlated with gene expression profiling of preimplantation human embryo development. The researchers discovered that success in human development to the blastocyst stage can be predicted with high sensitivity and specificity via dynamic, non-invasive imaging parameters that are observed prior to embryonic genome activation (on day 2). These parameters can be reliably measured using automated image analysis, confirming that successful development follows a set of carefully orchestrated and predictable events. Moreover, image phenotypes reflected underlying molecular programs of the embryo and individual

blastomeres. Single-cell gene expression analysis revealed that blastomeres can develop cell-autonomously, with some cells advancing to embryonic genome activation while others arrest. The dynamic morphological parameters of embryo development reflect underlying molecular program status, which provides a promising platform for early assessment of embryo viability. The time-lapse technology may supply the additional predictive information of embryo viability, thus enable embryologists to better select embryo(s) for transfer and improve implantation rates. A higher success rate, such as that seen with blastocyst transfers, may be achieved for day 2 embryo transfers; therefore, the adverse events associated with extended culture may be avoided.

In most time-lapse technologies, the embryos are imaged frequently inside the incubator. It is reasonable to raise concerns about the potential risk of exposing embryos to imaging light energy throughout the development process. Fortunately, the calculated light exposure due to time-lapse technology is increased only slightly compared to what the embryos are exposed during routine IVF procedures. Several reports have shown the equivalent embryo development, implantation rates and pregnancy rates between the embryos that are imaged vs. not imaged. Nevertheless, additional studies may be necessary to ensure the safety of imaging procedures.

In summary, new technologies have enabled dynamic embryo development evaluation leading to improved embryo selection. Such non-invasive technologies may promote the acceptance of single embryo transfer at cleavage stage and broadly reduce multiple pregnancies, therefore, further enhance IVF success by improving birth outcomes.