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EMBRYO SELECTION: THE PLACE OF TIME-LAPSE IN SUB-SAHARAN AFRICA SETTING

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ABSTRACT: *In the last two decades, IVF has rapidly evolved in sub-Saharan Africa, and has become the mainstay treatment modality for significant number of infertile couples. As in other parts of the world, implantation failure has remained the bane of successful outcome. To circumvent the challenge, routine grading of the embryo to determine its implantation potential is been practiced in most IVF Centre's. However, the conventional static observation of the embryo morphology is weakly predictive and associated with altering the condition of the culture system. As a result, an emerging technology Time-Lapse has been developed. It has the advantage of assessing the embryo's morphokinetic and enhance the probability of selecting of Euploid embryo without altering the condition of the culture system. Despite these perceived advantages, its optimal benefits may be dependent on the culture methodology adapted by the various IVF clinic. Time-Lapse safety profile has been established and may serve as a veritable tool towards improving embryo selection and IVF success rate.*

Keywords: Embryo, static observation, Time-Lapse, Morphokinetic.

INTRODUCTION

In-vitro fertilization (IVF) has evolved to become a major treatment modality for infertile couples (Wu *et al*., 2017). While the procedure has improved over the years, the efficacy is still very low with a success rate of about 30 – 40% (Kovics, 2014) Comparing many couples to have repeat sessions with the consequent health risk to the woman and as well as financial and emotional stress to the couple (Kaser and Racowsky, 2014). Attempt to maximize the chance of pregnancy has led to the concept of multiple embryo transfers that put the woman at risk of multiple pregnancy and associated complication to mother and baby (Montag *et al*., 2011). As a result, embryo selection becomes a very critical step during IVF, and it requires a critical evaluation of methods of embryo selection aim at determining the reliable type that can predict quality embryo with high implantation potential (Findikli and Oral, 2014). In this case a comparison between the static observation and dynamic method with Time-lapse technique.

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The Static Observation Method.

The static observation assessment of embryo morphology by light microscopy is the practice in most IVF laboratories. This method of embryo selection evaluates the morphological parameters to predict embryo viability (Desai *et al*., 2014). The evaluation guided by the grading system that varies between fertility clinics. In most laboratories, the parameters used in the cleavage stage are the degree of fragmentation, presence and number of pronuclei and the number, size, and symmetry of blastomeres per embryo (Ciray *et al*., 2012). The blastocysts evaluated by the expansion of the blastocoel and the cohesiveness of the cells. Based on these parameters, an international consensus was reached by ESHRE/ALPHA Scientist (ALPHA Scientist 2011). High-quality cleavage stage embryo at day two and three at four cell on day two and eight cell on day 3 with the minimal fragmentation of less than 20% with even size blastomeres and absence of multinucleation. The day 4 to compaction involving the entire embryo and the blastocyst fully expanded with both ICM and TE having many cells that can be easily discernable and cohesive. While the one cell stage based on the size, alignment, the number of pronuclei and the number and position of the pronucleolar precursor bodies. The assessment is often limited to once a day at the predetermined time point as repeated removal of the embryo from the incubator for observation may compromise the desired temperature and PH in the culture system (Desai *et al*., 2014).

Though the method is inexpensive, it is characterized by several drawbacks. These include subjectivity, the need for expertise and high level of inaccuracy (Kaser and Racowsky, 2014). Furthermore, embryo development is a dynamic event and the removal of the culture dish with the embryo from the incubator once a day at a specific time to assess the cleavage morphology only give a snapshot of the dynamic process(Desai *et al.*, 2014). Also, attempt to prevent compromise to the optimal culture condition, restrained the embryologist on the number of observations made on the developing embryo(Kovacs, 2014). The process renders the static observation method limited and static information omitting the dynamic and exact timing of the early mitotic division (Findikli and Oral 2014). Therefore, the assessment of embryo with this method does not give enough information and it has been shown that about 20-40% of the embryo selected this way will implant (Kirkegaard, 2014). Also, several reports on the morphological assessment revealed that embryo that develops to the stage of the blastocyst is not likely to be aneuploidy with higher implantation rate (Kovacs, 2014). This concept heralded the idea of extended culture to the blastocyst stage with its attendant's consequences of cost and potential risk of epigenetic changes to the developing embryo.

In the static observation method, the scoring of the pronuclear is done between 16-18 hours, the first cleavage between 25-26 hours and the embryo day 2 and day 3 scored between 40-42 hours (Kovacs, 2014). This scoring often taken as a representative score for the embryo. Though, this scoring approach widely criticized in the literature (Hlinka *et al*., 2012). The increasing practice of extended culture to blastocyst stage did not create the need for alternative methods(Ciray *et al*., 2012). The introduction of legislation against multiple embryo transfer in many European countries usher in the drive for more suitable methods for predicting viable embryo with high implantation potential, and time- lapse technique noted as a useful option

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(Montag *et al*., 2011). With time-lapse, most of the events scored with the static observation had occurred earlier and may even change within a short time (Desai *et al*., 2014, Kaser and Racowsky 2014). These changes often influence the scoring and prognostic values of these markers. Explains why the majority of the literature did not see any benefit in scoring the pronuclear morphology with the static observation methods (Montag *et al*., 2011, Kirkegaard *et al*., 2014.).

The Dynamic Method.

The dynamic method uses the time-lapse technology to monitor embryos without removing them from the incubator. It operates by taking pictures of the embryos at preset intervals, and a video is made through the relevant software to depict the various stages of the embryo development(Wu *et al*., 2017). As a result, more information made available on the timing of the cleavage and other dynamic morphological changes(Kovacs, 2014). The use of the time-lapse technique has enhanced detailed observations on developmental embryo kinetics *(*Desai *et al*., 2014). Several studies (Ciray *et al*., 2012, Basile *et al*., 2013, Mio, 2014) have suggested that embryo's implantation potential can best be determined by the precise timing of specific events such as pronuclear formation, syngamy, early cleavage, cell cycle interval, the synchronicity of cell division and initiation of blastulation (Desai *et al*., 2014).The effort to monitor embryo towards these benchmarks may result in the selection of the best embryo for uterine transfer(Montag *et al*., 2011).

The two widely used technologies are the Primo vision (Vitrolife) and Embryoscope (Fertilitech) systems. These uses bright field technology while the EEVA (Early Embryonic Viability Assessment, AUXOGYN) system uses dark field technology. All the systems have incorporated digital inverted microscope and take pictures of the embryo every 5-20 minutes intervals. The concern over embryo exposure to light has been shown significantly reduced when compared to the static observation light microscope (Kovac, 2014). The EEVA system uses the dark field, and it exposes the embryo to higher light illumination but its observation is mainly on the blastomeres membranes with less information on the intracellular morphology, and it cannot follow embryo beyond day two. Also, the system can mistake large fragments for blastomeres that often affect its precision (Findikli and Oral 2014). Despite this shortcoming, EEVA system has software that can predict embryos most likely to develop to the blastocyst stage from the early markers of day 2 (Montag *et al*., 2011). With time-lapse, the mitotic events are uniform time-patterning of cleavage clusters and interphase that provide a global information on the cleavage activity of the embryo as a whole (Kaser and Racowsky, 2014). In the process, it able to exclude good looking but incompetent developmental embryo in a non- invasive and contactless manner. The selective power to determine the good quality embryo tends to increase with the number of rated cell cycles and unlay the swift transitory post- mitotic changes associated with embryonic cell number (Ciray *et al*., 2012, Desai *et al*., 2014).

Several studies (Patel *et al*., 2016, Wu et al., 2017, Zaninovic *et al*., 2017) on the merits and demerits of static observation and the dynamic method with time-lapse technique have noted a marked variation in the timing of polar Body extrusion and pronuclear formation with time-lapse technique. Also a significant difference between the oocytes that develop the good quality embryo

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and those that form poor embryo by day three (Iwata and Mio, 2016). Also, an embryo that implanted were faster and synchronous with the appearance of the nuclei of the first cleavage(Kaser and Racowsky 2014). The embryo at four cell stage by day two had the faster disappearance of pronuclei while those with more than four cells had faster cleavage (Desai *et al*., 2014). The various kinetic parameters predicted the embryo's tendency to develop blastocyst (Kovacs, 2014). Furthermore, the first cytokinesis has a mean time of approximately 14.3minutes and the first mitosis and the second mitosis is 11.1 hours while the time between the second and the third is approximately 1 hour (Patel *et al*., 2016). These kinetic markers have been used to develop embryo cleavage time (t2, t3, t4, t5) and the cycle parameters CC2 (2-3 Cell division) and S2 (3-4 Cell division). The morphokinetic parameters now constitute the Know Implantation Data (KID) and as well as form the basis of the hierarchical model for embryo selection. (Hlinka *et al*., 2012). However, these early parameters could not predict implantation except the CC3 (Findikli and Oral 2014); that is the time to reach the eight- cell stage. Studies have shown that implanted blastocyst required a shorter time to reach the eight- cell stage (Kovacs, 2014, Patel *et al*., 2016, Wu *et al*., 2017). While the limitation of the early kinetic markers to predict implantation may be due to maternal genomic expression as embryonic genome activation occurs later between the 4-8 cell stages (Desai *et al*., 2014, Kovacs, 2014). Also, implantation may be determined by other factors like the age, oocyte quality, endometrial receptivity and sperm factor as well as laboratory culture condition influencing blastocyst quality and ploidy (Kaser and Racowsky, 2014)

Impact on Embryo Selection.

Aneuploid embryos are prone to develop implantation failure resulting in early pregnancy loss (Patel *et al*., 2016). While the static observation method has limited ability to identify such embryos (Desai *et al*., 2014), several studies have proposed time-lapse parameters as predictive of aneuploidy (Ciray *et al*., 2012, Findikli and Oral 2014, Zaninovic *et al*., 2017). Though these findings are subject to debate (Kirkegaard, 2014), euploid embryos are more likely to fall within the optimal ranges for t5, CC3 and t5-2 (Kaser and Racowsky, 2014). While these early parameters could not predict aneuploidy embryo, most of the embryos outside the optimal range were associated with fragmentations suggestive of chromosomal anomalies (Desai *et al*., 2014). Furthermore, trophectoderm biopsies with array comparative genomic hybridization (aCGH), suggests the start blastulation (tSB) and time to reach full blastulation (tBL) may be used to select blastocyst at risk for aneuploidy (Desai *et al*., 2014, Kirkegaard *et al*., 2014). Despite the perceived benefits of the time-lapse technique, there are controversies on whether these reported observations are universal without recourse to the culture methodology at the various IVF clinic(Ciray *et al*., 2012, Desai *et al*., 2014, Kaser and Racowsky, 2014). It has showed that embryos in a single step media had accelerated the first mitosis through to the five- cell stage than their counterpart in the sequential media (Cirey *et al*., 2012). Therefore, to validate its clinical use, each laboratory needs to characterize its optimal growth pattern for embryo within its in-vitro culture system.

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CONCLUSION

The time-lapse technique allows the monitor of the dynamic events as it happens in embryo development unlike the snapshots evaluation associated with the static observation. Several events associated with early embryonic development that if the observation made once a day, might miss some of the important changes. Thus, result in failure to identify the best embryo for uterine implantation. It could be useful in areas of research and help to revolutionize quality control in the IVF laboratories. Furthermore, various markers have been used to design hierarchical model with varying degree of predictive ability for implantation and blastocyst formation. However, there is little data on its predictive ability for clinical pregnancy and live birth. The time-lapse technique has been proven to be safe. Instead of being seen as a competing technology, it should engender interest on how it could compliment other methods to improve embryo selection during IVF to enhance success rate.

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