Human Reproduction, Vol.38, No.5, pp. 799–810, 2023
Advance Access Publication on April 4, 2023 https://doi.org/10.1093/humrep/dead063

human reproduction

MINI-REVIEW *Developments in reproductive biology and medicine*

Noninvasive metabolic profiling of cumulus cells, oocytes, and embryos via fluorescence lifetime imaging microscopy: a mini-review

Marta Venturas [1,2,*,†], Xingbo Yang [1,3,†], Denny Sakkas [2], and Dan Needleman [1,4]

[1]Molecular and Cellular Biology and School of Engineering and Applied Sciences, Harvard University, Cambridge, MA, USA [2]Boston IVF-The Eugin Group, Waltham, MA, USA [3]Cluster of Excellence Physics of Life, TU Dresden, Dresden, Germany [4]Center for Computational Biology, Flatiron Institute, New York, NY, USA

*Correspondence address. Boston IVF-The Eugin Group, 130 2nd Ave, Waltham, MA 02145, USA. E-mail: martaventu22@gmail.com
https://orcid.org/0000-0003-3977-0152

Submitted on December 21, 2022; resubmitted on March 14, 2023; editorial decision on March 20, 2023

ABSTRACT: A major challenge in ART is to select high-quality oocytes and embryos. The metabolism of oocytes and embryos has long been linked to their viability, suggesting the potential utility of metabolic measurements to aid in selection. Here, we review recent work on noninvasive metabolic imaging of cumulus cells, oocytes, and embryos. We focus our discussion on fluorescence lifetime imaging microscopy (FLIM) of the autofluorescent coenzymes NAD(P)H and flavine adenine dinucleotide (FAD+), which play central roles in many metabolic pathways. FLIM measurements provide quantitative information on NAD(P)H and FAD+ concentrations and engagement with enzymes, leading to a robust means of characterizing the metabolic state of cells. We argue that FLIM is a promising approach to aid in oocyte and embryo selection.

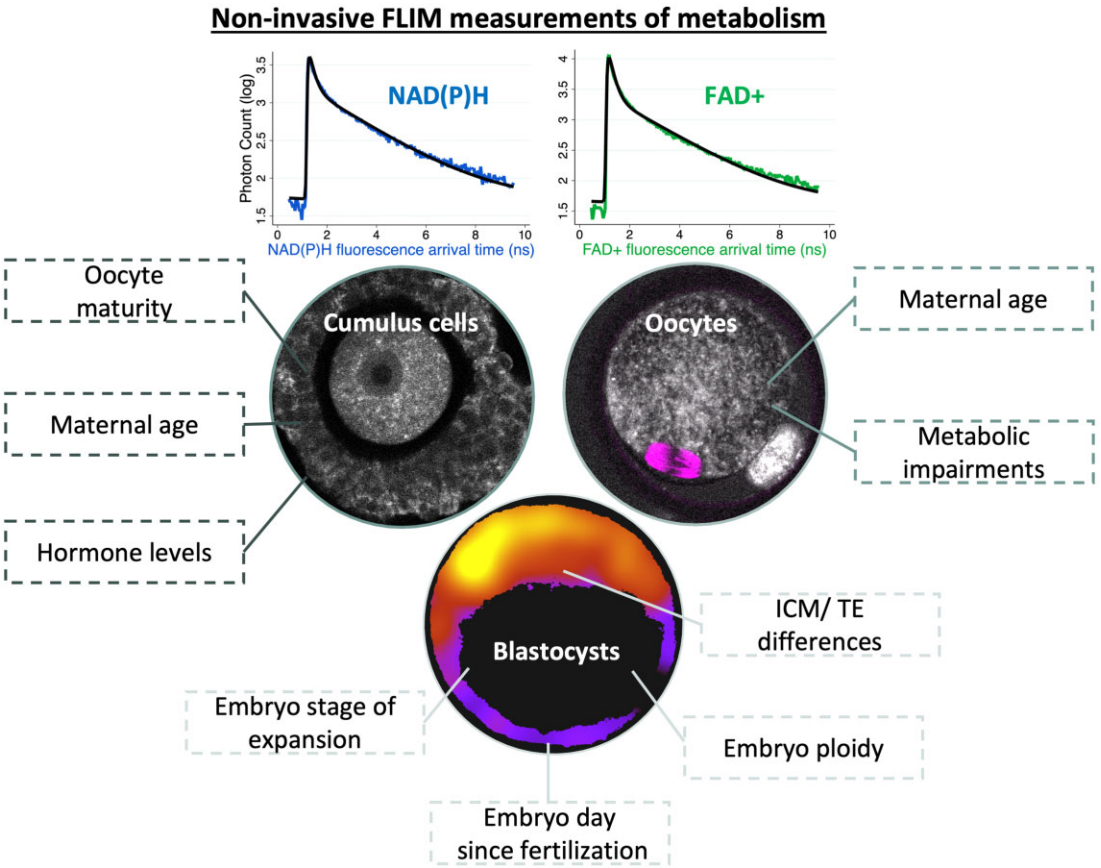
Key words: metabolism / noninvasive / assessments / fluorescence lifetime imaging microscopy / embryo selection

Downloaded from https://academic.oup.com/humrep/article/38/5/799/7104033 by guest on 08 May 2023

[†]The authors consider that the first two authors should be regarded as joint First Authors.

© The Author(s) 2023. Published by Oxford University Press on behalf of European Society of Human Reproduction and Embryology. All rights reserved.
For permissions, please email: journals.permissions@oup.com

GRAPHICAL ABSTRACT



Noninvasive measures of metabolism of cumulus cells, oocytes, and preimplantation embryos using fluorescence lifetime imaging microscopy. ICM: inner cell mass; TE: trophectoderm; FAD+: flavine adenine dinucleotide.

Introduction

A major goal in ART is to select the single embryo with the highest developmental potential from within a patient's embryo cohort (Gardner and Sakkas, 2003; Kirkegaard *et al.*, 2015; Kovacs and Lieman, 2019; Zaninovic and Rosenwaks, 2020). Selecting a single high-quality embryo to transfer reduces the number of potential embryos implanted and, in turn, decreases the number of embryo transfers a patient must undergo (Gardner and Sakkas, 2003), alleviating the risks associated with multiple pregnancies (Dudenhausen and Maier, 2010).

Currently, the most common method used to assess oocyte and embryo quality is to evaluate their morphology at discrete time points (Schoolcraft *et al.*, 1999; Tesarik and Greco, 1999; Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011). More detailed information can be obtained by timelapse microscopy (Goodman *et al.*, 2016), which can be analyzed more objectively with machine learning algorithms (Fernandez *et al.*, 2020; Leahy *et al.*, 2020; Zaninovic and Rosenwaks, 2020). However, it is still unclear if timelapse microscopy is beneficial for embryo selection (Ahlström *et al.*, 2022) and approaches based on morphology or morphokinetics fail to provide information on embryo physiological or genomic state (Wong *et al.*, 2014). Preimplantation genetic testing for aneuploidies (PGT-A) has increasingly been used to evaluate the ploidy of blastocysts (Forman *et al.*, 2013; Munné, 2018). However, PGT-A is invasive and its use remains controversial (Cornelisse *et al.*, 2020; Simopoulou *et al.*, 2021). Thus, improved methods for embryo selection would be beneficial for improving ART success.

The importance of metabolism for oocyte and embryo development

Cumulus–oocyte complex

Granulosa and cumulus cells (CCs) are specialized somatic cells that enclose the oocytes (Zhou *et al.*, 2016). At early stages of oogenesis, there is a crosstalk between granulosa and CCs and the surrounded oocytes (Sutton *et al.*, 2003; Richani *et al.*, 2021) that helps support

Downloaded from https://academic.oup.com/humrep/article/38/5/799/7104033 by guest on 08 May 2023

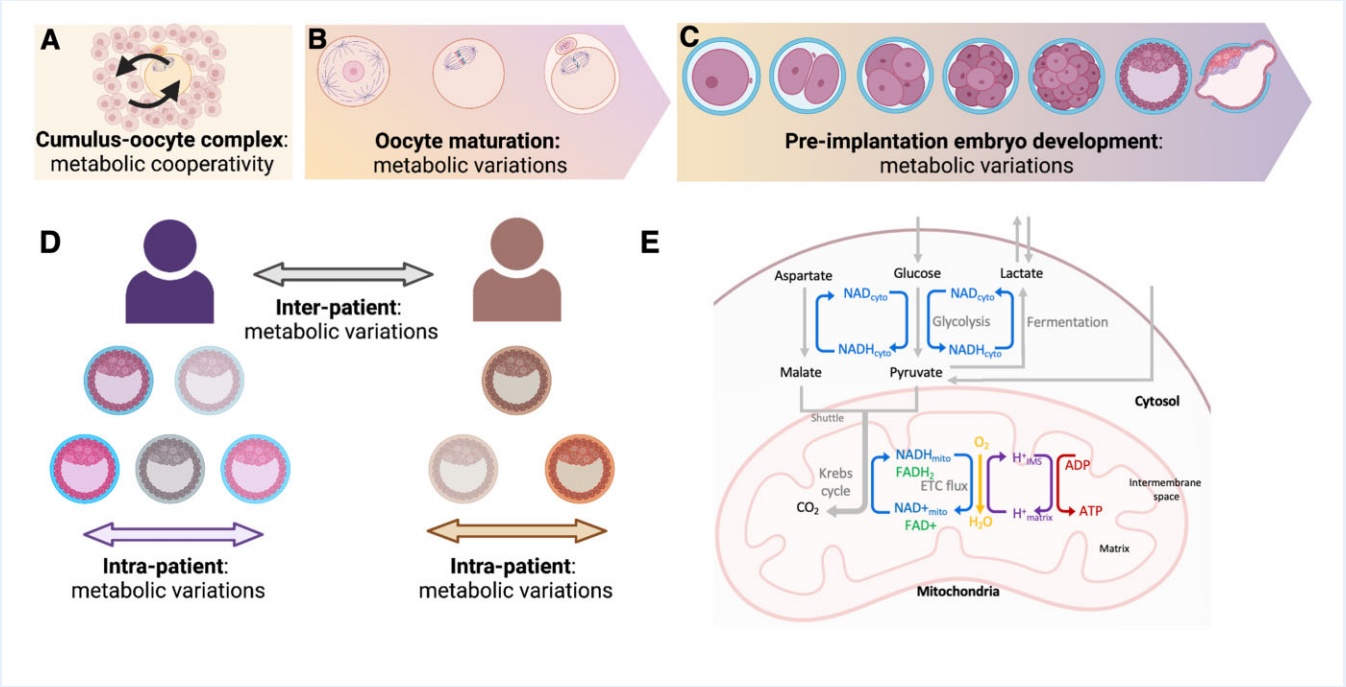


Figure 1. Metabolic cooperation and variability in oocyte maturation and embryo development. (**A**) Depiction of metabolic cooperativity between the oocyte and the enclosing cumulus cells. (**B**) Variations in metabolism are observed through human oocyte growth and maturation and (**C**) throughout preimplantation embryo development. (**D**) Metabolic variations are also observed between embryos from the same patient and between patients. (**E**) Endogenous coenzymes, such as NAD(P)H and flavine adenine dinucleotide (FAD+), are natural biomarkers of metabolism involved in pathways including glycolysis, fermentation, and mitochondrial respiration. Their autofluorescent properties enable noninvasive imaging of the metabolic state of cells. Created using BioRender.com.

oocyte growth (Downs *et al.*, 2002; Huang and Wells, 2010; Richani *et al.*, 2021), maturation (Dumesic *et al.*, 2015), and enable CCs differentiation (Sutton *et al.*, 2003; Gilchrist *et al.*, 2008) (Fig. 1A). Throughout this article we will focus on CCs, as they are coupled with the oocyte via gap junctions and paracrine signals (Anderson and Albertini, 1976). CCs mitochondrial activity, using measures of mitochondrial DNA copy number, immunofluorescent probes, or measures of membrane potential via flow cytometry, have been linked with oocyte maturation (Anderson *et al.*, 2018; Lan *et al.*, 2020) and the acquisition of developmental competence (Eppig, 1991; Albertini *et al.*, 2001; Lu *et al.*, 2022). Therefore, measurements of CCs metabolic state might provide a means to assess oocyte quality (Ogino *et al.*, 2016; Desquiret-Dumas *et al.*, 2017; Fontana *et al.*, 2020).

Oocyte metabolism, in particular mitochondria, plays key roles in supplying energy and metabolic precursors to support oocyte maturation and further embryo development (Hoshino, 2018; Fabozzi *et al.*, 2021) (Fig. 1B). Oocyte mitochondrial dysfunction has been linked with decreased quality and implantation potential (Wilding *et al.*, 2001; Eichenlaub-Ritter *et al.*, 2011; Zhao and Li, 2012) and is known to impact spindle assembly and chromosome segregation during nuclear maturation, however, the mechanisms by which they do so remain unclear (Eppig, 1996; Tatone *et al.*, 2011). Hence, the assessment of oocyte metabolism may help elucidate these mechanisms and provide a measure of their quality (Tan *et al.*, 2022a).

Preimplantation embryos

As the oocyte matures and is fertilized, further intricate metabolic shifts occur. The preimplantation embryo subsequently undergoes metabolic changes through development (Lane and Gardner, 2000; Chason *et al.*, 2011; Gardner and Harvey, 2015; Harvey, 2019) that are necessary to produce developmentally competent embryos (Gardner *et al.*, 2001; Leese, 2012) (Fig. 1C) and are also involved in cell fate specification (Chi *et al.*, 2020; Zhu and Zernicka-Goetz, 2020). These dynamic variations in metabolic pathways are interwoven with the viability of the embryo (Van Blerkom *et al.*, 1995; Harvey, 2019; Gardner, 2015), which led to the development of the 'Quiet embryo hypothesis', suggesting that embryos that have a less active metabolism have higher developmental potential (Leese *et al.*, 2007; Leese, 2012; Santos Monteiro *et al.*, 2021). However, the validity of this hypothesis is still uncertain (Gardner *et al.*, 2011; Tejera *et al.*, 2012).

Additionally, metabolic state varies across embryos between different patients and within the cohort of embryos from the same patient (Venturas *et al.*, 2021, 2022) (Fig. 1D). It is unclear what determines these variations, but oocyte- or embryo-specific characteristics, such as stage, ploidy, or time since fertilization, appear to influence their metabolic profiles (Gardner and Sakkas, 2003; Rosenwaks, 2017; Sanchez *et al.*, 2017; Shah *et al.*, 2020; Santos Monteiro *et al.*, 2021; Venturas *et al.*, 2022). To this end, measures of metabolism could aid

Downloaded from https://academic.oup.com/humrep/article/38/5/799/7104033 by guest on 08 May 2023

in selecting the embryo with the highest implantation potential from within a patient's cohort.

Assessments of metabolism

Measures of metabolism can be performed via the addition of fluorescent dyes to label structures like mitochondria (Gorshinova et al., 2017; Al-Zubaidi et al., 2019), or measures of mitochondrial DNA copy number (Fragouli et al., 2015; Kumar et al., 2021). However, these techniques are invasive and their utility in clinical IVF remains uncertain (Ogino et al., 2016; Desquiret-Dumas et al., 2017; Kumar et al., 2021).

Gene expression in CCs is a potentially noninvasive surrogate marker of oocyte metabolism and quality, but so far has not been predictive of clinical outcome (Hamel et al., 2010; Fragouli et al., 2014; Green et al., 2018; Racowsky and Needleman, 2018). More direct, noninvasive measures of metabolism quantify the uptake and secretion of metabolites in the media surrounding the embryos or oocytes (Conaghan et al., 1993; Urbanski et al., 2008), or via measures of embryo oxygen consumption levels (Lopes et al., 2010; Kurosawa et al., 2016). These methods require highly specialized skills and equipment, such as near-infrared or mass spectrometry, high-performance liquid chromatography, or microarrays. Despite the reported associations of levels of metabolites (Vergouw et al., 2008), proteins (Katz-Jaffe et al., 2006), and amino acids (Brison et al., 2004) with embryonic developmental potential, these methods have not yet been clinically useful (Vergouw et al., 2008; Hardarson et al., 2012).

Some intracellular molecules with integral roles in cellular physiology can be specifically probed with optical microscopy (Heikal, 2010; Cheng and Xie, 2012). Hence, several groups have focused on developing methods to optically measure intracellular metabolic function using techniques such as Raman spectroscopy, confocal imaging, and hyperspectral (McLennan et al., 2020; Tan et al., 2022a) or fluorescence lifetime imaging microscopy (FLIM) (Sanchez et al., 2019; Shah et al., 2020; Venturas et al., 2022; Tan et al., 2022b), with the aim to develop them for clinical use. These methods are noninvasive, avoiding the potential interference with biological functions associated with exogeneous dyes. A broad array of autofluorescent molecules involved in metabolic functions can be probed with hyperspectral microscopy (Sutton-McDowall et al., 2017; Santos Monteiro et al., 2021; Tan et al., 2022a,b). Hyperspectral microscopy is showing some promise for its potential clinical application (Sutton-McDowall et al., 2017; Tan et al., 2022a) and has recently been used to measure the association between embryo metabolic state and ploidy (Santos Monteiro et al., 2021).

NADPH, NADH, and flavine adenine dinucleotide (FAD+) are some of the most abundant, autofluorescent metabolites. These molecules have received particular attention because of their strong association with mitochondrial function (Heikal, 2010). These molecules are endogenous electron carriers involved in many metabolic pathways, including glycolysis, fermentation, and mitochondrial respiration (Chance and Williams, 1955) (Fig. 1E). Hence, these coenzymes have a diagnostic potential as noninvasive biomarkers of the cellular metabolic state and mitochondrial anomalies (Klaidman et al., 1995; McLennan et al., 2020; Tan et al., 2022b). Measurements of their fluorescence intensities are correlated with their concentrations (Heikal, 2010) and have been used to assess mitochondrial function (Klaidman et al., 1995; Dumollard et al., 2009; Santos Monteiro et al., 2021).

Fluorescence lifetime imaging microscopy

Besides fluorescence intensity, the advanced microscopic technique of FLIM enables additional measurements of the molecule's fluorescence lifetime (Heikal, 2010; Becker, 2012): the time it takes for a fluorescent molecule to return to ground state after excitation (Jablonski, 1933) (Fig. 2A). The fluorescence lifetime of a molecule is independent of its concentration, but depends on its molecular conformation, which can be altered by its environment (Suhling et al., 2004; Ghukasyan and Heikal, 2014). Both NAD(P)H and FAD+ have short and long lifetime components, depending on whether or how these molecules are engaged with an enzyme (Lakowicz, 2006). Changes in metabolic states can be measured by the change of their fluorescence lifetimes (Skala et al., 2007). Taken together, FLIM provides a quantitative characterization of cellular metabolic states in terms of eight metabolic parameters, including intensities, fluorescence lifetimes, and enzyme engagement of NAD(P)H and FAD+ (Becker, 2012; Ma et al., 2019; Sanchez et al., 2019) (Fig. 2B).

FLIM, like all light microscopy techniques, requires exposing the sample to illumination, raising the concern of potential damage. Indeed, it is well known that excessive light exposure in conventional microscopy (Masters and So, 2008) or laser pulses during biopsy (Bradley et al., 2017) can harm biological material. However, using low levels of illumination can eliminate such adverse effects (Nakahara et al., 2010; Scott et al., 2013) and it has been shown that FLIM illumination exposure during single or timelapse FLIM imaging does not disrupt the viability of mouse embryos or increase the levels of reactive oxygen species (Sanchez et al., 2018; Seidler et al., 2020). Despite these findings, safety in mouse embryos does not necessarily generalize to human embryos. Additionally, FLIM timelapse illumination does not appear to produce changes in FLIM parameters during human blastocyst expansion (Venturas et al., 2022), or impact maturation rates of human oocytes when compared to control (Pietroforte et al., 2022). However, in order to use this technique in a clinical setting, additional studies are needed to demonstrate its safety in human oocytes or preimplantation embryos. Laser intensity, time of exposure, and frequency of imaging should all be carefully studied.

Potential applications of FLIM in ART

FLIM has previously been applied in other fields, and its utilization in clinical ART is showing great promise (Sanchez et al., 2018; Ma et al., 2019; Venturas et al., 2021, 2022; Yang et al., 2021) (Fig. 2C).

Noninvasive FLIM assessments of cumulus cell metabolic state

Recent studies have evaluated the potential application of noninvasive measurements of CCs metabolism as a surrogate for oocyte quality

Downloaded from https://academic.oup.com/humrep/article/38/5/799/7104033 by guest on 08 May 2023

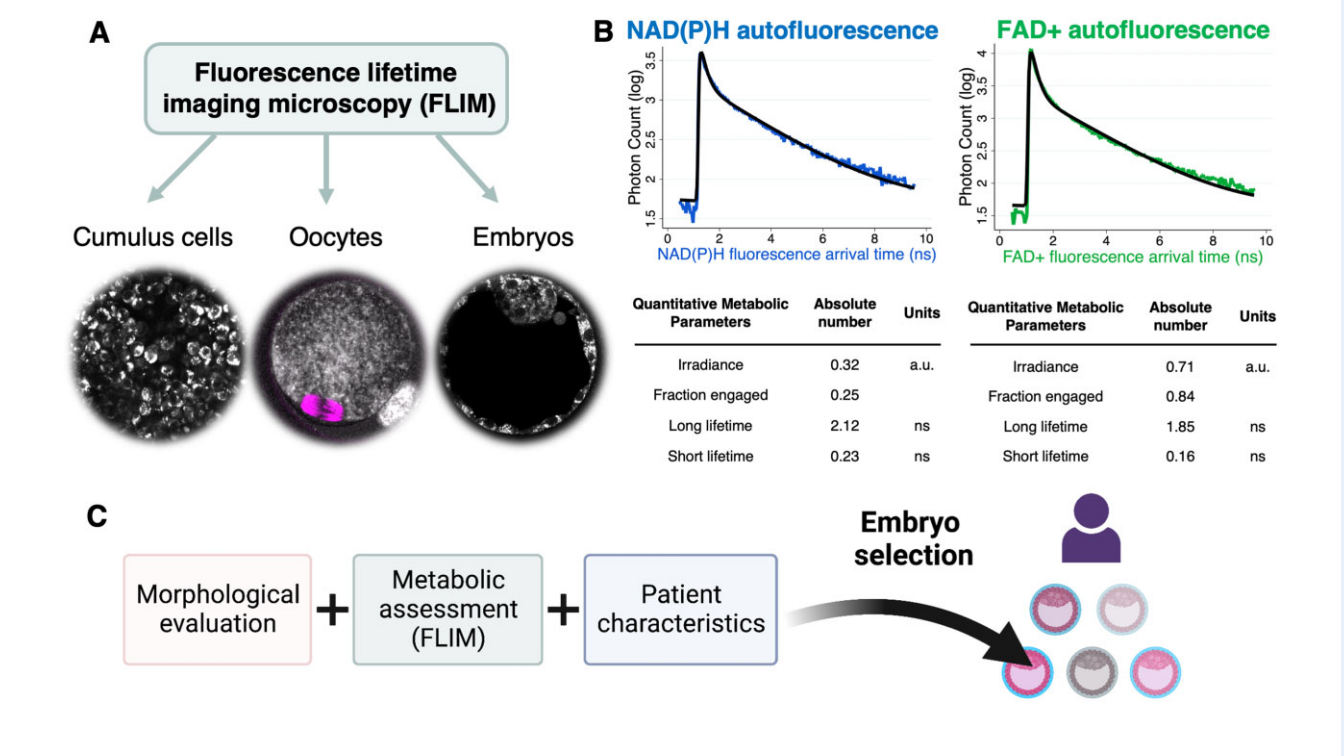


Figure 2. Noninvasive assessments of intracellular metabolic state from fluorescence lifetime imaging of NAD(P)H and FAD+. (**A**) Fluorescence lifetime imaging (FLIM) of cumulus cells, oocytes, and embryos. FLIM can be used to noninvasively measure metabolic state of cumulus cells, oocytes, and embryos. (**B**) Fluorescence decay curves from FLIM. Fitting the fluorescence decay curves to a double exponential function provides information on the fluorescence intensity, lifetime, and enzyme engagement of NAD(P)H and flavine adenine dinucleotide (FAD+), with a total of eight metabolic parameters. (**C**) Evaluation of cellular morphological features and patient clinical characteristics could potentially be used in synergy with quantitative measurement of the metabolic state of cumulus cells, oocytes, or embryos in order to help select the best embryo from within a patient's cohort. Ns: nanoseconds; au: arbitrary units. Created using BioRender.com.

(Richani *et al.*, 2021; Venturas *et al.*, 2021; Tan *et al.*, 2022b). Measuring the metabolic state of CCs using noninvasive methods offers several advantages. First, CCs are frequently removed or trimmed and discarded when performing ART treatments, therefore measuring their metabolic state is completely noninvasive. Additionally, the greater variance observed between oocytes from the same patient than between patients (Venturas *et al.*, 2021) implies that quantitative measures of CCs metabolism could be used to assess oocyte quality from within a patient's cohort. These metabolic changes might be associated with oocyte-specific characteristics, such as their maturity. In this regard, metabolic profiles of CCs characterized by FLIM have been associated with oocyte maturation status (Anderson *et al.*, 2018; Venturas *et al.*, 2021). CCs showed distinct FLIM parameters depending on whether they enclosed immature oocytes or mature metaphase II (MII) oocytes (Venturas *et al.*, 2021). Additionally, patient-specific factors, like maternal age, and hormone levels have been shown to also influence CCs and oocyte metabolic state (Venturas *et al.*, 2021; Lu *et al.*, 2022). These observations suggest that patient clinical characteristics should be factored in when developing prediction algorithms for oocyte viability. However, because CCs undergo a process of expansion and detach from the oocyte during maturation (Nikoloff, 2021), measurements of their metabolism are a less direct assessment of oocyte physiology. Whether CCs metabolic profile is associated with oocyte viability has yet to be determined.

Noninvasive FLIM assessments of oocyte metabolic state

Oocyte metabolic state has long been linked with their physiological state and quality (Dumollard *et al.*, 2007; Sanchez *et al.*, 2018; Scott *et al.*, 2018; Richani *et al.*, 2021). However, robust and quantitative techniques to measure oocyte metabolic state noninvasively have yet to be established. Recent studies using FLIM to measure oocyte metabolism in mice demonstrated that FLIM can be used to identify oocytes with metabolic impairments (Sanchez *et al.*, 2018) and showed the impact of age on oocytes metabolic state (Sanchez *et al.*, 2018). Maternal age, among other factors, negatively impacts oocyte quality, in particular because of increased rates of aneuploidy (Eichenlaub-Ritter *et al.*, 2011; Cimadomo *et al.*, 2018). However, the precise relation between oocyte metabolism and correct chromosome segregation has not been established. Therefore, noninvasive measurements of metabolism could provide a means to study this relation (Sanchez *et al.*, 2017, 2018; Scott *et al.*, 2018; Yang *et al.*, 2021). Preliminary work has found distinct FLIM parameters in oocytes that mature and those that do not (Pietroforte *et al.*, 2022). FLIM could

Downloaded from https://academic.oup.com/humrep/article/38/5/799/7104033 by guest on 08 May 2023

help improve the understanding of the interconnection between nuclear and cytoplasmic maturation of the oocytes or perhaps help predict which eggs will mature (Tan et al., 2022a). Additionally, media and conditions of culture can also affect metabolic pathways and nutrient utilization pathways (Gardner et al., 2011; Kleijkers et al., 2015). FLIM measurements of the intracellular metabolic state provide a means to study the metabolic relation of the oocyte with its environment, how this is related to the acquisition of developmental competence (Tan et al., 2022a) and potentially aid in improving culture conditions (Bertoldo et al., 2020; Pollard et al., 2021). Measuring the metabolic profile of single oocytes from a patient's cohort could help in selecting the oocyte with the highest implantation potential.

Noninvasive FLIM assessments of embryo metabolic state

It has long been known that embryo metabolism, in particular glucose uptake, is associated with embryo viability (Renard et al., 1980; Van Blerkom et al., 1995; Gardner et al., 2001; Leese et al., 2007; Gardner, 2015); however, it is technically challenging to noninvasively measure embryo metabolism with the sensitivity and robustness that would be required for ART clinical applications. FLIM of NAD(P)H and FAD+ is promising in this regard, as recent work shows that it is capable of measuring intricate metabolic shifts throughout preimplantation development in mouse (Ma et al., 2019; Sanchez et al., 2019) and human blastocysts throughout blastocyst expansion and hatching (Shah et al., 2022; Venturas et al., 2022). It was found that embryo metabolic profiles not only change throughout development but also vary between blastocysts from the same patient and between patients. These profiles are associated with the day of development but are not associated with embryo morphological grades (Venturas et al., 2022), which can suggest that both of these assessments may provide synergistic information, aiding separately in embryo selection (Tejera et al., 2012). Additionally, blastocyst metabolism was also associated with the ploidy status in human embryos (Shah et al., 2022). Whether assessment of blastocyst metabolism via FLIM is associated with embryo implantation should be further explored.

Mechanistic studies and interpretation of FLIM measurements

One of the challenges of using FLIM measurements to help select oocytes and embryos based on their metabolic state is to understand what physiological information is encoded by FLIM measurements and to what extent they are predictive of their developmental competence. The majority of FLIM studies so far are correlative, demonstrating the sensitivity of FLIM parameters to metabolic perturbations or changes in cell physiology (Sanchez et al., 2018, 2019; Ma et al., 2019; Venturas et al., 2021, 2022; Shah et al., 2022), but how to relate FLIM measurements to activities of specific metabolic pathways remains largely unknown. Knowing the relation between FLIM parameters and specific metabolic activities will better inform the interpretation of the correlations between FLIM measurements and patient characteristics that have been observed in clinical data (Venturas et al., 2021). The goal is to quantitatively interpret FLIM measurements of NAD(P)H and FAD+ in terms of activities of specific metabolic pathways, such as respiration, glycolysis, and fermentation in oocytes, CCs, and embryos, and to understand mechanistically how metabolic defects impact developmental competence in these systems. This would provide more relevant information on how to use these noninvasive FLIM measurements to select oocytes and embryos in ART.

Biophysical models provide useful tools to interpret FLIM measurements by mapping FLIM parameters into biologically meaningful quantities. Biophysical models have enabled mechanistic interpretations of FLIM of NADH in MII mouse oocytes. MII oocytes remain in a quasi-metabolic steady state with constant FLIM parameters for many hours, making quantitative metabolic perturbations easier to interpret, hence providing an ideal system to relate FLIM parameters to specific metabolic activities. A coarse-grained biophysical model of NADH redox reactions has enabled the prediction of metabolic fluxes, i.e. the turnover rate of metabolites, within single oocytes from FLIM measurements of NADH (Yang et al., 2021). Specifically, this model enables the prediction of mitochondrial oxygen consumption rate (OCR) for single oocytes from noninvasive FLIM imaging of NADH. Previously, it has been proposed that OCR correlates with oocyte viability (Scott et al., 2008; Tejera et al., 2011), but it is unclear what cellular processes control OCR. Prediction of OCR from FLIM measurements has demonstrated that the OCR of oocytes is insensitive to perturbations in cellular energy demand and nutrient supply, despite significant sensitivity of NADH FLIM parameters (intensity, fluorescence lifetimes, enzyme engagement) to these perturbations. In contrast, an oocyte's OCR is sensitive to direct mitochondrial perturbations. These results show that OCRs of oocytes are determined by intrinsic properties of mitochondria, rather than by cellular energy demand or nutrient supply (Yang et al., 2021). This apparent OCR homeostasis also implies the existence of an unknown mechanism of metabolic regulation that maintains the global metabolic flux at the expense of redistribution of specific metabolic fluxes. Combining NADH redox modeling with detailed biophysical models of mitochondrial metabolism will help identify the rewiring of metabolic fluxes in the oocytes, providing biological insights into how metabolic perturbations impact oocyte viability.

Recent work has revealed subcellular metabolic heterogeneity, including spatial variations in the mitochondrial membrane near the meiotic spindle in mouse oocytes (Al-Zubaidi et al., 2019). Such subcellular metabolic heterogeneity may be associated with oocyte viability. For example, abnormal distributions of mitochondria correlate with a decrease in oocyte developmental competence (Yu et al., 2010; Liu et al., 2016), highlighting the potential utility of probing subcellular metabolic heterogeneity to predict oocyte viability. In addition to single-cell averaged OCR, NADH redox modeling also enables prediction of OCR at different locations within the same cell by taking advantage of the subcellular resolution of FLIM measurements. A subcellular OCR gradient exists within a single oocyte, where mitochondria closer to the oocyte periphery display a higher OCR than those at the center of the oocyte (Yang et al., 2021) (Fig. 3A). This metabolic gradient is caused by enhanced proton leak in peripherally located mitochondria, suggesting the existence of distinct subpopulations of mitochondria within a single oocyte. However, it is unclear how these metabolic variations arise and how they impact the viability and developmental competence of the oocyte. Do mitochondria of different intrinsic activities move into different locations of the oocyte during maturation or are mitochondria

Downloaded from https://academic.oup.com/humrep/article/38/5/799/7104033 by guest on 08 May 2023

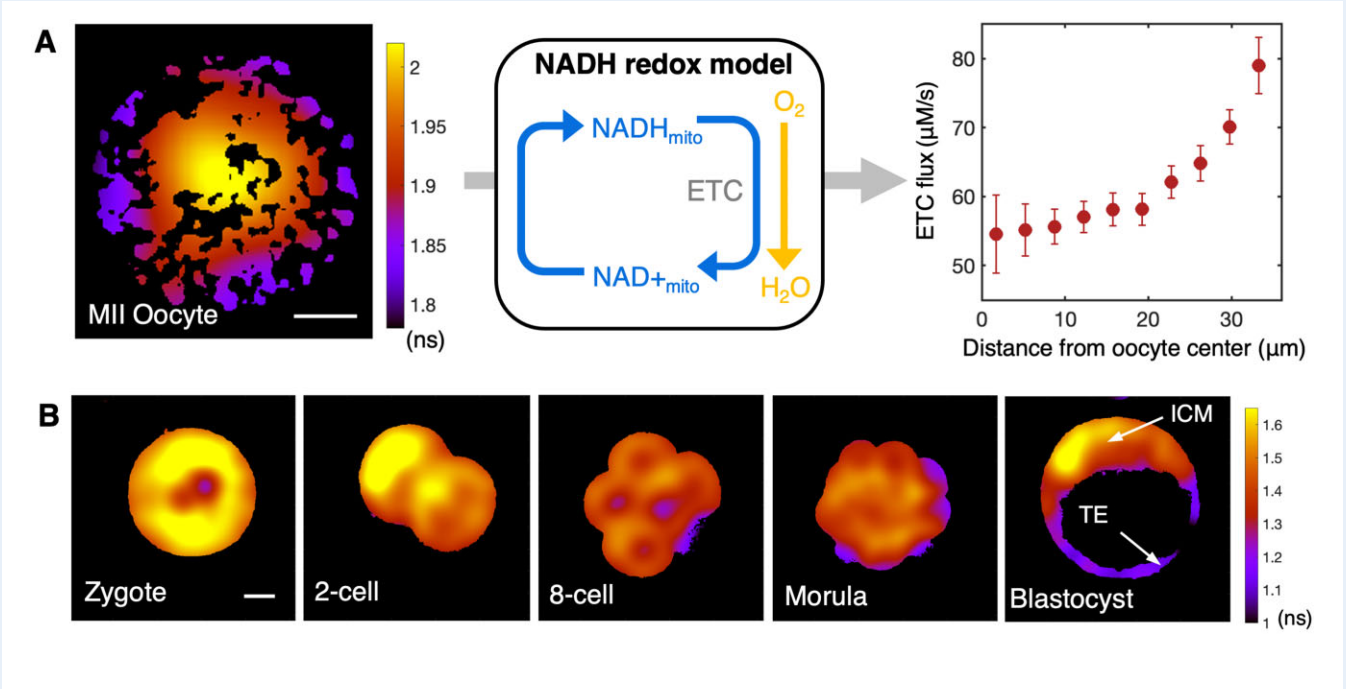


Figure 3. FLIM of NAD(P)H reveals spatiotemporal metabolic variations in mouse oocytes and developing embryos. (**A**) Fluorescence lifetime imaging (FLIM) of NADH reveals a spatial gradient of the average NADH fluorescence lifetime in mouse metaphase II (MII) oocytes (left). An NADH redox model (middle) is used to interpret FLIM measurements and predicted a subcellular gradient of flux through the mitochondrial electron transport chain (ETC), or equivalently oxygen consumption rate (right). (**B**) FLIM of NAD(P)H reveals spatiotemporal metabolic variations in terms of the average NAD(P)H fluorescence lifetime during mouse preimplantation embryo development. Inner cell mass (ICM) and trophectoderm (TE) display different metabolic states.

responding to heterogeneous local signals? Since cumulus–oocyte crosstalk is crucial in oocyte maturation, it is natural to ask how CCs impact these metabolic heterogeneities and regulate oocyte viability. Previous work has shown that oocytes matured with or without CCs *in vitro* display different developmental competence (Zhang *et al.*, 2012). Combining biophysical modeling of metabolic crosstalk and high-resolution imaging of cumulus–oocyte complexes by FLIM during oocyte maturation provides a method to address these questions.

Developing embryos display complex spatiotemporal metabolic dynamics. Understanding these variations may help guide embryo selection based on metabolic profiles. Recent work has highlighted the importance of spatiotemporal control of mitochondrial metabolism in oogenesis (Rodríguez-Nuevo *et al.*, 2022) and early embryo development (Nagaraj *et al.*, 2017). As discussed above, FLIM metabolic profiles of human blastocysts are associated with their developmental stage, but not with their morphological assessment (Venturas *et al.*, 2022). Understanding these correlations will require relating FLIM measurements to metabolic activities of the embryo. Earlier work has shown that embryo metabolism transitions from a respiration dominant mode to a hybrid mode of respiration and fermentation at the blastocyst stage, which provides a starting point to interpret variations in FLIM parameters. Mouse embryos provide a model system to study metabolic variations. FLIM of NAD(P)H and FAD+ has revealed intricate spatiotemporal dynamics throughout mouse preimplantation embryo development (Fig. 3B) (Sanchez *et al.*, 2019). Notably, a striking metabolic heterogeneity between the inner cell mass and trophectoderm has been observed in both mouse (Fig. 3B) and human blastocysts (Venturas *et al.*, 2022), suggesting a potential connection between metabolic variation and cell fate specification (Kumar *et al.*, 2018; Chi *et al.*, 2020). Single-cell and spatial transcriptomics have helped elucidate cell lineage specification in early embryos (Peng *et al.*, 2020; Meistermann *et al.*, 2021). Combining FLIM with transcriptomics and metabolic perturbations should help elucidate the causes and consequences of these metabolic variations, their role in cell fate specification (Peng *et al.*, 2020; Zhu and Zernicka-Goetz, 2020), and guide embryo selection in ART. It is well known that different cells within an embryo can exhibit different ploidies (Popovic *et al.*, 2019; Capalbo *et al.*, 2021). Since FLIM can provide information on the metabolic state of individual cells within an embryo, it would be interesting to use this technique to determine if this genetic mosaicism leads to metabolic heterogeneity.

Conclusion

It remains an open challenge to select oocytes and embryos with the highest developmental competence in ART. Extensive studies (Gardner *et al.*, 2011; Thompson *et al.*, 2016) have revealed associations between metabolic state and embryo developmental competence. Since FLIM of NAD(P)H and FAD+ can be used to

Downloaded from https://academic.oup.com/humrep/article/38/5/799/7104033 by guest on 08 May 2023

quantitatively characterize the metabolic states of CCs, oocytes, and embryos in a label-free and noninvasive manner (Ma et al., 2019; Sanchez et al., 2019; Venturas et al., 2022), it is a promising tool for selecting oocytes and embryos. Furthermore, recent studies on CCs, oocytes, and embryos have demonstrated that FLIM can sensitively detect metabolic variations not only across samples between different patients but also within samples from the same patient (Venturas et al., 2021, 2022). In addition, metabolic variations in oocytes and embryos have been associated with oocyte maturity, ploidy status (Shah et al., 2022), and embryo developmental stages but not with embryo morphology (Venturas et al., 2022). These results suggest that metabolic characterizations can be combined with patient clinical characterization and morphological evaluations to provide a synergistic approach for the selection of oocytes and embryos. Initial work in mouse indicates minimal photodamage from FLIM measurements (Sanchez et al., 2018), but further safety studies on human oocytes and embryos will be necessary. Biophysical models aid the interpretation of FLIM measurements and will provide a mechanistic basis for oocyte and embryo selection (Yang et al., 2021). Establishing the potential predictive power that FLIM can have will ultimately require future studies determining the extent of association between FLIM measurements and ART outcome.

Data availability

No new data were generated or analysed in support of this research.

Authors' roles

M.V. and X.Y. contributed to the conceptualization of the idea of the review and drafted the manuscript. D.S. and D.N. critical revision of the article.

Funding

National Institutes of Health (R01HD092550-01) and the National Science Foundation (PHY-2013874 and MCB-2052305).

Conflict of interest

D.N. is an inventor on patent US20170039415A1.

References

Ahlström A, Lundin K, Lind A-K, Gunnarsson K, Westlander G, Park H, Thurin-Kjellberg A, Thorsteinsdottir SA, Einarsson S, Åström M *et al.* A double-blind randomized controlled trial investigating a time-lapse algorithm for selecting Day 5 blastocysts for transfer. *Hum Reprod* 2022;**37**:708–717.

Albertini DF, Combelles CM, Benecchi E, Carabatsos MJ. Cellular basis for paracrine regulation of ovarian follicle development. *Reproduction* 2001;**121**:647–653.

Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. *Hum Reprod* 2011;**26**:1270–1283.

Al-Zubaidi U, Liu J, Cinar O, Robker RL, Adhikari D, Carroll J. The spatio-temporal dynamics of mitochondrial membrane potential during oocyte maturation. *Mol Hum Reprod* 2019;**25**:695–705.

Anderson E, Albertini DF. Gap junctions between the oocyte and companion follicle cells in the mammalian ovary. *J Cell Biol* 1976;**71**:680–686.

Anderson SH, Glassner MJ, Melnikov A, Friedman G, Orynbayeva Z. Respirometric reserve capacity of cumulus cell mitochondria correlates with oocyte maturity. *J Assist Reprod Genet* 2018;**35**:1821–1830.

Becker W. Fluorescence lifetime imaging–techniques and applications. *J Microsc* 2012;**247**:119–136.

Bertoldo MJ, Listijono DR, Ho W-HJ, Riepsamen AH, Goss DM, Richani D, Jin XL, Mahbub S, Campbell JM, Habibalahi A *et al.* NAD+ repletion rescues female fertility during reproductive aging. *Cell Rep* 2020;**30**:1670.

Bradley CK, Livingstone M, Traversa MV, McArthur SJ. Impact of multiple blastocyst biopsy and vitrification-warming procedures on pregnancy outcomes. *Fertil Steril* 2017;**108**:999–1006.

Brison DR, Houghton FD, Falconer D, Roberts SA, Hawkhead J, Humpherson PG, Lieberman BA, Leese HJ. Identification of viable embryos in IVF by non-invasive measurement of amino acid turnover. *Hum Reprod* 2004;**19**:2319–2324.

Capalbo A, Poli M, Rienzi L, Girardi L, Patassini C, Fabiani M, Cimadomo D, Benini F, Farcomeni A, Cuzzi J *et al.* Mosaic human preimplantation embryos and their developmental potential in a prospective, non-selection clinical trial. *Am J Hum Genet* 2021;**108**:2238–2247.

Chance B, Williams GR. Respiratory enzymes in oxidative phosphorylation: I. Kinetics of oxygen utilization. *J Biol Chem* 1955;**217**:383–393.

Chason RJ, Csokmay J, Segars JH, DeCherney AH, Armant DR. Environmental and epigenetic effects upon preimplantation embryo metabolism and development. *Trends Endocrinol Metab* 2011;**22**:412–420.

Cheng J-X, Xie XS. (eds) *Coherent Raman Scattering Microscopy*, 1st edn. Boca Raton: CRC Press, 2012. doi:10.1201/b12907.

Chi F, Sharpley MS, Nagaraj R, Roy SS, Banerjee U. Glycolysis-independent glucose metabolism distinguishes TE from ICM fate during mammalian embryogenesis. *Dev Cell* 2020;**53**:9–26.e4.

Cimadomo D, Fabozzi G, Vaiarelli A, Ubaldi N, Ubaldi FM, Rienzi L. Impact of maternal age on oocyte and embryo competence. *Front Endocrinol* 2018;**9**:327.

Conaghan J, Hardy K, Handyside AH, Winston RM, Leese HJ. Selection criteria for human embryo transfer: a comparison of pyruvate uptake and morphology. *J Assist Reprod Genet* 1993;**10**:21–30.

Cornelisse S, Zagers M, Kostova E, Fleischer K, Wely M Van, Mastenbroek S. Preimplantation genetic testing for aneuploidies (abnormal number of chromosomes) in in vitro fertilisation. *Cochrane Database Syst Rev* 2020;**9**:CD005291.

Desquiret-Dumas V, Clément A, Seegers V, Boucret L, Ferré-L'Hotellier V, Bouet PE, Descamps P, Procaccio V, Reynier P, May-Panloup P. The mitochondrial DNA content of cumulus granulosa cells is linked to embryo quality. *Hum Reprod* 2017;**32**:607–614.

Downloaded from https://academic.oup.com/humrep/article/38/5/799/7104033 by guest on 08 May 2023

Downs SM, Humpherson PG, Leese HJ. Pyruvate utilization by mouse oocytes is influenced by meiotic status and the cumulus oophorus. *Mol Reprod Dev* 2002;**62**:113–123.

Dudenhausen JW, Maier RF. Perinatal problems in multiple births. *Dtsch Arztebl Int* 2010;**107**:663–668.

Dumesic DA, Meldrum DR, Katz-Jaffe MG, Krisher RL, Schoolcraft WB. Oocyte environment: follicular fluid and cumulus cells are critical for oocyte health. *Fertil Steril* 2015;**103**:303–316.

Dumollard R, Carroll J, Duchen MR, Campbell K, Swann K. Mitochondrial function and redox state in mammalian embryos. *Semin Cell Dev Biol* 2009;**20**:346–353.

Dumollard R, Ward Z, Carroll J, Duchen MR. Regulation of redox metabolism in the mouse oocyte and embryo. *Development* 2007; **134**:455–465.

Eichenlaub-Ritter U, Wieczorek M, Lüke S, Seidel T. Age related changes in mitochondrial function and new approaches to study redox regulation in mammalian oocytes in response to age or maturation conditions. *Mitochondrion* 2011;**11**:783–796.

Eppig JJ. Coordination of nuclear and cytoplasmic oocyte maturation in eutherian mammals. *Reprod Fertil Dev* 1996;**8**:485–489.

Eppig JJ. Intercommunication between mammalian oocytes and companion somatic cells. *Bioessays* 1991;**13**:569–574.

Fabozzi G, Iussig B, Cimadomo D, Vaiarelli A, Maggiulli R, Ubaldi N, Ubaldi FM, Rienzi L. The impact of unbalanced maternal nutritional intakes on oocyte mitochondrial activity: implications for reproductive function. *Antioxidants (Basel)* 2021;**10**:91.

Fernandez EI, Ferreira AS, Cecílio MHM, Chéles DS, de Souza RCM, Nogueira MFG, Rocha JC. Artificial intelligence in the IVF laboratory: overview through the application of different types of algorithms for the classification of reproductive data. *J Assist Reprod Genet* 2020;**37**:2359–2376.

Fontana J, Martínková S, Petr J, Žalmanová T, Trnka J. Metabolic cooperation in the ovarian follicle. *Physiol Res* 2020;**69**:33–48.

Forman EJ, Hong KH, Ferry KM, Tao X, Taylor D, Levy B, Treff NR, Scott RT. In vitro fertilization with single euploid blastocyst transfer: a randomized controlled trial. *Fertil Steril* 2013;**100**:100–107.e1.

Fragouli E, Lalioti MD, Wells D. The transcriptome of follicular cells: biological insights and clinical implications for the treatment of infertility. *Hum Reprod Update* 2014;**20**:1–11.

Fragouli E, Spath K, Alfarawati S, Kaper F, Craig A, Michel C-E, Kokocinski F, Cohen J, Munne S, Wells D. Altered levels of mitochondrial DNA are associated with female age, aneuploidy, and provide an independent measure of embryonic implantation potential. *PLoS Genet* 2015;**11**:e1005241.

Gardner DK. Lactate production by the mammalian blastocyst: Manipulating the microenvironment for uterine implantation and invasion? *Bioessays* 2015;**37**:364–371.

Gardner DK, Harvey AJ. Blastocyst metabolism. *Reprod Fertil Dev* 2015;**27**:638–654.

Gardner DK, Lane M, Stevens J, Schoolcraft WB. Noninvasive assessment of human embryo nutrient consumption as a measure of developmental potential. *Fertil Steril* 2001;**76**:1175–1180.

Gardner DK, Sakkas D. Assessment of embryo viability: the ability to select a single embryo for transfer—a review. *Placenta* 2003;**24**:S5–S12.

Gardner DK, Wale PL, Collins R, Lane M. Glucose consumption of single post-compaction human embryos is predictive of embryo sex and live birth outcome. *Hum Reprod* 2011;**26**:1981–1986.

Ghukasyan VV, Heikal AA. *Natural Biomarkers for Cellular Metabolism: Biology, Techniques, and Applications*. Boca Raton, USA: CRC Press, 2014.

Gilchrist RB, Lane M, Thompson JG. Oocyte-secreted factors: regulators of cumulus cell function and oocyte quality. *Hum Reprod Update* 2008;**14**:159–177.

Goodman LR, Goldberg J, Falcone T, Austin C, Desai N. Does the addition of time-lapse morphokinetics in the selection of embryos for transfer improve pregnancy rates? A randomized controlled trial. *Fertil Steril* 2016;**105**:275–285.e10.

Gorshinova VK, Tsvirkun DV, Sukhanova IA, Tarasova NV, Volodina MA, Marey MV, Smolnikova VU, Vysokikh MY, Sukhikh GT. Cumulus cell mitochondrial activity in relation to body mass index in women undergoing assisted reproductive therapy. *BBA Clin* 2017;**7**:141–146.

Green KA, Franasiak JM, Werner MD, Tao X, Landis JN, Scott RT, Treff NR. Cumulus cell transcriptome profiling is not predictive of live birth after in vitro fertilization: a paired analysis of euploid sibling blastocysts. *Fertil Steril* 2018;**109**:460–466.e2.

Hamel M, Dufort I, Robert C, Léveillé M-C, Leader A, Sirard M-A. Genomic assessment of follicular marker genes as pregnancy predictors for human IVF. *Mol Hum Reprod* 2010;**16**:87–96.

Hardarson T, Ahlström A, Rogberg L, Botros L, Hillensjö T, Westlander G, Sakkas D, Wikland M. Non-invasive metabolomic profiling of Day 2 and 5 embryo culture medium: a prospective randomized trial. *Hum Reprod* 2012;**27**:89–96.

Harvey AJ. Mitochondria in early development: linking the microenvironment, metabolism and the epigenome. *Reproduction* 2019;**157**: R159–R179.

Heikal AA. Intracellular coenzymes as natural biomarkers for metabolic activities and mitochondrial anomalies. *Biomark Med* 2010;**4**: 241–263.

Hoshino Y. Updating the markers for oocyte quality evaluation: intracellular temperature as a new index. *Reprod Med Biol* 2018;**17**: 434–441.

Huang Z, Wells D. The human oocyte and cumulus cells relationship: new insights from the cumulus cell transcriptome. *Mol Hum Reprod* 2010;**16**:715–725.

Jablonski A. Efficiency of anti-stokes fluorescence in dyes. *Nature* 1933;**131**:839–840.

Katz-Jaffe MG, Gardner DK, Schoolcraft WB. Proteomic analysis of individual human embryos to identify novel biomarkers of development and viability. *Fertil Steril* 2006;**85**:101–107.

Kirkegaard K, Ahlström A, Ingerslev HJ, Hardarson T. Choosing the best embryo by time lapse versus standard morphology. *Fertil Steril* 2015;**103**:323–332.

Klaidman LK, Leung AC, Adams JD. High-performance liquid chromatography analysis of oxidized and reduced pyridine dinucleotides in specific brain regions. *Anal Biochem* 1995;**228**:312–317.

Kleijkers SHM, Eijssen LMT, Coonen E, Derhaag JG, Mantikou E, Jonker MJ, Mastenbroek S, Repping S, Evers JLH, Dumoulin JCM et al Differences in gene expression profiles between human preimplantation embryos cultured in two different IVF culture media. *Hum Reprod* 2015;**30**:2303–2311.

Kovacs P, Lieman HJ. Which embryo selection method should be offered to the patients? *J Assist Reprod Genet* 2019;**36**:603–605.

Downloaded from https://academic.oup.com/humrep/article/38/5/799/7104033 by guest on 08 May 2023

Kumar K, Venturas M, Needleman DJ, Racowsky C, Wells D. Extensive analysis of mitochondrial DNA quantity and sequence variation in human cumulus cells and assisted reproduction outcomes. *Hum Reprod* 2021;**37**:66–79.

Kumar RP, Ray S, Home P, Saha B, Bhattacharya B, Wilkins HM, Chavan H, Ganguly A, Milano-Foster J, Paul A *et al*. Regulation of energy metabolism during early mammalian development: TEAD4 controls mitochondrial transcription. *Development* 2018;**145**: dev162644.

Kurosawa H, Utsunomiya H, Shiga N, Takahashi A, Ihara M, Ishibashi M, Nishimoto M, Watanabe Z, Abe H, Kumagai J *et al*. Development of a new clinically applicable device for embryo evaluation which measures embryo oxygen consumption. *Hum Reprod* 2016;**31**:2321–2330.

Lakowicz JR. *Principles of Fluorescence Spectroscopy*. USA: Springer, 2006. https://www.springer.com/gp/book/9780387312781.

Lan Y, Zhang S, Gong F, Lu C, Lin G, Hu L. The mitochondrial DNA copy number of cumulus granulosa cells may be related to the maturity of oocyte cytoplasm. *Hum Reprod* 2020;**35**:1120–1129.

Lane M, Gardner DK. Lactate regulates pyruvate uptake and metabolism in the preimplantation mouse embryo. *Biol Reprod* 2000;**62**: 16–22.

Leahy BD, Jang W-D, Yang HY, Struyven R, Wei D, Sun Z, Lee KR, Royston C, Cam L, Kalma Y *et al*. Automated measurements of key morphological features of human embryos for IVF. *Med Image Comput Comput Assist Interv* 2020;**12265**:25–35.

Leese HJ, Sturmey RG, Baumann CG, McEvoy TG. Embryo viability and metabolism: obeying the quiet rules. *Hum Reprod* 2007;**22**: 3047–3050.

Leese HJ. Metabolism of the preimplantation embryo: 40 years on. *Reproduction* 2012;**143**:417–427.

Liu X-M, Zhang Y-P, Ji S-Y, Li B-T, Tian X, Li D, Tong C, Fan H-Y. Mitoguardin-1 and -2 promote maturation and the developmental potential of mouse oocytes by maintaining mitochondrial dynamics and functions. *Oncotarget* 2016;**7**:1155–1167.

Lopes AS, Lane M, Thompson JG. Oxygen consumption and ROS production are increased at the time of fertilization and cell cleavage in bovine zygotes. *Hum Reprod* 2010;**25**:2762–2773.

Lu X, Liu Y, Xu J, Cao X, Zhang D, Liu M, Liu S, Dong X, Shi H. Mitochondrial dysfunction in cumulus cells is related to decreased reproductive capacity in advanced-age women. *Fertil Steril* 2022; **118**:393–404.

Ma N, de Mochel NR, Pham PD, Yoo TY, Cho KWY, Digman MA. Label-free assessment of pre-implantation embryo quality by the Fluorescence Lifetime Imaging Microscopy (FLIM)-phasor approach. *Sci Rep* 2019;**9**:13206.

Masters BR, So P. Handbook of biomedical nonlinear optical microscopy. *J Biomed Opt* 2008;**14**:334–348.

McLennan HJ, Saini A, Dunning KR, Thompson JG. Oocyte and embryo evaluation by AI and multi-spectral auto-fluorescence imaging: Livestock embryology needs to catch-up to clinical practice. *Theriogenology* 2020;**150**:255–262.

Meistermann D, Bruneau A, Loubersac S, Reignier A, Firmin J, François-Campion V, Kilens S, Lelièvre Y, Lammers J, Feyeux M et al Integrated pseudotime analysis of human pre-implantation embryo single-cell transcriptomes reveals the dynamics of lineage specification. *Cell Stem Cell* 2021;**28**:1625–1640.e6.

Munné S. Status of preimplantation genetic testing and embryo selection. *Reprod Biomed Online* 2018;**37**:393–396.

Nagaraj R, Sharpley MS, Chi F, Braas D, Zhou Y, Kim R, Clark AT, Banerjee U. U. Nuclear localization of mitochondrial TCA cycle enzymes as a critical step in mammalian zygotic genome activation. *Cell* 2017;**168**:210–223.e11.

Nakahara T, Iwase A, Goto M, Harata T, Suzuki M, Ienaga M, Kobayashi H, Takikawa S, Manabe S, Kikkawa F *et al*. Evaluation of the safety of time-lapse observations for human embryos. *J Assist Reprod Genet* 2010;**27**:93–96.

Nikoloff N. The key role of cumulus cells in oocytes in vitro maturation protocols. *Fertil Steril* 2021;**116**:1663.

Ogino M, Tsubamoto H, Sakata K, Oohama N, Hayakawa H, Kojima T, Shigeta M, Shibahara H. Mitochondrial DNA copy number in cumulus cells is a strong predictor of obtaining good-quality embryos after IVF. *J Assist Reprod Genet* 2016;**33**:367–371.

Peng G, Cui G, Ke J, Jing N. Using single-cell and spatial transcriptomes to understand stem cell lineage specification during early embryo development. *Annu Rev Genom Hum Genet* 2020;**21**: 163–181.

Pietroforte S, Martins M, Zambelli F, Vassena R, Gulliford C, Sanchez TH, Sakkas D. Non-invasive prediction of oocyte maturation to metaphase II (MII) from germinal vesicle (GV) by mitochondrial function markers using fluorescence lifetime imaging microscopy (FLIM). *Fertil Steril* 2022;**118**:e146–e147.

Pollard C-L, Gibb Z, Hawdon A, Swegen A, Grupen CG. Supplementing media with NAD+ precursors enhances the in vitro maturation of porcine oocytes. *J Reprod Dev* 2021;**67**: 319–326.

Popovic M, Dhaenens L, Taelman J, Dheedene A, Bialecka M, De Sutter P, Chuva de Sousa Lopes SM, Menten B, Heindryckx B. Extended in vitro culture of human embryos demonstrates the complex nature of diagnosing chromosomal mosaicism from a single trophectoderm biopsy. *Hum Reprod* 2019;**34**:758–769.

Racowsky C, Needleman DJ. Cumulus cell gene expression as a potential biomarker for oocyte quality. *Fertil Steril* 2018;**109**:438–439.

Renard JP, Philippon A, Menezo Y. In-vitro uptake of glucose by bovine blastocysts. *J Reprod Fertil* 1980;**58**:161–164.

Richani D, Dunning KR, Thompson JG, Gilchrist RB. Metabolic co-dependence of the oocyte and cumulus cells: essential role in determining oocyte developmental competence. *Hum Reprod Update* 2021;**27**:27–47.

Rodríguez-Nuevo A, Torres-Sanchez A, Duran JM, De Guirior C, Martínez-Zamora MA, Böke E. Oocytes maintain ROS-free mitochondrial metabolism by suppressing complex I. *Nature* 2022;**607**: 756–761.

Rosenwaks Z. Introduction: biomarkers of embryo viability: the search for the "holy grail" of embryo selection. *Fertil Steril* 2017; **108**:719–721.

Sanchez T, Seidler EA, Gardner DK, Needleman D, Sakkas D. Will noninvasive methods surpass invasive for assessing gametes and embryos? *Fertil Steril* 2017;**108**:730–737.

Sanchez T, Venturas M, Aghvami SA, Yang X, Fraden S, Sakkas D, Needleman DJ. Combined noninvasive metabolic and spindle

Downloaded from https://academic.oup.com/humrep/article/38/5/799/7104033 by guest on 08 May 2023

imaging as potential tools for embryo and oocyte assessment. *Hum Reprod* 2019;**34**:2349–2361.

Sanchez T, Wang T, Pedro MV, Zhang M, Esencan E, Sakkas D, Needleman D, Seli E. Metabolic imaging with the use of fluorescence lifetime imaging microscopy (FLIM) accurately detects mitochondrial dysfunction in mouse oocytes. *Fertil Steril* 2018;**110**: 1387–1397.

Santos Monteiro CA, Chow DJX, Leal GR, Tan TC, Reis Ferreira AM, Thompson JG, Dunning KR. Optical imaging of cleavage stage bovine embryos using hyperspectral and confocal approaches reveals metabolic differences between on-time and fast-developing embryos. *Theriogenology* 2021;**159**:60–68.

Schoolcraft WB, Gardner DK, Lane M, Schlenker T, Hamilton F, Meldrum DR. Blastocyst culture and transfer: analysis of results and parameters affecting outcome in two in vitro fertilization programs. *Fertil Steril* 1999;**72**:604–609.

Scott L, Berntsen J, Davies D, Gundersen J, Hill J, Ramsing N. Symposium: innovative techniques in human embryo viability assessment. Human oocyte respiration-rate measurement–potential to improve oocyte and embryo selection? *Reprod Biomed Online* 2008;**17**:461–469.

Scott R, Zhang M, Seli E. Metabolism of the oocyte and the preimplantation embryo: implications for assisted reproduction. *Curr Opin Obstet Gynecol* 2018;**30**:163–170.

Scott RT, Upham KM, Forman EJ, Zhao T, Treff NR. Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial. *Fertil Steril* 2013;**100**:624–630.

Seidler EA, Sanchez T, Venturas M, Sakkas D, Needleman DJ. Noninvasive imaging of mouse embryo metabolism in response to induced hypoxia. *J Assist Reprod Genet* 2020;**37**:1797–1805.

Shah JS, Venturas M, Sanchez TH, Penzias AS, Needleman D, Sakkas D. Fluorescence lifetime imaging microscopy (FLIM) detects differences in metabolic signatures between euploid and aneuploid human blastocysts. *Fertil Steril* 2020;**114**:e76–e77.

Shah JS, Venturas M, Sanchez TH, Penzias AS, Needleman DJ, Sakkas D. Fluorescence lifetime imaging microscopy (FLIM) detects differences in metabolic signatures between euploid and aneuploid human blastocysts. *Hum Reprod* 2022;**37**:400–410.

Simopoulou M, Sfakianoudis K, Maziotis E, Tsioulou P, Grigoriadis S, Rapani A, Giannelou P, Asimakopoulou M, Kokkali G, Pantou A et al PGT-A: who and when? A systematic review and network meta-analysis of RCTs. *J Assist Reprod Genet* 2021;**38**:1939–1957.

Skala MC, Riching KM, Bird DK, Gendron-Fitzpatrick A, Eickhoff J, Eliceiri KW, Keely PJ, Ramanujam N. In vivo multiphoton fluorescence lifetime imaging of protein-bound and free nicotinamide adenine dinucleotide in normal and precancerous epithelia. *J Biomed Opt* 2007;**12**:024014.

Suhling K, Siegel J, Lanigan PMP, Lévêque-Fort S, Webb SED, Phillips D, Davis DM, French PMW. Time-resolved fluorescence anisotropy imaging applied to live cells. *Opt Lett* 2004;**29**:584–586.

Sutton ML, Gilchrist RB, Thompson JG. Effects of in-vivo and in-vitro environments on the metabolism of the cumulus-oocyte complex and its influence on oocyte developmental capacity. *Hum Reprod Update* 2003;**9**:35–48.

Sutton-McDowall ML, Gosnell M, Anwer AG, White M, Purdey M, Abell AD, Goldys EM, Thompson JG. Hyperspectral microscopy can detect metabolic heterogeneity within bovine post-compaction embryos incubated under two oxygen concentrations (7% versus 20%). *Hum Reprod* 2017;**32**:2016–2025.

Tan TCY, Brown HM, Thompson JG, Mustafa S, Dunning KR. Optical imaging detects metabolic signatures associated with oocyte quality. *Biol Reprod* 2022a;**107**:1014–1025.

Tan TCY, Mahbub SB, Campbell JM, Habibalahi A, Campugan CA, Rose RD, Chow DJX, Mustafa S, Goldys EM, Dunning KR. Non-invasive, label-free optical analysis to detect aneuploidy within the inner cell mass of the preimplantation embryo. *Hum Reprod* 2022b; **37**:14–29.

Tatone C, Heizenrieder T, Di Emidio G, Treffon P, Amicarelli F, Seidel T, Eichenlaub-Ritter U. Evidence that carbonyl stress by methylglyoxal exposure induces DNA damage and spindle aberrations, affects mitochondrial integrity in mammalian oocytes and contributes to oocyte ageing. *Hum Reprod* 2011;**26**:1843–1859.

Tejera A, Herrero J, de los Santos MJ, Garrido N, Ramsing N, Meseguer M. Oxygen consumption is a quality marker for human oocyte competence conditioned by ovarian stimulation regimens. *Fertil Steril* 2011;**96**:618–623.e2.

Tejera A, Herrero J, Viloria T, Romero JL, Gamiz P, Meseguer M. Time-dependent O2 consumption patterns determined optimal time ranges for selecting viable human embryos. *Fertil Steril* 2012; **98**:849–857.e3.

Tesarik J, Greco E. The probability of abnormal preimplantation development can be predicted by a single static observation on pronuclear stage morphology. *Hum Reprod* 1999;**14**:1318–1323.

Thompson JG, Brown HM, Sutton-McDowall ML. Measuring embryo metabolism to predict embryo quality. *Reprod Fertil Dev* 2016;**28**: 41–50.

Urbanski JP, Johnson MT, Craig DD, Potter DL, Gardner DK, Thorsen T. Noninvasive metabolic profiling using microfluidics for analysis of single preimplantation embryos. *Anal Chem* 2008;**80**: 6500–6507.

Van Blerkom J, Davis PW, Lee J. ATP content of human oocytes and developmental potential and outcome after in-vitro fertilization and embryo transfer. *Hum Reprod* 1995;**10**:415–424.

Venturas M, Shah JS, Yang X, Sanchez TH, Conway W, Sakkas D, Needleman DJ. Metabolic state of human blastocysts measured by fluorescence lifetime imaging microscopy. *Hum Reprod* 2022;**37**: 411–427.

Venturas M, Yang X, Kumar K, Wells D, Racowsky C, Needleman DJ. Metabolic imaging of human cumulus cells reveals associations among metabolic profiles of cumulus cells, patient clinical factors, and oocyte maturity. *Fertil Steril* 2021;**116**:1651–1662.

Vergouw CG, Botros LL, Roos P, Lens JW, Schats R, Hompes PGA, Burns DH, Lambalk CB. Metabolomic profiling by near-infrared spectroscopy as a tool to assess embryo viability: a novel, non-invasive method for embryo selection. *Hum Reprod* 2008;**23**: 1499–1504.

Wilding M, Dale B, Marino M, di Matteo L, Alviggi C, Pisaturo ML, Lombardi L, De Placido G. Mitochondrial aggregation patterns and activity in human oocytes and preimplantation embryos. *Hum Reprod* 2001;**16**:909–917.

Wong KM, Repping S, Mastenbroek S. Limitations of embryo selection methods. *Semin Reprod Med* 2014;**32**:127–133.

Downloaded from https://academic.oup.com/humrep/article/38/5/799/7104033 by guest on 08 May 2023

Yang X, Ha G, Needleman D. A coarse-grained NADH redox model enables inference of subcellular metabolic fluxes from fluorescence lifetime imaging. In Finley LWS, editor. *eLife* 2021;**10**: e73808.

Yu Y, Dumollard R, Rossbach A, Lai FA, Swann K. Redistribution of mitochondria leads to bursts of ATP production during spontaneous mouse oocyte maturation. *J Cell Physiol* 2010;**224**:672–680.

Zaninovic N, Rosenwaks Z. Artificial intelligence in human in vitro fertilization and embryology. *Fertil Steril* 2020;**114**:914–920.

Zhang A, Xu B, Sun Y, Lu X, Niu Z, Chen Q, Feng Y, Xu C. The effect of human cumulus cells on the maturation and developmental potential of immature oocytes in ICSI cycles. *J Assist Reprod Genet* 2012;**29**:313–319.

Zhao J, Li Y. Adenosine triphosphate content in human unfertilized oocytes, undivided zygotes and embryos unsuitable for transfer or cryopreservation. *J Int Med Res* 2012;**40**:734–739.

Zhou C-J, Wu S-N, Shen J-P, Wang D-H, Kong X-W, Lu A, Li Y-J, Zhou H-X, Zhao Y-F, Liang C-G. The beneficial effects of cumulus cells and oocyte-cumulus cell gap junctions depends on oocyte maturation and fertilization methods in mice. *PeerJ* 2016;**4**:e1761.

Zhu M, Zernicka-Goetz M. Living a sweet life: glucose instructs cell fate in the mouse embryo. *Dev Cell* 2020;**53**:1–2.

Downloaded from https://academic.oup.com/humrep/article/38/5/799/7104033 by guest on 08 May 2023