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Mitochondrial genetics in *in vitro* fertilization outcomes

Le rôle de la génétique mitochondriale dans les résultats cliniques de la fécondation *in vitro*

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Résumé. Le bon fonctionnement des mitochondries est un déterminant majeur de l'issue d'une fécondation *in vitro* (FIV), et le métabolisme mitochondrial, notamment, contribue à la qualité de l'ovocyte et au développement de l'embryon. Pour autant, le rôle exact des mitochondries dans la fertilité et l'infertilité demeure méconnu. Au-delà des mécanismes par lesquels les mitochondries sont impliquées dans le développement précoce, de nombreuses équipes cherchent à identifier des marqueurs mitochondriaux de la qualité ovocytaire et embryonnaire, en vue d'améliorer le résultat des FIV. Nous aborderons ici ces deux aspects, à savoir les connaissances actuelles sur l'implication des mitochondries dans le développement des gamètes et l'infertilité, et, par ailleurs, les marqueurs mitochondriaux déjà identifiés, ainsi que les perspectives d'amélioration du fonctionnement mitochondrial dans le champ de la reproduction.

Mots clés: activité mitochondriale, génome mitochondrial, développement de gamète, qualité de l'embryon

Abstract. Mitochondrial activity has been identified as a key feature for successful IVF outcomes, and mitochondrial metabolism is known to be a very important contributor in determining oocyte quality and embryo development. Despite this, the exact role of mitochondria in (in)fertility has not been elucidated yet. In addition, aside from the basic biology uncovering mitochondrial role in early development, many studies tried to identify mitochondrial markers able to predict the quality of oocytes and embryos, and use them to improve the clinical practices in IVF. In this review, we will present the basic knowledge about the contribution of mitochondria to gamete development and infertility. In addition, we will describe the most recent mitochondrial markers developed for the improvement of IVF outcomes, and show the most promising techniques for improving mitochondrial activity in reproduction.

Key words : mitochondrial activity, mitochondrial genome, gamete development, embryo quality

Mitochondrial biology

Mitochondria are often described as the powerhouse of the cell, meaning that these cytoplasmic organelles are responsible for the production of ATP through oxidative phosphorylation (OXPHOS). Despite this being true, it became clear during the years that mitochondria carry out several functions, ranging from calcium homeostasis to fatty acid metabolism and oxidative stress response. These organelles are also responsible for a variety of signalling cascades that regulate multiple biological processes within the cell [1].

Energy production, despite not being the only function of mitochondria, still represents the predominant activity, and the capacity to produce ATP through OXPHOS define mitochondrial maturity. Mitochondria are enclosed in a double membrane, semi permeable to ions, with a compartment in between the two: the intermembrane space. The components of the OXPHOS chain are anchored to the internal membrane, and some of them possess transmembrane domains that allow the passage of ions. The internal membrane is folded in numerous cristae, which allows for maximising the exposure of the OXPHOS complexes to the intermembrane space. During respiration, the OXPHOS complex I-IV act as pumps, moving the protons from

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the inner matrix to the intermembrane space, and creating a chemical imbalance that is then used by the ATPase complex (V) to synthesize ATP. The production of energy is also associated with the creation of Reactive Oxygen Species (ROS), which include hydrogen peroxide (H_2O_2), the superoxic anion (O^{2-}) and hydroxyl radicals (OH^-). These molecules have high reactivity to different biological targets and can induce damage to DNA, lipids and proteins, ultimately leading to cell death. Despite high concentrations are detrimental for cells, it has become clear how the variation in the levels of ROS can trigger a variety of cellular responses and regulate numerous important physiological processes [2].

Mature mitochondria are characterized by an elongated shape and a high number of cristae, and in normal conditions they are able to produce ATP to fulfil the cell's need [3]. When they are not active (or less active), they show a more globular shape with a reduced size and fewer cristae, and they are defined immature. Mitochondrial biogenesis is the process responsible for the production of new mitochondria, while mitophagy is responsible for their degradation. The activity and homeostasis of these organelles is defined not only by the ratio between newly synthesized mitochondria and degraded ones, but also fission and fusion play an important role in their complex regulation. In somatic cells, a high level of biogenesis and fusion forms networks of tubular mitochondria, and is normally observed in cells with a high demand of energy [4]. Conversely, in cells with a reduced metabolism, or those that rely mainly on glycolysis for their energy needs, there are both high levels of fission and increased mitophagy [5].

Mitochondria are thought to originate from the incorporation of a prokaryotic entity during evolution, and for this reason they retain a part of their own DNA, the mitochondrial DNA. The mitochondrial genome has numerous differences when compared to the nuclear genome: it is very small, around 16.5 kb, circular and does not contain introns. In addition, it is not bound by histones and does not possess the extensive machinery for DNA repair present in the cell nucleus. Nevertheless, this minute DNA is present in multiple copies within every single cell, and each of them contains 37 genes. These 37 genes encode for 13 proteins of the oxidative phosphorylation complexes, 2 ribosomal RNAs active within the mitochondria, and 22 tRNAs [6]. The multicopy nature of the mitochondrial genome gives rise to two different situations, named heteroplasmy and homoplasmy: in the case of homoplasmy, all the copies of the mtDNA within a single cell/tissue/individual will be exactly the same. If a genetic variant is present only in a proportion of the mtDNA molecules, there will be a coexistence of different genomes, i.e. heteroplasmy. In this situation, a variant is present at a certain frequency in the population examined, and the relative proportion of mutated/wildtype molecules will greatly influence the pathogenic consequences of a given mutation [7].

Despite possessing its own genome, mitochondria are completely dependent on the nucleus for their survival and activity. During evolution, in fact, many mitochondrial genes migrated from the mitochondrial to the nuclear genome, that now encodes for the great majority of the factors involved in OXPHOS. This is thought to be the consequence of a higher mutation rate of the mitochondrial DNA, which forced the reduction of the organelles' genome size in favour of the more "stable" nuclear genome [8]. Despite the mtDNA lost the majority of the genes necessary for their function, mtDNA is of fundamental importance, and mutations (both single nucleotide variants and structural rearrangements) or reduction in the number of copies of this genome have been associated to a wide variety of diseases [9].

It has been long thought that mtDNA is inherited exclusively from the maternal lineage, while the paternal contribution is limited to fertilization and initial embryo divisions, before its degradation during the 4-8 cell stage [10]. Despite this dogma, it has been very recently shown that this might not be true, or at least be partially incorrect. In a seminal paper published at the end of 2018, Luo and colleagues observed in some families the biparental inheritance of mtDNA. The authors analysed individuals with high heteroplasmy levels from different families. What they found was a surprising co-segregation of both maternal and paternal mtDNA, resulting in a high level of heteroplasmy in the children. This was observed only in some individuals of the family, while other relatives showed a "classical" unimaternal inheritance [11]. One previous report found a similar situation in one patients [12], but the work of Luo represents the first to extensively document this phenomenon, and might open the way to a whole new field of investigation. Regardless of the inheritance pattern, the mitochondrial genome undergoes a so-called bottleneck during Primordial Germ Cell (PGC) specification. During the bottleneck, only a few mtDNA molecules will segregate to the fully-grown oocyte, and these will be extensively amplified during oocyte growth (*figure 1*) [13]. This occurs to reduce the levels of heteroplasmy, which otherwise will accumulate and amplify through generations, and also to promote the variance in a (normally) uniparentally inherited genome. In addition, the increase of homoplasmy ensured by the reduced amount of transmitted mtDNA molecules will select out detrimental mutations, which will not allow competent oocytes to develop if their frequency is too high [8].

Mitochondria in development

Gamete development, for both females and males, is a complicated process that relies on a fine genetic

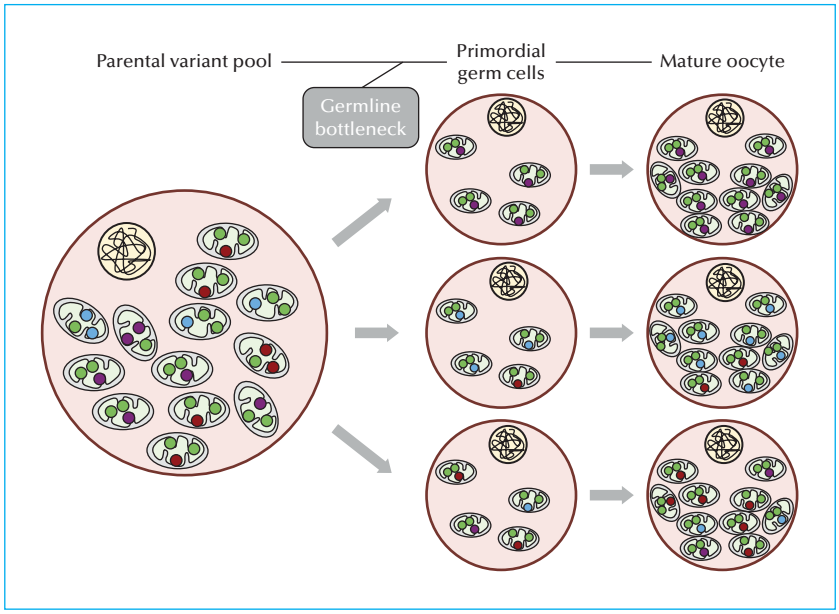

Figure 1. Schematic representation of the mitochondrial germline bottleneck. The parental pool possesses a number of mtDNA variants (colored circles). During PGC specification, mitochondrial replication stops and the subsequent cellular divisions lead to a significant reduction of the amount of mitochondria in the PGC. This reduction in number is defined germline bottlenck, and it will modify the frequency of variants in the single PGCs and in the deriving oocytes. This will give rise to situations where variants are lost, acquired, become homoplasmic, or remain substantially unchanged in frequency in different oocytes from the same cohort.
PGC= Primordial Germ Cells.

regulation to generate haploid cells able to form a zygote and develop into a healthy human being. Oocyte and sperm development is greatly affected by the metabolic capacity of the cells, and in the next section we will discuss how mitochondria can influence the capacity of oocytes and sperm to acquire developmental capacity and drive the initial phases of development.

Mitochondria in oocyte developmental competence

Studies of mitochondrial metabolism during oocyte development have been historically challenging, due to the difficulty in obtaining human developing PGCs and early-stage human oocytes. For this reason, the main knowledge about the metabolic events in the earliest stages of oocyte development comes from animal models, in particular murine, bovine and porcine. During mouse oocyte maturation, the oocyte must produce all the transcripts and mtDNA molecules that will be used during the latest phases of development and early embryo division. This high energetic requirement is met thanks to the collaboration between the oocyte and the surrounding cumulus cells (CCs). CCs are responsible for the conversion of Glucose into Pyruvate, which is transported into the oocyte metabolised. Mitochondria in oocytes are generally in an immature state, with globular shapes and a reduced presence of cristae. Despite being present in an immature state, they have been shown to participate in ATP production [14]. As shown in both animal models and humans, higher ATP levels are positively correlated with better reproductive outcomes [15], and oocyte that fail to fertilize are characterized by a lower amount of ATP [16]. Moreover, the inhibition of OXPHOS in mouse oocytes reduces the blastocyst rates of the derived embryos [17].

Regarding mtDNA, it has been observed how fertilized oocytes possess higher mtDNA copy number than oocytes that fail to fertilize [18, 19]. Accordingly, oocytes from older women or women with diminished ovarian reserve express lower levels of mtDNA [20, 21].

Mitochondria in reproductive aging

One of the main problems that western society is facing nowadays is the progressive aging of first time mothers. As already known for a long time, aging is associated with infertility, and women over 40 years of age have a limited capacity of achieving a live birth. The infertility caused by advanced maternal age is mainly due to oocyte quality, which rapidly decreases in women over 35 years of age. General aging affects mitochondria, and also in oocytes the same situation is observed: mitochondria from aged individuals are swollen, present vacuolization and structural alterations [22]. This is mirrored by mitochondrial membrane potential [23, 24] and ATP production

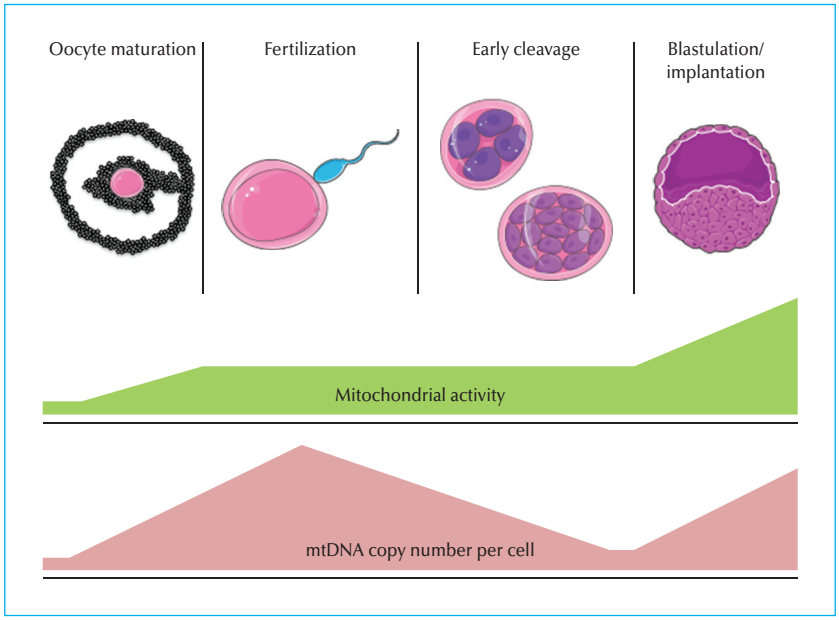

Figure 2. Schematic representation of the changes in mitochondrial activity and mtDNA copy number levels during oocyte maturation and embryo development.

[25], which also decrease with age in both humans and mice. Mitochondrial dysfunction has also been speculated to cause an increase in chromosomal aneuploidies and more dysfunctional meiotic spindles, and thus represent an important player in causing the aged phenotype [26, 27].

Mitochondria and male factor infertility

Mitochondrial shape and activity changes throughout the whole process of spermatogenesis: in spermatogonia mitochondria are present in a less active and "orthodox" form (as defined in [28]), while in spermatids mitochondria are more condensed and active. In mature sperm, the progressive loss of cytoplasm during maturation decreases the number of mitochondria: it was estimated that around 22-75 mitochondria are present in the mature sperm, organized in tubular structures and stored in the midpiece [29, 30]. The role of mitochondria in sperm has been debated, since it is not clear what source of energy is used for sperm motility, the most energy consuming process. Nevertheless, mitochondria play an important role in sperm biology; the expression of proteins of the electron transport chain has in fact been correlated with sperm quality [31]. Also, a deregulation of the activity of mitochondrial enzymes has been associated to infertile phenotypes, in specific asthenozoospermia (AZS) [32-34]. In AZS patients, in addition, a lower expression of specific mtRNA and mitochondrial genes has been reported [35]. Regarding mtDNA, mutations and structural rearrangements can alter cellular fitness and result in fertility issues, in both animal models and patients [36, 37]. Higher mtDNA copy number has been seen in infertile men [38], and in sperm with a reduced fertilization capacity [39]. Also, a higher prevalence of specific polymorphisms in the *MT-ATP6*, *MT-CYB*, *MT-ND4* and *MT-TL1* genes was detected in sperm of fertilization failure patients and infertile Chinese men [40, 41]. However, the impact of these variants on sperm quality still needs to be addressed by functional experiments.

From all this evidences, it is clear how mitochondria and their activity is involved in sperm maturation and function. Despite this, a clear understanding of the functional contribution of mitochondria remains to be investigated.

Mitochondria in embryos

Metabolic activity in embryos has been extensively debated. In the past, the mitochondrial contribution to preimplantation embryo development has been neglected, since it was thought that embryos relied mostly on glycolysis for their development, and mitochondria are normally present in a globular and immature state. Despite this, more and more evidences advocated in favour of an important role played by mitochondria [42]. During early embryo cleavages, the levels of ATP and oxygen consumed are substantially constant, with a small increase during the last cleavages. From morula stage onwards, the metabolic pattern of the embryos changes. Glucose becomes the primary source of energy, with ATP production and oxygen consumption also rising significantly at the blastocyst stage, indicating active OXPHOS processes [43] (*figure 2*).

Mitochondrial changes in shape and activity during development become very clear when comparing ICM and TE cells. The two different cell types in the blastocyst, in fact, have very different energy requirement, with the ICM not requiring a high amount of energy, and the TE requiring higher number of ATP molecules to progress through implantation and invasion. The mitochondria in these cells reflect exactly this situation, and in the ICM it is observed a low Mitochondrial Membrane Potential (MMP), a low number of mtDNA copies, and an overall low activity. On the contrary, mitochondria in TE cells are highly polarized, with a higher number of mtDNA copies, a higher MMP and a high production of ATP [44]. This was recently confirmed in humans by showing how embryo oxygen consumption remains basically constant during the first cell divisions, while it significantly increases from the morula stage onwards, with the highest increase observed in TE cells [45].

Properly developing embryos have been shown to have a higher ooplasmic volume and, as a consequence, a higher amount of mtDNA copies then arrested embryos [46]. mtDNA levels have not been strictly related to the implantation potential of embryos, but embryos with a lower mtDNA levels had a reduced developmental capacity after the implantation stage [16]. In addition, trough the modulation of the mitochondrial transcription factor A (TFAM), it was estimated that a minimal number of 40000-50000 copies of mtDNA are required to support embryonic development in mice [47].

mtDNA as a marker for IVF outcomes

The fundamental contribution of mitochondria to gamete and embryo development pushed researcher towards the identification of mitochondrial features that can be analysed in a non invasive way, and that will allow to predict, select or intervene in the active improvement of IVF outcomes. In this section, we will discuss the more recent advances in the field with a special focus on mitochondrial genetics.

Mutations in mitochondrial genes

Mitochondrial haplogroups have been investigated in different populations and linked to possible reproductive dysfunction: in a Chinese Han population, the haplogroup Z was found to be more present in couples with IVF failures, and other point mutations appeared at a higher frequency in the same group when compared to fertile controls [48]. A different study on 200 Caucasian women, on the contrary, found a lower incidence of haplogroup JT in women with diminished ovarian reserve, and suggested a possible protective role of this set of variants [49]. Haplogroups studies, however, need very large numbers for their intrinsic intra-individual variability, and up to now no study reached numbers large enough to strongly associate haplogroups to specific phenotypes.

mtDNA in polar bodies

Polar bodies have been extensively studied and used for the prediction of the chromosomal content in oocytes. Being an ideal source for genetic testing in oocytes, mtDNA copy number in PBs have been investigated to assess the correspondence with oocytes mtDNA levels: the reports presented so far indicate a poor correspondence between PBs and oocyte, in both mice and humans [50]. The lack of association is also observed while detecting sequencing variants [51, 52], pointing towards the need of identifying other non-invasive sources to investigate oocyte mtDNA.

mtDNA in peripheral blood

Recently, the levels of mtDNA in peripheral blood are being extensively investigated, since they represent the ideal source for non-invasive sampling of material. Lower amounts of mtDNA have been found in the blood of women presenting POI [53], and decreased levels of mtDNA have been associated to an unfavourable IVF outcome [54]. Despite this, other studies failed to observe a significant correlation between mitochondrial copy number in blood and gamete quality, limiting the predictive potential of this analysis [55, 56]

mtDNA in cumulus cells

Cumulus cells (CC) represent an important by product obtained after oocyte pick up, and they have been studied as a proxy to obtain information on embryo quality in a non-invasive way. The content of mitochondrial DNA in CC has been associated to embryo quality, but not to oocyte developmental capacity or fertilizability [57]. In the same study, the authors also observed an association of mtDNA levels to smoking and BMI, with a high BMI and smoking correlated to diminished levels of the mitochondrial genome (as reported also in [58]). The same French group also reported a significantly a lower mtDNA content and expression of *PPARGC-1A* in the CC of women with diminished ovarian reserve, again pointing towards the capacity of CC mtDNA to mirror the metabolic state and help predicting ovarian fitness [59]. Despite this, different studies found no association between metabolic activity and mtDNA levels in follicular cells [56, 60]. In a study on granulosa cells of 149 patients, Liu and colleagues found a significantly lower mitochondrial activity and ATP production in CC of older women, but didn't detect differences in the levels of mtDNA [56]. In addition, a study on CC of patients with endometriosis also highlighted a difference in the ATP production between the different groups, with an impaired metabolism characterizing the

endometriosis group. But again, no difference was found in the abundance of mtDNA [60].

Overall, despite cumulus cells might be promising indicators of a higher developmental capacity of oocytes, their effectiveness in clinical practices must be assessed through rigorous prospective controlled clinical trials, with a thorough validation of the techniques used to analyse them.

mtDNA in cleavage-stage/blastocysts

mtDNA copy number in embryos and blastocyst represents one of the most studied topic in recent years. In two different studies analysing the mtDNA copy number in single blastomeres and blastocyst biopsies, the authors observed a higher mitochondrial DNA count in embryos that fail to implant compared to embryo that did implant. One study [61] was based on a retrospective analysis, while the other [62] performed both a retrospective and a prospective study that showed an implantation rate of 74.3% in the embryos with normal or low mtDNA content, while zero out of 33 embryos with high levels did implant. These promising results have been challenged in a study by Victor and colleagues, which criticised the normalization method employed. They claimed, in fact, that Fragouli and colleagues did not take into account the differential nuclear content of aneuploid embryos. Victor then reanalysed the same dataset originally published by Fragouli, but correcting for the chromosomal content of the different embryos. What they found was a complete lack of association between mtDNA levels and implantation potential [63]. Nevertheless, the group of Wells and Fragouli retrospectively analysed the levels of mtDNA in euploid embryos, therefore not subject to the detection bias suggested by Victor. Also in this group of embryos, the authors were able to show a very good negative prediction potential of their test, since above a certain threshold they had zero euploid embryos implanting [64]. Finally, another group performed the evaluation of the predictive potential of mtDNA in trophectoderm biopsies with qPCR. Their experimental design tried to avoid patient specific confounding factors, by retrospectively analysing the mtDNA amount of embryos that were transferred together and that resulted in a singleton pregnancy. Again, the authors failed to detect any predictive capacity of the analysis [65].

From these conflicting evidences it seems that up to now there are no clear evidences to support one of the two different theories, and more data are required to have conclusive answers on the clinical potential of this analysis.

Mutations in POLG

Mutations in the gene encoding for the DNA polymerase gamma (*POLG*), the only able to replicate the mitochondrial genome, have been associated with the generation of multiple deletions and a higher prevalence of single nucleotide variants. These mutations normally cause severe pathogenic phenotypes, and lead to a range of neurological conditions [66]. More recently, specific mutations in the POLG gene were correlated to the development of premature ovarian insufficiency (POI)[67], but a more robust analysis on a bigger number of samples failed to confirm this link [68, 69]. Despite a lack of association, it is still possible that sub pathogenic variants in this gene might influence the reproductive fitness, since genome-wide association studies reported POLG as one the 11 top candidates for determining the age of natural menopause [70].

PGT-M for mitochondrial diseases

As described before, mtDNA mutations can give rise to a wide variety of pathological phenotypes. As a consequence, many efforts have been done in the last decade to perform a Preimplantation Genetic Testing (PGT) for mitochondrial mutations. This technique have been successfully applied for the prevention of the transmission of different mitochondrial mutations, such as the ones inducing MELAS [71], Leigh syndrome, LHON [72, 73] and NARP [74]. Despite the successes obtained in this field, there is still an on-going debate regarding the segregation of mtDNA molecules in the different cells of a blastocyst [75-77]. It is very important, in fact, that each cell of an embryo carries the same mtDNA content to allow an accurate selection of a mutation-free embryo. A major point of discussion regards the quantification methods used to evaluate the heteroplasmy levels in the single cells, since various groups used different techniques, such as ARMS-qPCR [78] or semi-quantitative fluorescent PCR followed by restriction enzyme digestion [79, 80]. Further studies are needed to identify the most reliable technique for this purpose, and standardize this type of analysis for a wide clinical application.

Finally, it is important to notice that PGT can be performed only in the case of heteroplasmic mutations; in the case of pathogenic mutations present in homoplasmy, in fact, a selection of a mutation-free embryo is not possible, and the only option up to now is represented by mitochondrial replacement therapies, discussed in the following chapter.

Increase mitochondrial activity to improve reproductive fitness

Ooplasmic transfer

Transfer of cytoplasm from a healthy oocyte to a less competent one has been tried already 30 years ago [81].

This was performed with the clear purpose of transferring competent and active mitochondria able to fulfil the energy requirement of the oocyte and allow its maturation. In the first trials performed, about 5-15% of the ooplasm from oocytes of young women was transferred to oocytes from reproductively aged women [82]. This technique, despite conceptually promising, carried a several issues. First, transferred mitochondria induce a condition of heteroplasmy, which is detrimental in several systems [83]. In addition, not only mitochondria are transferred, but also RNAs, proteins and structural complexes produced in the healthy oocyte are carried along, and this may cause unwanted reactions in the host cell. Ooplasm transfer was still used in patients, and babies were born. However, out of the few pregnancies obtained, aneuploidies were present in a significant proportion and one child was affected by metabolic syndrome at less than five years of age [84, 85]. For the risks connected to this procedure [86], ooplasm transfer was abandoned in the clinics and prohibited by the Food and Drug Administration in the USA.

Mitochondrial injection

An alternative to ooplasmic transfer is represented by the direct supplementation of the oocyte with isolated mitochondria. This has been mainly developed in pig models, where it has been shown how mitochondrial injection in metabolic deficient oocyte could significantly improve the fertilization rate and embryonic development [87, 88]. The beneficial effect of mitochondria supplementation, however, was not observed in metabolically competent oocytes, suggesting that a minimal threshold of active mitochondria should be reached to move though embryo development, but an artificial increase of mitochondria in good quality oocyte does not yield any effect.

Very recently, an attractive technique claimed to perform "oocyte rejuvenation" through autologous mitochondrial injections. The mitochondria are isolated from the "ovarian stem cells" present in the ovary, and since they belong to young cells, they will be active and able to rescue compromised oocytes. The group that developed the technique reported a huge increase in clinical pregnancies and live birth rates when the AGUMENT treatment (this is the name of the patented process) was performed in patients with a history of repeated IVF failures (from 5.2% to 25.7% for clinical pregnancy; from 1.3% to 18.1% for live birth rates) [89]. Despite the attractiveness of the data, this technique received criticisms for both the experimental design used to evaluate the efficacy, and also the lack of safety testing. Finally, a recent prospective clinical trial using AGUMENT in a more controlled setup failed to identify any association between the treatment and an improvement of IVF outcomes [90].

Chemical modulators

Another strategy to improve oocyte quality is represented by the use of compounds to stimulate mitochondrial activity and protect the cells against oxidative damage. The Coenzyme Q10 (CoQ) represents one of the most studied supplements, because of its role in mitochondrial respiration and its antioxidant capacity. In mice, it has been shown that dietary supplementation of CoQ could rescue the aged phenotype of oocytes, both in terms of mitochondrial activity and incidence of chromosomal abnormalities [24]. A similar recovery of mitochondrial activity was observed in obese mice supplemented with CoQ, although no effect was seen in terms IVF outcomes [91]. CoQ supplement was also analysed in patients, where a single clinical trial evaluated the aneuploidy rates in polar bodies of women who received a dietary supplement of CoQ. Unfortunately, the trial was prematurely terminated because of a detrimental effect of polar body biopsy on embryo development [92]. Other chemicals have been used to boost mitochondrial function in oocytes for different purposes: resveratrol, for example, has been used in the treatment of oocytes to improve their developmental competence, both as a supplement during IVM of fresh cycles [93], and as an adjuvant during vitrification/warming [94]. In these studies, resveratrol treatment increased the developmental competence of the oocytes, and increased ATP production, membrane potential and mtDNA levels. More clinical trials are currently ongoing, and only when the results will become available it will be possible to know the real effectiveness of these promising treatments.

Mitochondrial transfer

The most novel and appealing technique to manipulate the mitochondrial composition of a cell is represented by Mitochondrial Replacement Therapy (MRT) [95]. This procedure allows the transfer of the nucleus of an oocyte into a second enucleated oocyte that will further develop into a blastocyst, and will display the cytoplasm of the recipient oocyte and the nuclear content of the donor. Nuclear transfer (NT) is currently performed either by transferring pronuclei, metaphase spindles or polar bodies (followed by intracytoplasmic sperm injection), and it is developed to prevent the transmission of mitochondrial diseases [96]. This technique has proven to be feasible and the first three-parent-baby is recently born in Mexico [97]. In the United Kingdom, the fertility regulators already gave the green light for the start of the application of these techniques to prevent severe mitochondrial diseases, and many more three-parent children are expected in the coming years. Nevertheless, the efficacy and the safety of the technique still has to be investigated in depth [98], and the newborns will have to be extensively followed up to assess the long-term effects of these procedures.

MitoTALEN

Another fascinating approach to eliminate detrimental mutations and improve reproductive success in IVF is represented by genome editing strategies, where very promising results have been reached with mitochondria-targeted nucleases. Both transcription activator-like effector nucleases (TALENs) and zinc finger nucleases (ZFNs) have been successfully applied for the degradation of pathogenic mitochondrial DNA molecules, causing a significant shift of the heteroplasmic levels in somatic cell cultures [99, 100] and in the germ line [101]. These systems have been proven to be more effective, at the present moment, than CRISPR/Cas9 based approaches, which are limited by the difficulties in importing RNAs into mitochondria [102].

Conclusions

In conclusion, mitochondrial function and mitochondrial genetics are emerging as crucial factors for a correct gamete maturation and embryo development. For these reason, and for the concomitant innovations brought by Next Generation Sequencing technologies, the study of mitochondrial DNA became a very promising tool to predict pregnancy outcomes. Despite this, prospective randomized clinical trials must be performed before the wide implementation of such analyses in the clinical practice, and the moment these tests should be considered as experimental.

Liens d'intérêt : Les auteurs déclarent n'avoir aucun lien d'intérêt en rapport avec cet article.

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