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Review article

Time-lapse embryo imaging and morphokinetic profiling: Towards a general characterisation of embryogenesis

A.A. Mandawala[a], S.C. Harvey[a], T.K. Roy[b], K.E. Fowler[a,*]

[a] School of Human and Life Sciences, Canterbury Christ Church University, Canterbury, CT1 1QU, UK
[b] Genea Biomedx UK Ltd., Discovery Park, Sandwich, UK

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ABSTRACT

In vitro fertilisation is an effective method of assisted reproductive technology in both humans and certain non-human animal species. In most species, specifically, in humans and livestock, high *in vitro* fertilisation success rates are achieved *via* the transfer of embryos with the highest implantation and subsequent developmental potential. In order to reduce the risk of multiple gestation, which could be a result of the transfer of several embryos per cycle, restrictive transfer policies and methods to improve single embryo selection have been implemented. A non-invasive alternative to standard microscopic observation of post-fertilisation embryo morphology and development is time-lapse technology; this enables continuous, uninterrupted observation of embryo development from fertilisation to transfer. Today, there are several time-lapse devices that are commercially available for clinical use, and methods in which time-lapse could be used to improve embryology are continually being assessed. Here we review the use of time-lapse technology in the characterisation of embryogenesis and its role in embryo selection. Furthermore, the prospect of using this technology to identify aneuploidy in human embryos, as well as the use of time-lapse to improve embryological procedures in agriculturally important species such as the pig and cow are discussed.

Contents

* Corresponding author.
E-mail address: katie.fowler@canterbury.ac.uk (K.E. Fowler).

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1. Introduction

In vitro fertilisation (IVF) is one of several well-established methods of assisted reproductive technology (ART) used in clinics globally and the technique gives parents facing fertility disadvantages the chance to have offspring. According to the Human Fertilisation and Embryology Authority (HFEA) 2013, fertility problems affect one in six couples in the United Kingdom and to date 2% of babies born here are conceived *in vitro* (HFEA, 2013). Since the introduction of IVF in the 1970s, this approach has been very successful, but a 100% success rate is still some way from being achieved. IVF is now also making a major impact in various livestock species, and is particularly important in the cattle industry. Unfortunately, the commercialisation of this technique in some other agriculturally important species, such as the pig, is still some way off.

Central to successful IVF in both human and agricultural situations is the transfer of embryos that will implant and develop correctly. In human IVF, a significant risk is multiple gestation pregnancies (Kovacs, 2012) that result, in most cases, from the transfer of several embryos per cycle in order to compensate for comparatively low implantation rates. For example, in 2009, out of 400,000 intracytoplasmic sperm injection (ICSI) IVF cycles, 80,000 resulted in live births; a success rate achieved primarily due to the transfer of two or more embryos in over 75% of the cycles (Ferraretti et al., 2013); of these deliveries, a significant 20% were multifetal pregnancies. Over the decades, the incidence of multiple gestations has led to an increase in maternal and neonatal morbidity/mortality rates (Kovacs, 2012). As such, restrictive transfer policies have now been implemented in a number of European countries following the implementation of the One Child at a Time report by the Human Fertilisation & Embryology Authority (HFEA). Procedures to develop single embryo selection techniques that could potentially eliminate the incidence of multiple gestations (with the exception of monozygotic twinning) are also being investigated (Practice et al., 2013). Such procedures require assessment of the outcome of fertilisation and assessment of embryo quality. This is routinely done by observing the embryo microscopically to confirm that fertilisation has occurred and that development is proceeding as expected. An alternative to this is the use of time-lapse monitoring.

Traditionally, embryo monitoring subsequent to IVF involves removing embryos from the culture environment, at least once per day, to evaluate embryo morphology and developmental progression. This method only provides embryologists with one still image, per day, of a dynamic process. Additionally, culture conditions such as temperature, pH and humidity are disrupted which in turn has been shown to have a detrimental impact on the embryo's development (Campagna et al., 2001). Time-lapse technology, is a non-invasive, alternative to this approach that permits continuous, uninterrupted, observation of embryo development from the point of fertilisation to transfer (Kirkegaard et al., 2012). Under time-lapse imaging, the culture conditions are far less disturbed and embryologists are provided with additional, more detailed information such as cleavage patterns, the timing of cell divisions and changes to embryo morphology. Taken together, consideration of these parameters allows selection of embryos with higher implantation potential (Azzarello et al., 2012). The first use of time-lapse technology in embryology was in 1929 to map the development of rabbit embryos (Massip and Mulnard, 1980). The first reported use of time-lapse in human IVF however, was not until several decades later in 1997, when the technology was used to map the development of human embryos that had been fertilised by ICSI (Payne et al., 1997). Since then, several time-lapse devices have been devised, and many studies have been performed to assess how time-lapse technology could improve prospects in embryology for both human and non-human animal species. This review will focus on the use of time-lapse technology in the characterisation of embryogenesis and its role in embryo selection in several species including the human, mouse and some agriculturally important livestock species. Furthermore, the prospect of using the technology to identify aneuploidy in embryos has been discussed.

2. Time-lapse analysis of embryogenesis

Time-lapse embryo imaging enables non-invasive observation of key developmental markers such as polar body extrusion, pronuclear formation, cleavage timings and patterns as well as enabling identification of fragmentation throughout the developmental process (Leary et al., 2015). Time-lapse analyses of *in vitro* embryo development have been conducted in several species, which include but are not limited to mouse (Beraldi et al., 2003; Togashi et al., 2015; Yamazaki et al., 2007), hamster (Gonzales et al., 1995) and zebrafish (Cooper and D'Amico, 1996). This approach has also been successfully used in the analysis of embryogenesis in the invertebrate model organisms *Drosophila melanogaster* and *Caenorhabditis elegans*. These systems demonstrate the experimental power allowed when, for example, hundreds of *Drosophila* embryos can be simultaneously and precisely oriented and imaged (Chung et al., 2011; Levario et al., 2013; Yanik et al., 2011). Similar to the situation in *Drosophila*, techniques have been developed that combine microfluidics, automated imaging and automation of image processing to allow the analysis of aspects of embryogenesis in the nematode *C. elegans*. For example, such systems have been used to quantify the effects of mutations on the timing of various stages of embryogenesis differences (Cornaglia et al., 2015).

When imaging embryos, both the duration of light exposure and the wavelength of the light should be considered; for example, it has been shown that low wavelength light (below 550 nm, approximately 15% of the light emitted from a standard microscope used for IVF) impedes embryo development (Oh et al., 2007; Takenaka et al., 2007), due to localised heating causing DNA damage or generating free-radical species in the blastomeres (Frigault et al., 2009; Wong et al., 2013). This is of particular importance when transitioning away from standard embryo monitoring (for example, taking an image once per day), to time-lapse microscopy, where in some cases, numerous Z-stack images are taken every few seconds or minutes; such procedures risk damage to the embryo, particularly