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Culture without the petri-dish

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Abstract

Automation of oocyte maturation and embryo production techniques is a new and exciting development in the field of reproductive technologies. There are two areas where increased automation is having an impact: in the area of embryo diagnostics and in the process of embryo production itself. Benefits include decreased staffing and skill requirements for production and assessment of embryos, as well as increasing quality management systems by removing the ''human'' factor. However, the uptake of new technologies is likely to be slow, as costs and the conservative nature of the Assisted Reproduction Technology industry to adopt new techniques.

Keywords: Automation of oocyte maturation; Embryo production techniques; Automation of embryological processes

1. Introduction

Automation of embryological processes which we currently undertake by hand in the laboratory, is a natural and logistical consequence of broad thinking about ensuring safety, quality and increasing efficiencies in laboratories without the burden of extra specialist technical support.

I define automation as the process of removing the ''human factor'' from the process. In embryology, this can then be largely defined into two areas: automation of embryo production/manipulation and automation of embryo selection. These are not necessarily mutually exclusive of each other though, as embryo production may also involve some form of selection pressure, in the process of production.

2. Identifying the "best embryo"

2.1. Image analysis

Time-lapse videomicroscopy is the earliest form of image analysis used to measure embryo development. Using such technology, accurate measurements of specific embryological events, such as pronuclei formation, early cleavage events, compaction and blastulation have been recorded (e.g. [1]). Use of videomicroscopy has yielded valuable information regarding the rate of cleavage and subsequent viability. Holm et al. [2] have used the technique to show that faster cleavage divisions during early cleavage in bovine embryos result in embryos more likely to reach the blastocyst stage.

With the advent of digital imaging and increased computer graphic analytical power, some researchers have begun to use image analysis in morphological assessment of embryos. Factors such as grey-scale pixelation (a measurement of light impedance), size and

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shape of early cleavage embryos is currently under investigation, although there is little evidence to suggest that such parameters actually identify embryos with improved developmental outcomes (see ''Embryoguard'' www.cryo-imt.com). Recently, the use of the ''Pol-Scope'', which combines innovations in polarization optics with novel image processing software, allowing measurement of birefringence at all points of the image, has also been suggested as useful in the development of non-invasive measurable morphological parameters correlated to embryo quality (e.g. [3,4]).

2.2. Biochemical analysis

Energy substrate utilization has long been targeted as a method of choice for determining the most ''viable'' embryo. Several studies have been undertaken to correlate viability with a metabolic parameter(s) [5–8]. The most widely studied substrates are the carboxylic acids (pyruvate and lactate) and glucose. Non-invasive measurement of these substrates has been conducted using ''Lowry'' type assays, which incorporates the reduction or oxidation of NAD(P)/H with substrate specific dehydrogenase enzymes [9]. NAD(P)H is fluorescent, whereas the reduced form is not. Thus, quantification of energy substrate depletion (or addition) from an incubation medium is measured by the production or loss of NAD(P)H and quantitative fluorometry. As such small volumes of medium are usually involved to incubate an embryo while it is undergoing substrate uptake or production, it is usual to measure fluorescence with a photometer attached to a microscope. There is evidence that glucose metabolism, especially the rate of glycolytic activity, can predict embryo quality. Recently, Gardner et al. have described that glucose uptake appears to be correlated to human blastocyst quality and this, in turn, is correlated to implantation success [6]. Renard et al. [10], using Days 10–11 bovine embryos, have observed a similar result. However, further development of such techniques are required, as ''Lowry'' based analyses requires an incubation period and therefore is not an immediate, real time assay.

Recently, Houghton et al. [7] have described that the profile of amino acids taken up by human Days 2–3 embryos predicts development to the blastocyst stage and Brison et al. [11] have followed amino acid turnover by embryos and found correlations with embryos survival following transfer. These analyses involve firstly incubation of embryos in culture medium to allow amino acid flux to occur, followed by chemical alteration of the amino acids to allow separation and then high-performance liquid chromatography. As with other metabolic substrate assays, this suffers from not being an immediate, real time assay.

Finally, oxygen consumption has also been mooted as indicative of embryo quality [12]. However, there are enormous practical difficulties in measuring O_2 consumption by a single embryo. Houghton et al. [13] developed a technique for measuring O_2 consumption using a non-invasive fluorometric method, which required several hours incubation. However, the sensitivity of the technique (approximately 0.5 nl/embryo/h) was too high to be used for single embryo assessment (the uptake of a bovine blastocyst is approximately 0.5–1.5 nl/embryo/h [14,15]. Recently, new microprobe technology has emerged which can measure either ions or molecules such as O_2 [16]. Measurement is rapid but requires specific skills and equipment. Furthermore, these authors (as have others) have revealed that O_2 consumption is problematic, as there is a considerable amount that is not linked to ATP-production, most likely utilized by oxidases on the surface of the embryo. A custom-built sensor approach to measure O_2 consumption in embryos is now being offered by Unisense (http://www.unisense.com) and validation of the process has been conducted [17].

The development of thin-film sensors, which can be used in lamination with plastics and permeable polymers offers a new and exciting alternative for real time, rapid measurement of temperature, gases, small molecular weight molecules and proteins. Protein sensing usually involves some aspect of ligand protein-binding protein interaction (such as an immunoglobin selective for a particular ligand protein), which is in turn bound to a reactive material that responds to ligand interaction, allowing the detection of proteins in solution.

Already this technology is making its mark in food and health safety areas. For example, gas detection using thin-film technology makes for the production of cheap portable carbon monoxide or ozone detectors, to measure such gases at very low levels in the atmosphere (parts per billion).

3. Automation of embryo production and manipulation

3.1. Laser manipulations

Lasers have been used for several years in human embryology, especially in the field of ''assisted hatching'' and ''pre-implantation genetic diagnosis''. High-energy lasers are used to partially or fully dissect