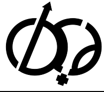

Metabolism in the pre-implantation oocyte and embryo

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Abstract

An understanding of oocyte and embryo metabolism is critical to understanding and developing *in vitro* culture systems. In the last 60-70 years there has been a constant evolution in the way metabolism studies have been conducted. This includes a change from studying the metabolism of the oocyte alone *vs*. as a whole cumulus oocyte complex. The study of *in vivo* environments has lead to the creation of defined sequential culture systems, resulting in overcoming developmental blocks and improved embryo development. And techniques for studying metabolism have evolved from the use of radiolabelled isotopes to increasingly specific fluorescence probes and metabolomics, allowing for large, integrative profiles. Metabolism is a potential diagnostic for selecting the most likely embryos to implant. We envisage the future of metabolism will involve the ability to measure 'more-in-less' (more substrates, less volumes) and allow for a holistic approach to understanding the relationship between metabolism and developmental competence, as it is unconceivable that a single metabolic output will be able to assess health and/or quality.

Keywords: embryo, *in vitro* embryo production, metabolism, oocyte.

Introduction

Fifty years ago Robert Edwards discovered that mechanical release of an oocyte from the ovarian antral follicle could initiate the final stages of oocyte maturation (Edwards, 1965). Since then, *in vitro* oocyte maturation (IVM), *in vitro* fertilisation (IVF) and culture of embryos post-fertilisation (*in vitro* embryo culture, IVC); collectively known as *in vitro* embryo production (IVP), has been widely utilised for the study of pre-implantation oocyte and embryo development and is increasingly utilised in livestock animal production and human assisted reproduction.

An understanding of the metabolism of cumulus oocyte complexes (COCs) and embryos is critical, not only to enable the creation of improved culture systems, resulting in the development of healthier *in vitro* produced embryos, but metabolism is a potential marker of developmental competence, determining which embryos are the healthiest and thereby have the highest chance of implantation and a healthy pregnancy.

There are numerous excellent review articles covering metabolism of the COC (Sutton *et al.*, 2003b; Thompson *et al.*, 2007, 2014; Sutton-McDowall *et al.*, 2010; Krisher, 2013) and the embryo (Bavister, 1995; Thompson, 2000; Leese *et al.*, 2008; Leese, 2012). With this in mind, the focus of this review is to present a brief synopsis of changes in pre-implantation metabolism through development, limitations to the current metabolic diagnostics used and possible future directions for determining metabolism of COCs and pre-implantation embryos. Furthermore, while we acknowledge that the COC and embryo utilise many energy sources such as lipids (Sturmey *et al.*, 2009; Dunning *et al.*, 2014) and amino acids (Wale and Gardner, 2012), this review will focus on the metabolism of carbohydrates and downstream signalling molecules.

Metabolism: timing (and stage) is everything

The peri-conception period, covering the final stages of oocyte maturation through to pre-implantation embryo development, is a highly dynamic period, with the COC and pre-implantation embryo exposed to several different micro-environments, ranging from the highly vascular, hence highly perfused ovarian follicle to the low oxygen levels (Tervit *et al.*, 1972; Maas *et al.*, 1976; Fischer and Bavister, 1993) and more mucus environment of the uterus. It is well established that the metabolism of the COC and pre-implantation embryo varies (Fig. 1) and this is largely reflective of the *in vivo* environment (Krisher, 2013).

In an attempt to improve IVP success, culture systems have been formulated based on the composition of the *in vivo* environment (reviewed by Summers and Biggers, 2003; Sutton *et al.*, 2003a; Table 1), resulting in significantly higher rates of developmental competence and pregnancy success rates. Indeed, pioneering work by Tervit and colleagues used the composition of sheep oviductal fluid (characterised by Restall and Wales, 1966) to create synthetic oviductal fluid (SOF) and performed culture in low oxygen concentrations (Tervit *et al.,* 1972), a system that is still widely utilised, with modified versions used throughout

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IVP in larger animals (Gandhi *et al.*, 2000).

However, due to the static, yet highly chemically defined nature of culture systems, *vs.* the highly perfused and complex environments *in vivo*, there is room for improvement and consequently the compositions of IVP media suites are constantly evolving. To date, the most successful media suites include sequential media to accommodate changing metabolic needs (Summers and Biggers, 2003; Lane and Gardner, 2007), although this is challenged within the human IVF field, suggesting that single media systems are suitable (Cohen *et al.*, 2008; Paternot *et al.*, 2010).

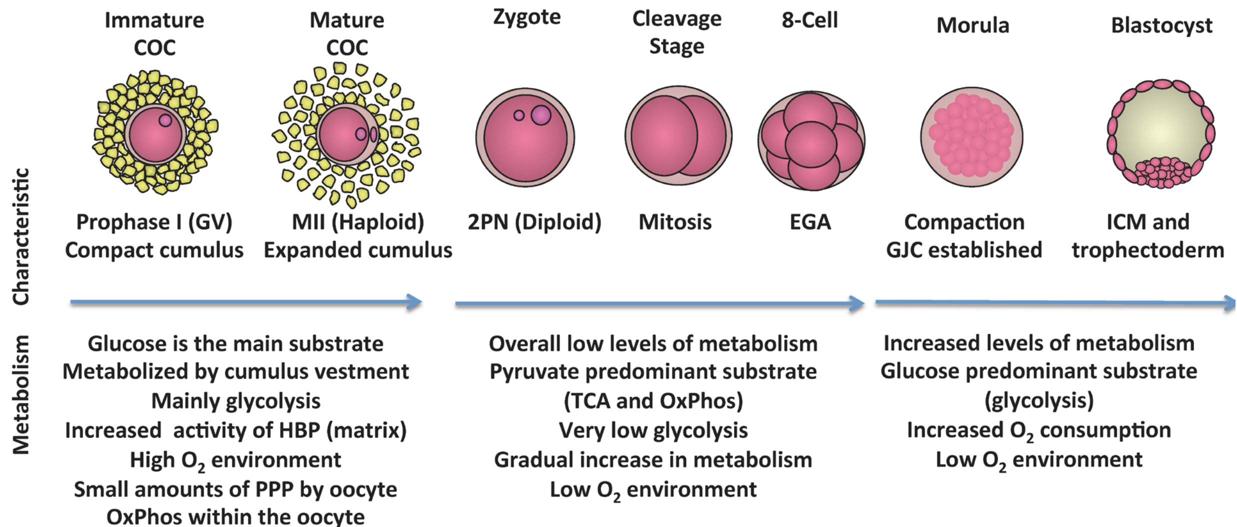

Figure 1. Changes in the metabolism of cumulus oocyte complexes (COCs) and preimplantation embryos. 2PN = 2 pronuclei; GJC = gap junction communication; GV = germinal vesicle; HBP = hexosamine biosynthetic pathway; ICM = inner cell mass; OxPhos = oxidative phosphorylation and TCA cycle = tricarboxylic acid cycle.

Table 1. Carbohydrate composition of the *in vivo vs. in vitro* environments that cumulus oocyte complexes (COCs) and embryos are exposed to.

	COC		Fertilisation		Embryo		
	In vivo	*In vitro*	*In vivo* (Oviduct)	*In vitro*	*In vivo* (Uterus)	*In vitro* (Cleavage)	*In vitro* (Post-Compaction)
Glucose (mM)	1.4-2.3[1] 2-3.8 [2]	1.5 (SOFM) 5.6 (M199)	2.4-3[3] 0.5-3.11[4]	2.8 (HTF) 0 (Fert TALP)	0.5[5] 0.02-0.04[6] 3.15 [4]	1.5 (SOFC1) 0.5 (G1.2)	3 (SOFC2) 3.2 (G2.2)
Lactate (mM)	3-6.4[1] 5-14.4[2]		2.5[7] 4.9-10.5[4]	21.4 (HTF) 10 (Fert TALP)	8.6[5] 5.9[4]	10.5 (G1.2)	5.9 (G2.2)
Pyruvate (mM)	0.4 [1]	0.33 (SOFM) 0.2 (M199)	0.2[7] 0.24[4]	0.33 (SOFM) 0.3 (HTF) 0.2 (Fert TALP)	0.17[5] 0.1[4]	0.33 (SOFC1) 0.32 (G1.2)	0.33 (SOFC1) 0.1 (G2.2)

SOF = Synthetic Oviductal Fluid; HTF = Human Tubal Fluid (Quinn *et al.,* 1985); Fert TALP = Modified Tyrode's Medium (Gardner *et al.,* 2004); G1.2/G2.2 (Lane *et al.*, 2003). [1]Sutton-McDowall *et al.*, 2005; [2]Leroy *et al.,* 2004; [3]Lippes *et al.,* 1972; [4]Gardner *et al.,* 1996; [5]Dickens *et al.*, 1995; [6]Carlson *et al.*, 1970 and [7]Lopata *et al.,* 1976.

Pre-ovulation: the cumulus oocyte complex

Historically, the carbohydrate metabolism of the oocyte has been described (Biggers *et al.*, 1967; Rieger and Loskutoff, 1994; Bavister, 1995; Krisher and Bavister, 1999; Spindler *et al.*, 2000). However, in the last decade, the importance of the cumulus cells supplying the oocyte with nutrients and substrates to achieve developmental competence has emerged (Dumesic *et al.*, 2015), as a consequence of understanding the importance of the bi-directional communication between the oocyte and cumulus vestment (Eppig, 1991; Albertini *et al.*, 2001; Matzuk *et al.*, 2002). Thus, characterisation of the metabolic profile of the COC as a whole is essential in our view. However, the COC contains two distinct cell types with

different metabolic profiles: the oocyte predominantly undergoes oxidative phosphorylation and the cumulus vestment has a high rate of glycolytic activity (Thompson *et al.,* 2007). The primary substrate of the COC is glucose and is metabolised via numerous pathways to provide energy and substrates for extracellular matrix formation and cumulus mucification, nucleic acid synthesis and plays a major role as a stress/fuel sensing molecule (reviewed by Sutton-McDowall *et al.,* 2010). With the progression of COC maturation, metabolism increases steadily, with increases in glucose, pyruvate and oxygen consumption observed (Sutton *et al.,* 2003a).

The environment in which a COC is exposed to during maturation, both *in vivo* and *in vitro*, largely impacts its developmental competence (Sutton *et al.*, 2003c; Krisher, 2013; Dumesic *et al.,* 2015). For example, maternal hyperglycaemia and hyperlipidemia compromise COC health, embryo development and pregnancy outcomes (Chang *et al.*, 2005; Leroy *et al.*, 2008; Robker, 2008; Purcell and Moley, 2011; Van Hoeck *et al.*, 2011). To date, the technology to measure the metabolism of oocytes and COCs within the ovarian follicle does not exist, with measurements performed *ex vivo* and usually with some degree of further *in vitro* manipulation. This includes physical removal from the follicle, exposure to culture media, sometimes combined with hyperstimulation to retrieve adequate numbers of COCs. This begs the question as to the influence of even brief exposure to *in vitro* conditions on the metabolism of *in vivo* derived COCs. We have reported that even a brief exposure (1 h) of immature mouse COCs to "collection" media containing different concentrations of glucose can have a dramatic effect on post-fertilisation embryo development (Frank *et al.*, 2013). Aspiring to determine the precise differences between the metabolism of *in vivo* and *in vitro* matured COCs is not possible, as *in vivo* derived COCs must be removed to measure their metabolism.

Over the past decade, improvements in IVP success have largely been attributed to improved IVM culture systems, by creating environments that more closely mimic *in vivo* conditions. Systems that are more *in vivo*-like provide clues as to which metabolic parameters are associated with improved developmental competence; these are emerging from studies with media additives that improve COC development. An example is the addition of exogenous oocyte secreted factors (OSF), specifically recombinant bone morphogenetic protein 15 (BMP15) and growth differentiation factor 9 (GDF9), resulting in improved developmental competence (Gilchrist and Thompson, 2007). While OSFs promote the distinct cumulus cell phenotype such as mucification and proliferation (Buccione *et al.*, 1990; Salustri *et al.*, 1990a, b); steriodogenesis (Vanderhyden and Macdonald, 1998) and prevention of cumulus cell apoptosis (Hussein *et al.*, 2005), OSF also promote cumulus cell metabolism, as both glycolysis and *de novo* cholesterol biosynthesis is compromised within cumulus cells of oocytectomised complexes (OOX, a COC in which the oocyte is surgically removed). The activity of these pathways can be restored with the addition of exogenous OSFs (Sugiura and Eppig, 2005).

The complex nature of COC metabolism associated with enhanced developmental competence is well demonstrated by examining the impact of BMP15 and FSH supplementation *in vitro*. In the absence of FSH, cattle COCs treated with BMP15 alone consume less glucose and produce less lactate compared to FSH treatment alone, this is a predictable consequence of little cumulus expansion compared to standard IVM conditions, which utilize FSH. Yet both groups have similar rates of glycolytic activity (Sutton-McDowall *et al.*, 2012). Within the oocyte, BMP15 treatment promotes oxidative phosphorylation and tricarboxylic acid (TCA) cycle activity (FAD and NAD(P)H, respectively) and as a consequence, higher levels of antioxidants (reduced glutathione, GSH) and reactive oxygen species levels (ROS, H_2O_2; Sutton-McDowall *et al.,* 2012, 2015; Sudiman *et al.*, 2014) were detected. In comparison, FSH stimulates glucose consumption by cumulus cells, with increasing levels of glucose utilised via the hexosamine biosynthetic pathway for cumulus expansion towards the end of IVM (Sutton-McDowall *et al.*, 2005). Significantly, both these independent treatments improved developmental competence. Hence, BMP15 and FSH promote distinct metabolic pathways within the different compartments of the COC. When combined, FSH and BMP15 stimulate a metabolic equilibrium (Sutton-McDowall *et al.,* 2012, 2015), in which the metabolic effect of each was "masked", yet this combined treatment yielded the highest developmental competence (blastocyst rates).

Metabolism pre- and post-compaction

The first stage of oocyte-embryo transition is oocyte activation following sperm penetration. This includes the cortical granule reaction and hardening of the zona pellucida to prevent polyspermy, resumption of meiosis, pronuclear formation and syngamy. These events are initiated by cytoplasmic release of small signalling ions such as calcium and zinc (Wang and Machaty, 2013; Que *et al.*, 2014), with minimal gene transcript and energy demand. Zygotes and cleavage-staged embryos rely on the oxidation of carboxylic acids such as pyruvate and lactate via the TCA cycle and oxidative phosphorylation within the mitochondria, with minimal glycolytic activity as the demand for ATP is low (Fig. 1; Thompson, 2000). Post-compaction, in morula and blastocyst stage embryos, overall metabolism increases, with glycolysis becoming the predominant source of ATP, a pattern seen in mouse (Houghton *et al.*, 1996), cow (Thompson *et al.*, 1996), pig (Swain *et al.*, 2001; Sturmey and Leese, 2003) and

human (Gott *et al.*, 1990) embryos. In addition, oxygen consumption, TCA cycle and oxidative phosphorylation also increase (Thompson, 2000).

Development of improved embryo culture systems was driven by the inability to overcome the specific cell-cycle developmental block induced by an unsupportive culture environment. Early development in the presence of high levels of glucose and substrates results in Crabtree-like metabolism (increased glycolytic activity and depression of oxidative phosphorylation). Such conditions induce a developmental block coinciding with embryonic genome activation; namely a 2-cell block in mouse (Lawitts and Biggers, 1991) and at the 8-cell stage in ruminants (Thompson *et al.*, 1992; Gardner *et al.*, 1997; Summers and Biggers, 2003). As mentioned previously, the development of sequential culture systems, adapted to reflect the metabolic needs of COCs and embryos (i.e. reduced substrate concentrations in the pre-compaction period), has resulted in significant improvements in the developmental outcomes of IVP embryos, overcoming the developmental blocks.

How to measure metabolism

Metabolism can be measured in two ways, either direct measurement of metabolites (including associated proteins, genes or signalling molecules) within the COC and embryo, or sampling the surrounding environment, such as *in vivo* fluids or the culture media. Sampling of the *in vivo* environment has been critical in formulating culture systems based on the metabolic profiles of COCs and embryos and has resulted in improved embryo development (Summers and Biggers, 2003).

Direct measures within the COC, oocyte or embryo

PCR (mRNA), western blots (protein levels and post-translation modifications), direct enzyme assays and immunohistochemistry (localisation) have been used to study the presence and relative activities of key metabolic enzymes and downstream targets. However, a large proportion of the initial metabolism experiments were performed using radiolabelled substrates. The Hanging Drop assay involves culturing oocytes or embryos in ~3 µl of culture media containing cold and hot (radiolabelled) substrates. This drop was suspended in the lid of a centrifuge tube (or similar vessel) containing a solution of sodium hydroxide or sodium bicarbonate (the latter requiring CO_2 gassing), which acts as an isotope “trap” and provides humidification of the chamber (O'Fallon and Wright, 1986). Depending on which carbon/hydrogen was labelled, the production of labelled CO_2 or H_2O indicated the proportion of the substrate metabolised via particular pathways. For example, the production of $^{14}CO_2$ from [1-^{14}C] glucose measured activity through pentose phosphate pathway (PPP) and TCA cycle. Likewise, the production of $^{3}H_2O$ from [5-^{3}H] glucose is indicative of glycolytic activity. A summary diagram of the metabolism of labelled glucose isotopes is available in Downs and Utecht (1999).

Widely used in the 1980s-1990s (O'Fallon and Wright, 1986; Rieger and Guay, 1988; Downs and Utecht, 1999), the advantages of the Hanging Drop method included the radiolabelled products amplifying the metabolic signal, resulting in high sensitivity and the ability to measure metabolic pathway activity in single oocytes and embryos (Bavister, 1987). Classed as non-invasive, embryo transfers could be performed at the completion of the assay period (O'Fallon and Wright, 1986). However, this assay could not be used in conjunction with embryo transfer in human embryos due to the use of radiolabelled substrates. Furthermore, the availability of commercially available assays that allows absolute concentrations of substrates to be determined has increased. Examples of commercially available kits include ADP/ATP kits (Sutton-McDowall *et al.,* 2012; Zeng *et al.*, 2013; Richani *et al.*, 2014) or clinical chemical analysers for pyruvate, lactate and glucose.

The influence of metabolism on development can be studied using inhibitors and/or stimulators of specific enzymes within metabolic pathways. Oocytes and embryos are cultured in the presence of the antagonists/agonists and outputs such as nuclear maturation and developmental stage would then be assessed (Downs, 1997; Downs and Mastropolo, 1997; Downs *et al.*, 1998; Downs and Utecht, 1999; Sutton-McDowall *et al.*, 2006). In combination with other measurements of metabolism such as substrate turnover, the use of antagonists and agonists remains highly valuable in determining the impact of a metabolic pathway on oocyte and/or embryo competence.

More recently, the development of a variety of effective fluorescent probes that react with specific enzymes or substrate, combined with improved accessibility to confocal microscopy technology has improved the measurement of the metabolism at the single oocyte and embryo level as it has the capacity to combine information on quantification and localisation of activity. Unlike traditional labelling, such as immunohistochemistry, where cells need to undergo extensive processing, such as fixation and permeabilisation, a large proportion of these newer probes are designed for use in live cells. For example, glucose uptake into a COC can be measured using 6-(*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-6-deoxyglucose (6-NBDG), a fluorescent glucose analogue that is non hydrolyzable (Sutton-McDowall *et al.,* 2010; Wang *et al.*, 2012a, b), and this method of studying glucose uptake complements measures of expression of glucose transporter genes (Wang *et al.,* 2012a, b).

Improved and increased accessibility to

commercially available probes has been particularly advantageous to the study of mitochondria. Since mitochondrial health and functionality is dependent on multiple factors such as density, localisation and distribution, maturity and activity (Babayev and Seli, 2015), the following paragraphs will use mitochondrial labelling as an example of how probes target different characteristics.

The most commonly used mitochondrial probes are JC-1 and Mitotracker probes. JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide) is a dual emission, ratio-metric probe that has been utilized to measurement changes in mitochondrial membrane potential ($\Delta_{\Psi m}$) in live mouse and human oocytes (Diaz *et al.*, 1999; Wilding *et al.*, 2001; Van Blerkom *et al.*, 2002, 2003; Zeng *et al.,* 2013). When $\Delta_{\Psi m}$ is low, JC-1 exists as a monomer (green emission) and is converted to J-aggregates/dimers (red emission) with high $\Delta_{\Psi m}$. Hence, the ratio of red to green fluorescence indicates changes in $\Delta_{\Psi m}$ independent of mitochondrial size, shape and density. However, JC-1 has disadvantages, as it is very sensitive to concentration; with the use of too high JC-1 concentrations leading to false positives, is highly sensitive to other factors such as H_2O_2, requires a long incubation time and has poor cell retention (Perry *et al.*, 2011). While JC-1 works well in rodent oocytes and embryos, in our experience JC-1 has poor cellular permeability when incubated with cattle oocytes and embryos, requiring cell permeabilisation or removal of the zona pellucida; both processes may harm an oocyte and embryo, and therefore not favourable considering the probe is assessing cell function.

Alternatives to JC-1 are the Mitotracker range of probes: mildly thiol-reactive chloromethyl moieties that are lipophilic cations, hence are highly cell permeable and only fluoresce within cells. Furthermore, they are more robust than JC-1, with higher photostability, require less reaction time, have higher cell retainability and less cross-reactivity with other factors (Perry *et al.,* 2011). There are two main forms of Mitotracker probes; carboryanine or rosamine based. The fluorescence of carboryanine base probes, such as Mitotracker Green FM (MTG) are independent of $\Delta_{\Psi m}$, hence indicators of total mitochondrial mass in combination with localization, particularly useful in studies comparing mitochondrial biosynthesis in immature *vs*. mature oocytes (Stojkovic *et al.*, 2001; Sun *et al.*, 2001; Sturmey *et al.*, 2006; Gendelman and Roth, 2012). In comparison, rosamine based probes, such as Mitotracker CMXRos, (MTR) are oxidized within cells and sequestered within the mitochondria, hence indicators of $\Delta_{\Psi m}$ and activity (Castaneda *et al.*, 2013; Viet Linh *et al.*, 2013; Niu *et al.*, 2015; Sanchez *et al.*, 2015; Sutton-McDowall *et al.,* 2015). In a similar concept to JC-1, cells can be co-labelled with MTR and MTG to determine a ratio of active to total mitochondria (Pendergrass *et al.*, 2004), although to our knowledge, such a comparison has not been performed in oocytes or embryos.

With advancements in probe design, microscopy and imaging technology, image analyses has also evolved to measure different pixel attributes, such as distribution, co-localization and patterning, in addition to pixel intensity. This can improve the quality of information about the role of mitochondria under different states of oocyte and embryo health. Ultrasound sonography, dermatology and cancer research are fields that routinely use advanced imaging matrices to assess variations in patterns of pixel characteristics such as wrinkles, smoothness, uniformity and entropy (Castellano *et al.*, 2004; Alvarenga *et al.*, 2007; Mittra and Parekh, 2011) of images. In comparison, image analysis within the pre-implantation research field is largely limited to measurements of fluorescence intensity or visual assessment. We have recently utilized texture analyses (Haralick *et al.*, 1973; Murata *et al.*, 2001; Cabrera, 2006) to assess the influence of exposing cattle COCs to FSH and BMP15 on the distribution of MTR, monochlorobimane (MCB; indicative of reduced glutathione) and peroxyfluor 1 (PF1; measures levels of H_2O_2, a derivative of reactive oxygen species; Sutton-McDowall *et al.,* 2015). In addition to pixel intensity, textural analyses demonstrated an association with homogeneous localization of fluorescence with improved developmental competence (Sutton-McDowall *et al.,* 2015). As technology improves, the mechanisms through which outputs are measured will continue to evolve.

While fluorescent probe are of value to the study of metabolism, label-free and non-toxic methods for characterising metabolism and viability would be preferable, in particular as a potential diagnostic of oocyte and embryo health. Electron donors NADPH/NADH (NAD(P)H) and the electron acceptor FAD are endogenous fluorophores with different spectral properties and therefore can be measured simultaneously by confocal microscopy. NADH has both cytoplasmic and mitochondrial localisation, whereas FAD is exclusively localised to the mitochondria (Table 2). FAD and NAD(P)H are critical for energy homeostasis, hence measurement of levels indicates the redox state of cells (FAD: NAD(P)H; Skala and Ramanujam, 2010). Measurement of intra cellular autofluorescence has not been widely exploited for investigations into cellular metabolism of embryos. However, Dumollard *et al.*, 2007a, b) utilised autofluorescence as a method for label-free localisation of mitochondria (Dumollard *et al.,* 2007a) and to study the influence of energy substrates on redox state over time (Dumollard *et al.,* 2007b). Furthermore, autofluorescence measurements have demonstrated changes in redox ratios in COCs following IVM in the presence of OSF (Sutton-McDowall *et al.*, 2012, 2015; Sugimura *et al.*, 2014) and EGF-like peptides (Richani *et al.,* 2014).

Table 2. Parameters of autofluorescence molecules involved in metabolism.

	Electron	Localisation	Pathways	Excitation (nm)	Emission (nm)
NADH	Donor	Cytoplasm Mitochondria	Glycolysis TCA cycle Oxidative Phosphorylation	350	460
NADPH	Donor	Cytoplasm	PPP	350	460
FAD	Accepter	Mitochondria	Oxidative Phosphorylation	450	535

Sampling of the culture media

Standard techniques for measuring metabolites include mass spectrometry/chromatography and clinical chemical analysers (Sutton-McDowall *et al.,* 2012, 2014). Leese and colleagues devised fluorometric assays for measuring nano and pico litres of samples based on the oxidation and/or reduction of autofluorescence signalling molecules such as FAD and NAD(P)H (Leese and Bronk, 1972). Indeed, many of these assays are still used due to their high sensitivity and the ability to measure the metabolite turnover of a single COC and embryo.

Metabolomics is the newest member of the "omics" family and unlike other metabolic assays, brings a more holistic approach to profiles, as it allows not only measurement of substrate turnover but also changes in pathway activity and downstream targets (Krisher *et al.*, 2015). Metabolomics combines two technologies to separate (gas chromatography or high performance liquid chromatography) and detect (mass spectrometry, nuclear magnetic resonance or Raman spectrometry) larger numbers of metabolites within spent culture media compared to fluorometric assays and other analytical methods. Both quantitative or qualitative measurements can be performed with quantitative measures requiring the generation of standard curves, which limits the number of substrates that can be measured (Thompson *et al.,* 2014). Successful application of some metabolomics platforms for spent media analysis to measure embryo quality were initially favourable and indeed still pursued (Krisher *et al.,* 2015), but has since been abandoned for use in human IVF, as results were inconsistent and dependent on media formulations.

The future for metabolic measurement of oocytes and embryos

A massive knowledge gap remains in characterising the metabolome of COCs and embryos *in vivo* as the ability to measure this *in situ* is essentially non-existent. There is a need to create new technologies that allow for *in vivo* measurement of biochemical reactions, given that even short exposures to *in vitro* conditions can alter COC and embryo metabolism. The development of remote sensing diagnostics, such as micro optical fibres and nano-particles are options for remote sensing with minimal invasion. An ideal candidate is the adaptions of multiphoton endoscopes to micro-optical fibres to allow for *in vivo* measurement of autofluorescence, hence redox state of COCs and embryos (Helmchen, 2002).

Even *in vitro*, the metabolic requirements of COCs are dynamic, with high levels of plasticity, where as most measurements are taken at a single time point. Furthermore, numerous metabolic pathways are in play and differential activity can result in numerous downstream consequences. For this reason, the use of single measurements of single metabolic outputs is not sufficient. Platforms that allow multi sampling of different aspects of metabolism are critical for advancing our knowledge of COC maturation. This could be achieved using label-free technologies and non-toxic, reversible probes, allowing for repeated measurements and changes in metabolism, crucial for dynamic periods in development such as oocyte maturation, fertilisation and embryonic genome activation. Essentially measuring more in less. A long-term goal could involve the development of sensing probes and systems that could be integrated into incubators, allowing the constant monitoring of changes in metabolism and thereby predict oocyte and embryo health and quality.

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