Mitochondria: Their relevance during oocyte ageing

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ABSTRACT

The oocyte is recognised as the largest cell in mammalian species and other multicellular organisms. Mitochondria represent a high proportion of the cytoplasm in oocytes and mitochondrial architecture is different in oocytes than in somatic cells, characterised by a rounder appearance and fragmented network. Although the number of mitochondria per oocyte is higher than in any other mammalian cell, their number and activity decrease with advancing age. Mitochondria integrate numerous processes essential for cellular function, such as metabolic processes related to energy production, biosynthesis, and waste removal, as well as Ca²⁺ signalling and reactive oxygen species (ROS) homeostasis. Further, mitochondria are responsible for the cellular adaptation to different types of stressors such as oxidative stress or DNA damage. When these stressors outstrip the adaptive capacity of mitochondria to restore homeostasis, it leads to mitochondrial dysfunction. Decades of studies indicate that mitochondrial function is multifaceted, which is reflected in the oocyte, where mitochondria support numerous processes during oocyte maturation, fertilization, and early embryonic development. Disregulation of mitochondrial processes has been consistently reported in ageing and age-related diseases. In this review, we describe the functions of mitochondria as bioenergetic powerhouses and signal transducers in oocytes, how dysfunction of mitochondrial processes contributes to reproductive ageing, and whether mitochondria could be targeted to promote oocyte rejuvenation.

1. Introduction

Reproductive ageing in female mammals and women is characterised by a progressive decline of ovarian function, manifested by a decrease in the quantity and quality of oocytes with advancing age. The reproductive tract of women is one of the first organ systems to show hallmarks of ageing, in comparison to other organs. However, the molecular pathways that are involved in the phenomenon of oocyte ageing remain unelucidated (te Velde and Pearson, 2021; Selesniemi et al., 2008; Garg and Sinclair, 2015; Harrison et al., 2017). Most industrialised countries show an increasing number of women’s first pregnancies at what is considered an advanced reproductive age (≥35 years), caused by factors such as prolonged education and career ambitions, which has important implications for society (Remkes-Grottenthaler, 2003; Marshall et al., 2020; Patel et al., 2018). Difficulty conceiving and infertility are treated as taboo topics in most nations and lead to significant psychological stress for those experiencing them (Patel et al., 2018). Although assisted reproductive technologies (ART) such as cryopreservation (the freezing of oocytes, sperm, or embryos) and in vitro fertilization exist, they are not ubiquitously available or successful and require substantial financial investment (Katz et al., 2011). Furthermore, a fertilised oocyte derived from a woman of advanced age has a higher chance of resulting in miscarriage, and/or aneuploid offspring like trisomy of chromosome 21, commonly known as Down syndrome (Bittles et al., 2007). With advancing age, a decline in mitochondrial number and function is observed in oocytes. Mitochondria are exclusively maternally inherited and therefore the original population present in the oocyte will give rise to all future mitochondria in the offspring (Jansen and de Boer, 1998). Thus, elucidating the mechanisms underlying the loss of mitochondrial function with ageing and why oocytes age much earlier as compared to other organ systems, may lead to new therapeutic strategies to prolong oocyte fitness and fertility. Additionally, as oocyte ageing occurs earlier in life as compared to other organ systems, studying oocyte ageing could represent an important model to explain fundamental processes related to the maintenance of reproductive fitness.
contributing to ageing in general. Therefore, in this review we will first summarise the function of mitochondria in oocytes during physiological reproductive processes, followed by their changes during oocyte ageing, and finally, discuss new strategies to target mitochondrial function and slow down oocyte ageing.

2. Effect of ageing on female reproductive fitness

Among the female reproductive tract organs, the ovary is critical for maintaining fertility and endocrine homeostasis as the main producer of steroid hormones and by bearing the female germline cells, oocytes (Broekmans, 2009). The ovary exhibits early-onset ageing-associated dysfunction, with evident decline after the mid-thirties (Tilly and Sinclair, 2013; Klinkert, 2005). It is generally accepted that the age-related decline in fertilization outcome of oocytes is not as strongly dependent on the number of remaining oocytes in the mammalian ovary (termed ovarian reserve), but is more dependent on the quality of the oocyte and the microenvironment to which the oocyte is exposed (Chiang et al., 2011). Maternal ageing is known to stimulate several molecular changes that drive defects in chromatin separation, chromosome decondensation, and spindle detachment causing chromosomal misalignment (Liu and Keeffe, 2002). It has been reported that in 95 % of children born with Down’s syndrome, the extra chromosome 21 was provided by the maternal part, and in 80 % of cases, this occurred during the first meiotic division that takes place in the ovary prior to ovulation (Gaulden, 1992). Increased oxidative and energy stress within the ovary appear to contribute to the incidence of chromosomal failures in advanced age mothers (Agarwal and Allamaneni, 2004). A substantial body of work indicates that oocytes of advanced maternal age have decreased intracellular ATP content, which was shown in women (van Blerkom et al., 1995), rats, hamsters (Simsek-Duran et al., 2013), pigs (Sato et al., 2014), and mice (Igarashi et al., 2005), and correlates with the incidence of chromosomal failures.

Therefore, advanced maternal age affects oocyte maturation, meiotic divisions, and embryonic development (Timoteo-Ferreira et al., 2021; Sher et al., 2007; Llnoch et al., 2021; Di Emidio et al., 2014; Rambags et al., 2014; Sekhon et al., 2014). The risk of childlessness and stillbirth is much higher in advanced age mothers compared to younger mothers, defined as below 30 years of age (Selemani et al., 2014; Brion et al., 2008). The Barker hypothesis postulates that diseases arising in offspring can originate from the foetal developmental period (Barker et al., 1995), and diminished oocyte quality may therefore be a crucial risk factor for future disease (Ge et al., 2014). Menopause in women is the natural consequence of ovarian physiological ageing (Ge et al., 2014; Perry et al., 2015; Webb et al., 2017). Therefore, acquiring new insights and knowledge about the mechanisms driving ovarian ageing is of critical importance and this review describes how mitochondria contribute to oocyte ageing.

3. Mitochondrial function in oocytes and reproductive processes

Mitochondria provide energy and building blocks to support transcription and translation during oocyte maturation, fertilization, and early embryonic development. The quality of mitochondria in oocytes determines the quality of the oocyte and the developing embryo, since the paternal mitochondria are degraded after the sperm has entered the oocyte (Dumollard et al., 2007). Mitochondria coordinate numerous metabolic, epigenetic, redox, and calcium signalling processes that are essential for cellular function, and emerging studies highlight mitochondrial dysfunction as a key contributor to oocyte ageing. Therefore, we will briefly summarise mitochondrial functions in the following sections to provide the background for a better understanding of mitochondrial dysfunction in the ageing oocyte.

3.1. Mitochondrial metabolism

Mitochondria are widely recognised as the powerhouses of the cell (Spinelli and Haigis, 2018), but they also produce numerous precursor molecules that are the building blocks for protein, lipid, DNA, and RNA biosynthesis, maintain homeostasis of reducing equivalents, and manage metabolic waste products (Spinelli and Haigis, 2018). Glucose is catabolised via glycolysis in the cytosol, after which pyruvate is imported into the mitochondria to undergo oxidative phosphorylation in the tricarboxylic acid (TCA) cycle (Fig. 1). Electrons generated in the TCA cycle are donated to the electron transport chain (ETC) complexes located at the inner mitochondrial membrane by dedicated electron carriers (NADH, FADH2). The ETC complexes then transport electrons along the mitochondrial membrane, while pumping protons into the intermembrane space, in a process called oxidative phosphorylation (OXPHOS) (Siekevitz and Watson, 1957; Walsh et al., 2018; Letts and Sazanov, 2015; Watt et al., 2010). NADH and succinate generated in the TCA cycle are oxidised at complex I and II, respectively. Electrons are then passed to coenzyme Q and further to complex III, cytochrome C, and complex IV (Fig. 1). Simultaneously, protons are pumped into the intermembrane space by complexes I, III, and IV, which gives rise to the mitochondrial membrane potential. This electrochemical gradient is coupled to ATP generation from ADP by ATP synthase, which is otherwise an energetically unfavourable reaction. Importantly, the process of electron transport-coupled proton pumping is not fail-proof, and electron leakage occurs predominantly at complexes I and III. When these electrons react with molecular oxygen, they give rise to reactive oxygen species (Schieber and Chandel, 2014), which we will discuss in more detail in section 3.3. Besides glucose, glutamine and acetate are other important carbon sources, which are both metabolised in the mitochondria and serve as fuel for the TCA cycle or biosynthesis of other molecules (Yang et al., 2014; Fujino et al., 2002). The branched-chain amino acids (BCAAs) leucine, isoleucine, and valine can also feed into the TCA cycle (Ahn and Mettela, 2015) and lipid-derived fatty acids become an important fuel source when cells face nutrient stress (Röhrig and Schulze, 2016). In addition to the macromolecules described previously, mitochondria contribute to the synthesis of nucleotides, proteins, haeme, and glucose (Houten and Wanders, 2010; Newman and Maddocks, 2017; Hunter and Ferreiria, 2011; Bahli et al., 1997). They also maintain the cellular balance of redox equivalents (Spinelli and Haigis, 2018; Mrácek et al., 2013; Gnoni et al., 2009) and regulate cellular waste management by converting by-products of metabolism such as ammonia and hydrogen sulpheride (Morris and Kepka-Lenhart, 2002). Thus, besides their important role in energy homeostasis, mitochondria are key biosynthetic hubs that control energetic, metabolic, and redox homeostasis and thereby maintain cellular function.

Oocytes produce energy predominantly through oxidative phosphorylation (Dumollard et al., 2007; Leese and Barton, 1984). The bioenergetic state of the oocyte impacts its developmental competence, with previous research showing that mitochondrial membrane potential and oocyte ATP content affects embryonic implantation competence (van Blerkom et al., 1995; Igarashi et al., 2005; Di Emidio et al., 2014; Wilding et al., 2001). Mitochondrial membrane potential in oocytes is gradually altered upon ageing (Igarashi et al., 2005; Tilly, 2001) and it was demonstrated that mitochondrial activity was an indicator of oocyte developmental competence (Perry et al., 2015). Furthermore, interference with OXPHOS was reported to lead to arrested oocyte maturation and chromosome misalignment (Yakushiji et al., 2005; Theolier et al., 2006; Wyman et al., 2008). The maximum respiratory capacity of aged oocytes is significantly reduced, with lower ATP-linked respiration levels (Sugimura et al., 2012). Reduced ATP production leads to decreased metabolic activity and could also affect cell cycle regulation, mitotic spindle formation, chromosome segregation, fertilization, embryonic development, and implantation (van Blerkom, 1995; Van Blerkom, 2011; Eichenlaub-Ritter, 2012). Balanced energy consumption is crucial for successful oocyte maturation (Dumollard et al., 2007; Van
Fig. 1. Schematic illustration of central carbon metabolism in mitochondria. Pyruvate produced from glucose in glycolysis is imported into the mitochondria and further oxidised in the TCA cycle to yield the electron carriers NADH and FADH$_2$. The ETC complexes accept and transport electrons along the mitochondrial membrane, while pumping protons into the intermembrane space, which generates the electrochemical gradient that drives ATP production by ATP synthase. Acetate, glutamine, BCAA, and fatty acids can act as alternative carbon sources to pyruvate. Besides ATP, OXPHOS yields intermediates that can act as molecular building blocks that together fill the bioenergetic and biosynthetic needs of the cell. Abbreviations: MPC = mitochondrial pyruvate carrier, SucCoa = succinyl-CoA, OAA = oxaloacetate, α-KG = alpha-ketoglutarate, CoQ = coenzyme Q, Cyt c = cytochrome c.

Fig. 2. Schematic illustration of the spindle formation in metaphase II oocytes of young and advanced age women. The spindle formation during metaphase is an energy-driven process. Therefore, an adequate ATP supply by mitochondria is essential. There is a higher percentage of disrupted spindle, higher percentage of abnormal tubulin placement from the metaphase plate during the second meiotic division, and higher percentage of chromosome segregation failure in oocytes from advanced age women when compared to younger counterparts. Due to an insufficient energy supply by the mitochondria in oocytes of advanced age women, the regulatory mechanisms responsible for assembly of the meiotic spindle are significantly altered, which leads to a higher prevalence of aneuploidy if compared to oocytes of younger women.
and inappropriate mitochondrial functionality has recently been associated with a stress-related reduction in oocyte developmental competence (Roth, 2018). In summary, optimal mitochondrial energy production is crucial for the functional competence of an oocyte and the developing embryo after fertilization and is compromised by mitochondrial dysfunction. The importance of an adequate energy supply during the spindle assembly in an oocyte will be described in the following section.

3.2. Mitochondrial energy supply during the spindle assembly

Chromosome segregation errors are the main cause of oocyte aneuploidy in humans and have been shown to increase as women age. Mitochondrial dysfunction is one key factor responsible for chromosomal anomalies during the meiotic divisions of mammalian oocytes (Schon et al., 2000; Desler et al., 2007), which has been linked to the incidence rate of Down’s syndrome due to advanced maternal age (Duncan et al., 2012). Moreover, it is known that the incidence rate of meiotic division errors is higher in oocytes of advanced maternal age, which is correlated with extrusion failure of the first polar body, shorter spindles, irregular disjunction of chromosomes followed by aneuploidy (Zhang et al., 2006) (Fig. 2). Although the spindle assembly checkpoint may prevent such events by detecting the presence of unattached chromosomes, several components involved in this signalling cascade seem to be compromised in aged mammalian oocytes (Eichenlaub-Ritter et al., 2004, 2011). These spindles are formed by microtubules (MT), which are highly dynamic tubulin polymers (Compton, 2000). The spindles in oocytes appear in a barrel-like shape containing flat spindle poles and only several astral microtubules (Eichenlaub-Ritter et al., 2004). The activity of motor proteins is required for the process of spindle formation and chromosome segregation, specifically proteins of the kinesin superfamily are crucial for the spindle assembly during meiosis (Eichenlaub-Ritter et al., 2004). In this process, kinesins hydrolyse about 125 ATP molecules for each microtubule binding event, meiosis (Eichenlaub-Ritter et al., 2004). In this process, kinesins hydrolyse about 125 ATP molecules for each microtubule binding event, meiosis (Eichenlaub-Ritter et al., 2004). Although the spindle assembly checkpoint may prevent such events by detecting the presence of unattached chromosomes, several components involved in this signalling cascade seem to be compromised in aged mammalian oocytes (Eichenlaub-Ritter et al., 2004, 2011). These spindles are formed by microtubules (MT), which are highly dynamic tubulin polymers (Compton, 2000). The spindles in oocytes appear in a barrel-like shape containing flat spindle poles and only several astral microtubules (Eichenlaub-Ritter et al., 2004). The activity of motor proteins is required for the process of spindle formation and chromosome segregation, specifically proteins of the kinesin superfamily are crucial for the spindle assembly during meiosis (Eichenlaub-Ritter et al., 2004).

3.3. Redox co-factor balance and metabolic homeostasis

ROS trigger cellular signalling cascades by activating proteins indirectly or through protein oxidation and are produced as a by-product of various enzymatic reactions, or directly by NADPH oxidase enzymes (Davies, 2016; Lambeth and Neish, 2014). Similarly, the gasotransmitter nitric oxide (NO) can give rise to various reactive nitrogen species (RNS) and nitric oxide (NO) can give rise to various reactive nitrogen species (RNS) that can also act as signalling molecules by nitrosylating proteins. As discussed in section 3.1, mitochondrial OXPHOS is a biochemical reducing and oxidizing (redox) process that produces ROS as a by-product. In quiescent cells, mitochondrial metabolism is the main process during which ROS is generated (Saharwal and Schumacker, 2014; Wong et al., 2017). Neutralizing ROS and RNS is important because, in excess, they can damage cellular DNA, lipids, and proteins. In absence of sufficient buffering capacity or repair mechanisms, progressive accumulation of oxidative damage causes cellular decline and associated pathologies. Mitochondrial protection mechanisms against electron leakage and excess ROS include the superoxide dismutase enzymes, which convert superoxide (O2•−) to hydrogen peroxide (H2O2), with SOD1 located in the mitochondrial intermembrane space and SOD2 in the matrix (Fig. 3). Hydrogen peroxide can also give rise to a hydroxyl radical (•OH) when partially reduced. Cellular hydrogen peroxide can be degraded to water by catalase, glutathione peroxidases (GPX), and peroxiredoxins. However, catalase is absent in mitochondria and only a single splice variant of GPX4 has been reported to be present in mitochondria (Lubos et al., 2011; Casanas-Sánchez et al., 2015). Mitochondria rely on the combined activities of peroxiredoxins 3 and 5 (PRDX3, PRDX5), thioredoxin 2 (TXN2), and thioredoxin reductase 2 (TXR2) to decompose the locally generated hydrogen peroxide and reduce oxidised proteins (Ismail et al., 2019). Mitochondrial proteins are specifically susceptible to oxidation and oxidative damage (van der Reest et al., 2016). When compared to the concentration of the antioxidant glutathione (GSH), mitochondria display a higher ratio of exposed protein thiols to GSH as compared to the cytosol. Mitochondria also have a slightly higher pH compared to the cytosol, which increases reactivity of protein cysteine residues by deprotonating them to cysteine thiolates (Requejo et al., 2010). Thus, although cellular and mitochondrial redox states are tightly connected, the compartmentalised mitochondrial milieu presents unique challenges to maintain redox-metabolic homeostasis. Opposed to the damaging roles of ROS, they also play an important role as signalling molecules, and mitochondria are increasingly recognised as redox signalling hubs. Stressors such as hypoxia, changes in mitochondrial membrane potential, cytokine stimulation, and nutrient availability are the causes for the generation, sequestration, and interconversion of ROS in mitochondria (Martínez-Reyes et al., 2016). As potent mitogen signalling agents, ROS foster proliferation, differentiation, and migration through the oxidation of protein cysteine residues (Aggarwal et al., 2019; Truong and Carroll, 2013). Recent results indicate that protein cysteine oxidation can help cells adapt to redox stress and that metabolic and mitochondrial proteins are particularly liable to oxidation (van der Reest et al., 2018).

Most cellular redox reactions utilise the co-factor nicotinamide adenine nucleotide (NAD), which exists in oxidised, reduced, and phosphorylated forms. NAD levels decline with age, which has been linked to several hallmarks of ageing and age-related diseases, specifically mitochondrial decline. In response, several research studies have now established that supplementation with NAD+ precursors or genetic approaches to boost NAD+ levels extend the lifespan in various model organisms, including worms, yeast, and mice. Together, this suggests that a loss of mitochondrial mass and function in ageing oocytes compromises the ability to meet cellular metabolic demand for ATP and maintain redox-metabolic homeostasis required for optimal function. Although it is known that oocyte quality is dependent on mitochondrial metabolism, ROS increase as a function of metabolic rate, and mitochondrial dysfunction compromises cellular redox-metabolic homeostasis; the sequence of events underlying oocyte ageing and its triggers remain unclear. It is essential to unravel these co-dependent processes to begin to explore therapeutic interventions to correct and prolong oocyte function during ageing.

3.4. Mitochondrial Ca2+ homeostasis in oocytes

Mitochondria may also influence oocyte activation and embryonic development through the modulation of Ca2+ signalling. Before fertilization, oocytes are arrested at metaphase II stage, and the sperm-triggered Ca2+ oscillations are required for several processes such as resumption of meiosis, polyspermy block, male chromatin decondensation, recruitment of maternal mRNAs, and pronuclear formation (Wang et al., 2018; Szpila et al., 2019) (Fig. 4A). The Ca2+ homeostasis in postovulatory oocytes depends on proper mitochondrial activity and function, as mitochondria-associated membranes facilitate the transfer
of Ca\(^{2+}\) ions from the endoplasmic reticulum (ER) (Liu and Zhu, 2017) (Fig. 4B). An excess of Ca\(^{2+}\) transfer can disrupt oxidative phosphorylation and redox homeostasis, or trigger the mitochondrial permeability transition pore to open, which further compromises mitochondrial function and can induce apoptosis. Conversely, low Ca\(^{2+}\) transfer from the ER to mitochondria contributes to bioenergetic crisis, indicating that maintaining cellular calcium homeostasis is a delicate balance (Cardenas et al., 2010). Aged oocytes have been shown to present lower amount of Ca\(^{2+}\) stored in the ER, altered cytoplasmic Ca\(^{2+}\) uptake due to decreased sarcoplasmic reticulum Ca\(^{2+}\) ATPases (SERCA2) expression, and diminished ATP availability secondary to altered mitochondrial functionality (Szpila et al., 2019) (Fig. 4B). It has been previously demonstrated that impaired mitochondrial oxidative phosphorylation and ATP production may affect ATP-dependent Ca\(^{2+}\) oscillations (Dumollard et al., 2007; Wang et al., 2018), and the use of L-carnitine improved mitochondrial function and Ca\(^{2+}\) oscillations in a mouse model (Siekevitz and Watson, 1957). Additionally, aging seems to reduce the expression of mitochondrial fission factor Drp1 which alters mitochondrial morphology and dynamics and, ultimately, the ER-mitochondria inter-organelle communication and Ca\(^{2+}\) transfer (Udagawa et al., 2014). Similarly, the ER-mitochondria Ca\(^{2+}\) controls multiple apoptotic routes during embryogenesis (Pinton et al., 2008). The deletion of mitofusin 1 in oocytes led to female infertility associated with mitochondrial dysfunction and increased expression of pro-apoptotic genes (Zhang et al., 2019a). Animal models also identified that ROS is produced through Ca\(^{2+}\)-induced waves in mitochondria of activated oocytes and that oscillating levels are necessary to control early embryogenesis (Han et al., 2018). In the context of assisted reproductive treatments and in vitro fertilization cycles, calcium ionophores have been used to perform artificial oocyte activation in different scenarios such as total fertilization failure and severe male factor infertility (Murugesu et al., 2017).

The uptake of Ca\(^{2+}\) ions into mitochondria occurs through the mitochondrial calcium uniporter complex (Rizzuto et al., 2012; Giorgi et al., 2018). This mammalian uniporter complex consists of four core constituents: the pore-forming mitochondrial calcium uniporter (MCU) protein (Fig. 4B), the proteins MICU1 and MICU2 that function as molecular gatekeepers and are connected by a disulphide bridge, and the Essential MCU regulator (EMRE) crucial for the Ca\(^{2+}\) transport (Kirkoch et al., 2004; De Stefani et al., 2011). A recent study reported that EMRE also controls pore opening by stimulating the dimerization of an MCU-EMRE complex (Wang et al., 2019). Another recent study has shown that a single MICU1–MICU2 heterodimer appears to be enough to gate an MCU–EMRE tetramer (Fan et al., 2020). Excessive Ca\(^{2+}\) influx can be harmful, which is prevented by proper MCU activity facilitated by MICU1–MICU2 heterodimers (Patron et al., 2014; Fetrungaro et al., 2015). Interestingly, it was shown that MICU1 appears to switch the uniporter into a resting position, instead of changing the pore conformation (Fan et al., 2020). The uniporter becomes active only when Ca\(^{2+}\) levels exceed a threshold (Mallickarjunan et al., 2012; Coerdas et al., 2013). Mitochondrial Ca\(^{2+}\) entry is stimulated upon the activity of the respiratory chain complexes and causes translocation of protons into the intermembrane space, increasing the mitochondrial membrane potential (Mammucari et al., 2018). Excessive Ca\(^{2+}\) influx can therefore elevate mitochondrial oxidative stress and lead to apoptosis (Peng and Jou, 2010), indicating that mitochondrial calcium homeostasis is essential to maintain mitochondrial metabolic function and its dysregulation can contribute to pathology.

### 3.5. Mitochondrial contributions to epigenetic regulation in oocytes

It is increasingly recognised that the DNA methylation status of an oocyte shows dynamics of re- and de-methylation during oocyte development (Fig. 5). There are two steps of genome-wide reprogramming in mammals, namely in germ cells and in preimplantation embryos (Reik et al., 2001; Matilainen et al., 2017). Further, this step of epigenetic reprogramming in germline cells is crucial for imprinting (Reik et al., 2001). The decline in oocyte quality observed in ageing occurs in part through epigenetic regulation. Mitochondrial metabolites are important intermediates that allow mitochondrial-nuclear communication through the generation and modification of nuclear epigenetic marks. Mitochondrial pyruvate, citrate, acetate, ketones, amino acids, and beta-oxidation of lipids can all yield acetyl-CoA, the substrate of histone acetyltransferase (HAT) enzymes (Wellen et al., 2009; Lee et al., 2014), with high energy and acetyl-CoA conditions leading to increased histone acetylation and gene transcription, whereas low levels favour chromatin condensation (Menzies et al., 2016). In contrast, the sirtuin family of histone deacetylases (HDACS) rely on NAD\(^+\) as a cofactor and mitochondrial pyruvate transfer from the cytosol to the matrix via the pyruvate carrier, allowing the production of acetyl-CoA by the pyruvate dehydrogenase complex. HDACs also control mitochondrial function by regulating the expression of genes involved in mitochondrial metabolism. Furthermore, HDACs are required for the regulation of mitochondrial biogenesis and function, as well as for the maintenance of mitochondrial membrane potential and ATP production. Additionally, HDACs are involved in the regulation of apoptosis, as they can modulate the expression of pro- and anti-apoptotic genes. In conclusion, the interplay between mitochondrial function and epigenetic regulation is crucial for the proper development of embryos.
Jumonji C domain demethylases (JMJD). LSDs are an important mitochondrial electron acceptor in the ETC and during Jumonji C domain demethylases (JMJD). LSDs are an important mitochondrial quality control of cellular quality through apoptosis, can be used as a strategy to improve oocyte fitness. It would be especially crucial to determine whether modulating mitochondrial quality control, or mitochondrial control of apoptosis during ageing (Matilainen et al., 2014). When the accumulation of primary damage can no longer be compensated for by repair mechanisms, the mitochondrial system becomes exhausted and can no longer support the metabolic demand of oocytes. Mitochondrial control of apoptosis is especially relevant in oocytes, considering that the number of oocytes that reach full maturation during each menstrual cycle is tightly controlled through the tightly controlled interplay between protein synthesis and degradation, subject to protein turnover mechanisms that balance protein synthesis, degradation, and recycling. Mitophagy is a selective form of autophagy, a process that progressively declines with ageing (Zhou et al., 2017). Together, these processes ensure the health of the mitochondrial network, and failure of each individual component can compromise mitochondrial health.

In addition to intrinsic mitochondrial processes that maintain quality within the organelle, the mitochondrial network is also crucial for maintaining cellular homeostasis through its role in governing apoptotic cell death. The extrinsic pathway relies on death receptor signalling, whereas the intrinsic (or mitochondrial) pathway can be activated by stimuli such as mitotic arrest and DNA damage (Bock and Tait, 2020). These cause activation of B cell lymphoma 2 (BCL-2), and BHE3-only protein family members, which suppress anti-apoptotic and activate pro-apoptotic BCL-2 proteins (Wei et al., 2001). Together, this leads to mitochondrial membrane permeabilization (MOMP) in which proteins residing in the mitochondrial intermembrane space are released, most notably cytochrome c (Bock and Tait, 2020). In turn, cytochrome c binds apoptotic peptide activating factor 1 (APAF1) to form the apoptosome (Dorstyn et al., 2018). This leads to the recruitment and activation of caspase 9, which in turn stimulates the activity of caspase 3 and 7 (intrinsic and extrinsic pathway converge) (Bock and Tait, 2020). These effector proteases will cleave hundreds of protein targets (Julien and Wells, 2017). In addition to cytochrome c, MOMP also releases proteins that block the caspase inhibitor XIAP, including OMI and SMAC, thereby further promoting apoptosis (Bock and Tait, 2020). Finally, MOMP can also cause caspase-independent cell death, as mitochondria in a cell generally undergo MOMP together (Goldstein et al., 2000) and progressive mitochondrial dysfunction causes metabolic catastrophe and ATP loss (Lartigue et al., 2009).

Mitochondrial dysfunction contributes to ageing and various cellular signalling pathways that lead to senescence (Theurey and Pizzo, 2018). As such, mitochondrial quality control is antagonistic to ageing because it promotes the health of the larger mitochondrial network, its metabolism, and its contribution to overall cellular function. However, ageing both accelerates the rate of primary mitochondrial alterations and compromises the effectiveness of quality control processes (Wohlgenuth et al., 2014). When the accumulation of primary damage can no longer be compensated for by repair mechanisms, the mitochondrial system becomes exhausted and can no longer support the metabolic demand of oocytes. Mitochondrial control of apoptosis is especially relevant in oocytes, considering that the number of oocytes that reach maturity during each menstrual cycle is tightly controlled through apoptosis, and fewer than one percent of all germ cells generated during oogenesis survive (Shen et al., 2021). It remains to be demonstrated whether modulating mitochondrial quality control, or mitochondrial control of cellular quality through apoptosis, can be used as a strategy to improve oocyte fitness. It would be especially crucial to determine a window of intervention that would allow for the enhancement of alterations of specific mitochondrial processes, yet overall cellular mitochondrial dysfunction only persists if quality control mechanisms fail. Mitochondrial quality is controlled through the interplay of mitochondrial biogenesis, dynamics (fusion and fission), proteostasis, and mitophagy (Picca et al., 2018). Mitochondrial biogenesis can be stimulated through stressors such as exercise and ROS, as these increase cellular metabolic demand (Sallam and Laher, 2016). Mitochondrial morphogenesis is controlled through the interplay between fission, where individual mitochondria are separated from the larger network, and fusion. Fission is governed by mitochondrial fission protein (FIS1) and dynamic-1-like protein (DNM1L), which ensure that damaged mitochondria are diluted out of the network and cleared to maintain the overall health of the organelle network. Fusion is governed by mitofusins (MFN1/2) and dynamic-like 120 kDa protein (OPA1). Dysfunctional mitochondria will then undergo membrane depolarization and are targeted for degradation through mitophagy. Proteostasis depends on the tightly controlled interplay between protein synthesis and degradation, subject to protein turnover mechanisms that balance protein synthesis, degradation, and recycling. Mitophagy is a selective form of autophagy, a process that progressively declines with ageing (Zhou et al., 2017). Together, these processes ensure the health of the mitochondrial network, and failure of each individual component can compromise mitochondrial health.

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mitochondrial functioning during the oocyte lifecycle, while still ensuring that dysfunctional organelle components, mitochondria, and cells would be removed and recycled.

4. Importance of mitochondrial DNA copy number, mtDNA defects, and mitochondrial dysfunction for oocyte ageing

Mitochondria contain their own circular, double-stranded DNA with a length of 15–17 kb, and mitochondrial dynamics during oocyte maturation, and cleavage events are strictly regulated (St John, 2014; Babayev et al., 2016; Rimon-Dahari et al., 2016; Van Blerkom et al., 2000). Impaired mitochondrial dynamics and decreased mitochondrial number in aged oocytes are suggested to play crucial roles in female fertility and the viability of oocytes (Spikings et al., 2007; St John et al., 2010; Van Blerkom et al., 2008). A deficiency in mitochondrial inheritance may cause diminished energy supply leading to arrested cell division (Van Blerkom et al., 2008; Allen and de Paula, 2013). The mitochondria of mammalian oocytes display a quiescent state especially before maturation, with little bioenergetic and transcriptional activity, which is thought to prevent heritable mtDNA mutations (de Paula et al., 2013; Bentov et al., 2011). Instead, energy to support oocyte maturation is provided primarily by the surrounding cumulus and granulosa cells (Collado-Fernandez et al., 2012). After fertilization, mitochondria become the primary energy source during embryonic development and they fully transition from quiescence to activation by the blastocyst stage (Hashimoto et al., 2017). As such, mitochondrial dynamics protect the oocyte from DNA damage, and a decline in mitochondrial number as observed in reproductive ageing may jeopardise it.

It is estimated that two to ten copies of mtDNA are present in each mitochondrion, which totals 10²–10⁴ copies in most somatic cells. In mammalian species, mtDNA comprises 37 protein-coding genes that give rise to twenty-two transfer RNAs and the small and large subunits of ribosomal RNA (Harman, 1956). The other genes encode ETC subunits, indicating that functional mitochondrial metabolism requires a coordinated interplay between nuclear and mitochondrial DNA transcription. Considering that the mitochondrion is the main site of ROS production in cells, the free radical theory of ageing considers mitochondria to be major contributors of ageing through ROS-induced mtDNA mutations (Harman, 1956). Free radical and non-radical oxidants can cause mtDNA damage by generating minor alterations to bases, producing 8-oxo-2′-deoxyguanosine (8-oxo-dG), formamidopyrimidines, and 8,5′-cyclo-2′-deoxynucleosides. In turn, further oxidation of 8-oxo-dG can give rise to highly mutagenic secondary lesions (Henderson et al., 2002). Additionally, oxidants can cause damage to the sugar backbone of DNA bases, creating abasic sites, oxidised deoxyribose rings, or aldehyde modifications, and ultimately can give rise to DNA single or double-strand breaks (De Bont and van Larebeke, 2004). Interestingly, abasic sites and strand breaks seem to be the major variants of ROS-induced mtDNA damage as compared to mutagenic base lesions (Shokolenko et al., 2009).

If these mtDNA modifications are left unrepaired, they can result in mutations and replication failures. It has been reported that the D-loop regulatory region of mtDNA is unstable during ageing (Rose et al., 2010) and that women with a proof-reading lack of PolgA, which is a catalytic subunit of mtDNA polymerase, display mtDNA mutations in oocytes (Llarena and Hine, 2020). Interestingly, a clinical study comparing patients with their healthy relatives showed that women with a proof-reading lack of PolgA, which is a catalytic subunit of mtDNA polymerase, display mtDNA mutations in oocytes (Llarena and Hine, 2020). In addition to proof-reading during replication, mitochondrial DNA is coated with histone-like proteins to guarantee mtDNA homeostasis and regulate replication and transcription. It has been shown that mitochondrial transcription factor A (TFAM) can coat the genome of mitochondria, and also plays a role in mtDNA repair (Campbell et al., 2012; Bestwick and Shadel, 2013), although it still needs to be elucidated how TFAM mediates DNA compaction. Previous studies showed that the number of mutations in the mitochondrial genome appears to be significantly higher when compared to the nuclear genome, due to its close localization to the ETC (Kasapoglu and Seli,
ROS are a major source of acquired mtDNA mutations associated with ageing (Kasapoglu and Seli, 2020; Luoma et al., 2004; Richter, 1995; Greaves et al., 2009) and indeed, an increase in acquired mtDNA mutations and a corresponding decline in mitochondrial function was reported with ageing (Payne and Chinnery, 2015). As a result, the mitochondrial unfolded protein response (mtUPR) is activated to ensure mitochondrial protein homeostasis (Jensen and Jasper, 2014). Similarly, mitochondrial CoQ10 biosynthesis and the expression of genes involved in mitochondrial metabolism decline with age, resulting in reduced antioxidant defense and impaired oocyte competence (Ben-Meir et al., 2015). In addition, studies in animal models have shown that an increased number of mtDNA mutations are significantly correlated with shorter lifespan and an ageing-related phenotype including frailty, diminished fertility, and a hematopoietic stem cell decline (Trifunovic et al., 2004; Kujoth et al., 2005).

Considering that mtDNA encodes for essential subunits of the ETC, mtDNA mutations can lead to an uncoupling of the respiratory chain (Subayev et al., 2016). During oocyte maturation a higher energy supply is required, leading to a shift from glycolysis to oxidative phosphorylation (Dell’Aquila et al., 2009; Spikings et al., 2006; Schafer et al., 2008). Therefore, the chronic accumulation of mtDNA mutations in oocytes will negatively influence the developmental competence of early-stage embryos (Arnhem and Cortopassi, 1992). Previous studies showed that the deletion ΔmtDNA4977 occurs frequently with advancing age, which was therefore suggested as a biomarker of ageing (Arnhem and Cortopassi, 1992). Interestingly, Keefe and colleagues reported that the deletion ΔmtDNA4977 was found in oocytes in 93 % of IVF patients among women over the age of 37 years, but in only 28 % of IVF patients among women of a younger age (Keefe et al., 1995). Clinically, the use of mitochondrial nutrients and growth hormones in aged women seems to improve oocyte quality by both increasing the number of functional mitochondria and improving mitochondrial activity (van der Reest et al., 2018; Requejo et al., 2010). In the following sections, strategies are discussed that can improve mitochondrial function and metabolic activity to delay or partially reverse the ageing process in oocytes.

5. Therapeutic strategies to restore or delay oocyte ageing

In the last two decades, it has emerged that female reproductive ageing constitutes one of the most critical factors that influences human reproduction, driven by decreasing ovarian reserve and diminished oocyte quality. Oocyte quality is affected by numerous environmental factors, such as diet and lifestyle, which influence the oocytes or their organelles with advancing maternal age. Previous studies elucidated that oocyte mitochondria contain the highest amount of maternal genetic material and also generate the vast majority of ATP required for oocyte function. Therefore, mitochondria in oocytes are crucial to support the processes of chromosomal segregation and fertilization. However, oxidative metabolism linked to ATP production generates ROS as a by-product, and with advancing maternal age, exposure to ROS accumulates during the time of prophase I arrest of oocytes. As such, future therapeutic strategies to improve, reverse or slow down oocyte ageing might include dietary, pharmacological, or modern biotechnological interventions to restore redox balance and boost oocyte metabolic and mitochondrial fitness. A breakthrough in human assisted reproductive technologies was the birth of the world’s first IVF baby in 1978. Today, there is a widespread use of various assisted reproductive technologies. In the following sections, possible oocyte rejuvenation strategies and the importance of mitochondria for these interventions will be reviewed.

5.1. Modulation of mitochondrial biogenesis

Mitochondrial biogenesis is crucial for the development of oocytes and the process of selective inheritance, which regulates the transmission of harmful mtDNA mutations (Hill et al., 2014). Disturbances in mitochondrial biogenesis have been reported to lead to severe disorders, including reproductive failures (St John, 2012). In oocytes and other mammalian cell types, mitochondrial biogenesis is regulated at the transcriptional, translational, and post-translational level. Mitochondrial biogenesis is further dependent on cellular energy supply and highly sensitive to environmental and developmental signals (Zhang and Xu, 2016). The peroxisome proliferator-activated receptor γ co-activator 1α (PGC1α) is suggested to be the master regulator of mitochondrial biogenesis (Zhang et al., 2019b). In addition, it has been reported that PPRC1, another member of the PGC family of transcriptional co-activators, and ROS regulate mitochondrial biogenesis in early porcine embryos (Zhang and Xu, 2016; Kageyama et al., 2021). However, specific modulation strategies to stimulate mitochondrial biogenesis in oocytes are not investigated sufficiently. For embryos, it was suggested that oxidative stress elevates mitochondrial biogenesis through the NRF2-PGC1α-TFAM pathway (Lee et al., 2002; Sulliman et al., 2003; St-Pierre et al., 2006). Interestingly, it was shown in renal tubular epithelial cells that overexpression of FOXO1 led to upregulation of PGC1α expression and improved mitochondrial dysfunction. Consequently, FOXO1 could be a potential target to modulate the expression of PGC1α and thereby mitochondrial biogenesis (Zhang et al., 2021). A recent study showed that resveratrol supported mitochondrial biogenesis in human granulosa cells, which surround oocytes inside the follicle (Ragonese et al., 2021). The compound sildenafil, which is a potent phosphodiesterase 5 inhibitor, led to upregulated mitochondrial biogenesis in endothelial cells (Corum et al., 2020). To further elucidate whether modulation of mitochondrial biogenesis is a viable approach to restore mitochondrial content in aged human oocytes, the other biological targets under control of PGC1α should be considered to ensure safety and absence of unintended side effects, especially enzymes involved in energy metabolism and factors crucial for the replication and transcription of mtDNA.

5.2. Mitochondrial supplementation of oocytes

One strategy in use to improve the fertilization outcome of aged oocytes is mitochondrial supplementation. One technique in use is the partial cytoplasm transfer, where oocytes are supplemented with mitochondria and other organelles, metabolic by-products, proteins, and RNAs from the donor cytoplasm (Ferreira et al., 2021; Bianchi et al., 2015; Grondahl et al., 2010; Reich et al., 2011; Chappel, 2013). This transfer can be done during intracytoplasmic sperm injection (ICSI), where around five to ten percent of donor oocyte cytoplasm is injected together with the sperm into the recipient oocyte (Spikings et al., 2006). This technique has shown successful results; however, concerns have arisen due to the heteroplasmy, which may lead to negative physiological consequences in the offspring (Labarta et al., 2019). Another technique is the total cytoplasm transfer, where the entire aged or pathologic cytoplasm of an oocyte is replaced (Labarta et al., 2019). As this includes mitochondria, this approach could prevent diseases linked to abnormal maternal mitochondria. In this procedure, either the metaphase spindle or the germinal vesicle is aspirated and transferred into the cytoplasm of a recipient oocyte (Zhang et al., 2017; Liu et al., 2003, 1999; Liu et al., 2000; Zhang et al., 1999). Building upon this approach, the human polar body nuclear transfer was developed (Ma et al., 2017). Due to the problem with heteroplasmy of the previously mentioned methods, there was a strong ambition to develop an autologous approach. The discovery of adult ovarian stem cells and oocyte precursor cells in ovaries enabled the concept of autologous germline mitochondrial energy transfer (AUGMENT) (Tilly and Sinclair, 2013; Cozzolino et al., 2019). This strategy is based on injecting mitochondria generated from oocyte precursor cells from the same patient during ICSI (Fig. 6), and has been used around the globe to boost oocyte quality (Tilly and Sinclair, 2013; Roznichenko et al., 2016). However, the clinical experience of the Augment procedure suggests that there are limitations to this technique and it is not effective in boosting blastocyst...
quality (Labarta et al., 2019). In a recent study, the AUGMENT technique was compared to the standard IVF protocol. This study included 59 patients with a mean age of 36.3 years and 2.5 previous unsuccessful IVF procedures, and the day-5 blastocyst rate was higher in the control group when compared to the AUGMENT group (Labarta et al., 2018). Overall, in 46% of the IVF patients, there was no blastocyst available for embryo transfer, although the AUGMENT protocol was used during the fertilization of their oocytes (Labarta et al., 2018). Based on these findings, it appears that the AUGMENT strategy does not improve embryo developmental competence (Labarta et al., 2018).

5.3. Mitochondrial replacement therapies

Mitochondrial replacement therapies (MRTs) have been proposed to overcome related to defective mitochondria (Sharma et al., 2020). The most developed procedures include polar body transfer, pronuclear transfer, and spindle transfer. Such approaches emerged from the need to overcome lethal mitochondrial disorders, but they have also been applied in different fields of modern medicine (Sharma et al., 2020). In the context of ART, MRT has recently been used in cases of low oocyte quality, such as reproductive senescence (Costa-Borges et al., 2020). Essentially, pronuclear transfer consists of producing zygotes using the oocyte from both the biological mother and the oocyte donor. Subsequently, the pronuclei of the biological parents are cautiously removed and transplanted into the donor’s zygote after removing its original pronuclei (Sharma et al., 2020; Tachibana et al., 2018). The spindle transfer is performed before fertilization and the maternal spindle complex is extracted from the biological mother’s oocyte followed by its transplantation into the enucleated donor’s egg (Sharma et al., 2020; Tachibana et al., 2018). Finally, polar body transfer can be performed using both the first and the second polar body, but its replication is more challenging to accomplish due to issues related to meiotic recombination (Tachibana et al., 2018). Unlike previous cytoplasm transfer techniques, the use of MRTs seem to exhibit mtDNA carryover and heteroplasmy rates below 2% (Cree and Loi, 2015; Wolf et al., 2015). Importantly, very few cases of genetic drift and restoration to the original maternal haplotype have been described in human oocytes and animal embryos (Kang et al., 2016; Yamada et al., 2016).

Mitochondrial donation techniques were approved by different countries to be performed legally in the context of mitochondrial diseases (Schandera and Mackey, 2016). The UK Parliament was the first to allow clinical applications of MRT in 2015. In 2016, the birth of the world’s first three-parent baby was reported in Mexico, with a successful application of maternal spindle transfer. A second three-parent baby was born after pronuclear transfer in Ukraine in 2017. Additionally, an infertile woman took part in an ongoing pilot clinical trial using maternal spindle transfer in Greece and gave birth to a healthy baby. This trial was initiated after the publication of a pre-clinical model aimed at enhancing oocyte developmental competence by the same group of researchers (Costa-Borges et al., 2020). There have been many criticisms regarding the use of MRTs outside the context of genetic diseases due to the lack of long-term follow-up (Cohen et al., 2020). Besides the issues regarding mtDNA carryover and heteroplasmy leading to a genetic bottleneck, there are difficulties related to technical challenges and absence of data on long-term health effects to the offspring. To date, MRT is still considered an experimental approach.

5.4. Modulation of cellular redox balance

As described, ROS-mediated oxidative stress is considered an important contributor to oocyte ageing. Key mouse studies first suggested that antioxidant therapies may help prevent or reverse oocyte ageing (Tarin et al., 1998). Nevertheless, antioxidant therapy to improve fertility in humans has been researched primarily in the context of male infertility (Barati et al., 2020). Supplementation with N-acetylcysteine (NAC), a precursor of the antioxidant glutathione, improved several parameters associated with male infertility in a three-month randomised controlled trial (Ciftci et al., 2009). In female mice, NAC treatment increased the quality of oocytes and embryonic development and led to larger litters compared to age-matched controls (Liu et al., 2012). Another mouse study supplementing the antioxidants vitamin C and E also observed increased quality and number of oocytes (Tarin et al., 2002). As ROS are implicated in many processes affecting human health, several antioxidants have been evaluated in large observational studies and human trials. Although some suggest benefits for specific diseases, meta-analyses have shown conflicting results with a significant increase of adverse effects in some studies (Bjelakovic et al., 2007; Biesalski et al., 2010). This can be explained by the increasing understanding that ROS...
do not only cause oxidative stress but also are crucial signal transducers and play a key role in regulating metabolic activity (Requejo et al., 2010). Systemic antioxidant treatment may therefore disrupt both pathological and physiological functions of ROS. The use of oral CoQ10 dietary supplementation has shown a positive influence on the ROS level of follicular fluid, although more research is needed to establish this method as a novel therapeutic strategy for women of advanced reproductive age (Giannubilo et al., 2018). Nevertheless, using CoQ10 supplementation in women undergoing ICSI-IVF treatment did not significantly elevate the euploidy or pregnancy rates (Bentov et al., 2014). The compound resveratrol (3,4′,5-trihydroxystilbene), which was discovered in different plants, fruits, nuts, and red wine, enhances sirtuin 1 (SIRT1) activity and was shown to have anti-aging properties related to its protective effect on mitochondria (Sims et al., 2021; Rodríguez-Varela and Labarta, 2020). Another compound tested as an antioxidant treatment for oocytes is melatonin (Carlomagno et al., 2011). A study comparing human follicular fluid to serum showed that the melatonin concentration is threefold higher in follicular fluid (Szrzejenski et al., 1987). This led to melatonin supplementation when women undergo controlled ovarian hyperstimulation (Tamura et al., 2013). Melatonin supplementation also led to elevated intra-follicular melatonin concentrations and to a higher progesterone production in the corpus luteum of infertile women suffering from luteal phase defect (Tamura et al., 2013). However, according to a recent systematic review, the use of vitamin E or C, melatonin, or omega 3 polyunsaturated fatty acids did not show an overall significant effect on the live birth rate (Showell et al., 2013).

5.5. Modulation of NAD+ levels

NAD+ is a metabolic cofactor required for thousands of enzymatic reactions and its age-related decline is well-documented (Rajman et al., 2018). It has been observed that oocytes from older mice have lower levels of NAD+ and reduced expression of a key enzyme in the NAD salvage pathway, nicotinamide mononucleotide adenyltransferase 2 (NMNAT2) (Wu et al., 2019). Specific deletion of NMNAT2 led to metabolic dysfunction and disturbed meiosis. Supplementation with a NAD precursor or overexpression of NMNAT2 in aged oocytes decreased ROS levels and chromosomal defects. Another study showed that wild-type mice lost about half of their NAD reserves by 32 months of age, whereas knockout mice deficient in CD38, a major mammalian NAD+ consumer, maintained their NAD+ levels (Barbosa et al., 2007; Escande et al., 2013). In contrast, overexpression of CD38 led to depletion of NAD levels, mitochondrial dysfunction, and metabolic rewiring (Camacho-Pereira et al., 2016). Studies in various model organisms have established that NAD levels regulate mitochondrial fitness in oocytes, and play an important role in ageing and longevity (Rajman et al., 2018; Imai, 2009). Modulating consumers, producers, or regulators of NAD levels may prove an attractive therapeutic strategy to restore the age-related decline in NAD and restore mitochondrial and metabolic function in oocytes, if sufficient specificity can be achieved. Three main strategies have been explored: supplementing NAD precursors, inhibiting NAD degradation, and activating NAD biosynthetic enzymes [recently reviewed in 215]. All three strategies were shown to increase NAD levels to varying degrees in different model organisms or human clinical trials, with observed benefits for specific health outcomes. Three major precursors are used to generate NAD: the amino acid tryptophan or two forms of vitamin B3, nicotinamide (NAM) and nicotinic acid (NA). Two recent complementary studies showed that supplementation with nicotinamide mononucleotide (NMN) rescued reproductive ageing by reversing the age-associated decline in oocyte quality (Miao et al., 2020; Bertoldo et al., 2020). NMN is produced endogenously as an intermediate of the NAD salvage pathway but is also readily taken up when supplemented. These studies showed that NMN supplementation restored NAD+ levels in ageing oocytes and rescued ROS-mediated functional decline in mitochondria, thereby increasing oocyte function, ovulation, and fertilization (Miao et al., 2020; Bertoldo et al., 2020). Although these studies are promising, adverse side effects have been observed for NA and NAM supplementation, and some strategies targeting enzymes that use, produce or degrade NAD and its precursors also induced toxicities (Rajman et al., 2018). Considering that over 500 enzymatic reactions rely on NAD as a cofactor (Ansari and Raghava, 2010), this complex system needs to be understood more thoroughly from a biological, pharmacological, and physiological perspective before safe and effective interventions can aid in increasing longevity and prevent or reverse oocyte ageing.

5.6. Caloric restriction

Caloric restriction (CR) has emerged as a key intervention to prolong lifespan and prevent or delay the onset of various age-related diseases in multiple animal models and in humans (Baroja et al., 2019; Most et al., 2017). A CR diet is defined by a 25–30% reduction in caloric intake in comparison to a standard diet, whereby no food groups are excluded (Most et al., 2017). Although there is no standardised CR diet recommendation, it is suggested to correspond to a daily food intake below 1800 kcal over a period of 15 years (Most et al., 2017). CR has been shown to attenuate the signalling activity of the insulin/IGF-1 (IIS) pathway, contributing to an extended lifespan (Mathew et al., 2017). Further, there are two crucial sensor systems in the IIS axis, AMPK and sirtuins. In the mammalian ovary, the PIE3/PIK/mTOR pathway is important for the regulation of dormant primordial follicles and their activation (Rosario and Anderson, 2021). In a recent study, the mTOR inhibitor rapamycin positively affected the in vitro maturation of bovine oocytes (Kordovizki et al., 2020). In mice, Bitto and colleagues (2016) showed that transient supplementation with rapamycin increased lifespan (Bitto et al., 2016). Furthermore, another study showed that rapamycin treatment specifically maintained the developmental potential of oocytes (Sato and Kawamura, 2020). Thus, the caloric restriction effect can be mimicked by compounds that modulate the signalling pathways implicated in CR-mediated lifespan extension, and a better understanding of these pathways may aid the development of more effective pharmacological strategies.

5.7. Gene editing of mitochondrial DNA

Recent advances in drug discovery and design, delivery, and gene-editing such as CRISPR technology may allow for more specific therapeutic interventions that target specific enzymes to modulate redox-metabolic enzymes and regulators. Considering the contributions of mitochondria to the ageing process of oocytes discussed earlier in this review, an exciting advance may be the recent discovery of mitochondrial genome editing (Mok et al., 2020). The combination of mitochondrial transfer therapy with the CRISPR-Cas9 gene-editing tool would open new therapeutic strategies to replace pathologic mitochondrial DNA in oocytes generated from patients in advanced maternal age (Fogleman et al., 2016; Jo et al., 2015). While CRISPR-Cas9 systems have greatly accelerated the molecular toolbox for genome editing, applying it to the mitochondrial genome requires delivery of guide DNA into mitochondria, which is challenging (Gammage et al., 2018). Recently, a bacterial enzyme was discovered that can be modified to edit double-stranded mitochondrial DNA (Lo and Parham, 2009). This is an important advance to previous mtDNA manipulation strategies, which rely on the elimination of mtDNA copies, as it allows for precise editing while maintaining endogenous mtDNA. Nevertheless, this approach will require extensive testing before its clinical application can be considered safe, effective, and ethical.

5.8. The use of ESC and iPSC to develop oocytes as an autologous source of mitochondria

Shinya Yamanaka’s discovery of induced pluripotency opened a new
AUGMENT in advanced age oocytes. If technical challenges concerning the collection, therefore, these OSCs could be adequate mitochondria donors for worth mentioning that these cells can only differentiate into oocytes. shape containing few cristae, and they are undifferentiated (Zhang et al., human oocytes in the metaphase II stage, namely they have a round mitochondrial activity can be modulated to restore oocyte fitness. Nevertheless, there is still a long way to go before autologous mitochondria supplementation is fully understood and validated in animal models, let alone clinical application in IVF clinics.

6. Conclusion

Mitochondria act as essential energy factories in oocytes to support the energetically demanding processes of maturation, fertilization, and embryonic development. As oocytes age, a concomitant decline in mitochondrial number and quality is observed, with increasing evidence that mitochondrial function is a critical determinant of oocyte and embryo fitness. This sparked interest within the reproductive field to better understand how mitochondria contribute to the relatively early onset ageing of oocytes as compared to other organ systems, and whether mitochondrial activity can be modulated to restore oocyte fitness. Several therapeutic strategies have been developed to halt, delay, or partially reverse symptoms of oocyte ageing. Additionally, technical advances have led to increasingly sophisticated assisted reproductive technologies. Nevertheless, the safe and ethical modulation of mitochondrial and oocyte quality requires more comprehensive understanding of their interplay. The identification of accessible and non-invasive biomarkers for mitochondrial and oocyte quality would be especially helpful to determine which therapeutic strategy is best suited to individual clinical cases. Taken together, elucidating the molecular mechanisms that contribute to the maturation and ageing of oocytes, the unique role of mitochondria herein, and careful identification of therapeutic targets to improve mitochondrial function and thereby oocyte health, can contribute to new strategies to enhance and prolong reproductive fitness.

Author contributions

J.v.d.R.: writing, review, editing, and funding acquisition; M.CH.: editing and funding acquisition; G.N.C.: writing and review editing; P.K.: writing, review editing, and funding acquisition.

Declaration of Competing Interest

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