

# Effects of The Mitochondrial Genome on Germ Cell Fertility: A Review of The Literature

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## Abstract

Infertility is one of the major problems faced in medicine. There are numerous factors that play a role in infertility. For example, numerous studies mention the impact of the quantity and quality of mitochondria in sexual gametes. This is a narrative review of the effects of the mitochondrial genome on fertility. We searched the PubMed, Science Direct, SID, Google Scholar, and Scopus databases for articles related to “Fertility, Infertility, Miscarriage, Mitochondria, Sperm, mtDNA, Oocytes” and other synonymous keywords from 2000 to 2020. The mitochondrial genome affects infertility in both male and female gametes; in sperm, it mainly releases free radicals. In the oocyte, a mutation in this genome can affect the amount of energy required after fertilisation, leading to gestation failure. In both cases, infertile cells have substantially less mitochondrial DNA (mtDNA) copies. The effects of mtDNA on gamete fertility occur via changes in oxidative phosphorylation and cellular energy production. Also, a reduction in the number of mtDNA copies is directly associated with sex cell infertility. Therefore, evaluation of the mitochondrial genome can be an excellent diagnostic option for couples who have children with neonatal disorders, infertile couples who seek assisted reproductive treatment, and those in whom assisted reproductive techniques have failed.

**Keywords:** Infertility, Mitochondria, Mitochondrial Genome, Oocyte, Sperm

**Citation:** Rahimi Darehbagh R, Khalafi B, Allahveisi A, Habiby M. Effects of the mitochondrial genome on germ cell fertility: a review of the literature. *Int J Fertil Steril.* 2022; 16(2): 70-75. doi: 10.22074/IJFS.2021.527076.1098.

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## Introduction

The mitochondrion has emerged from prokaryotic ancestors, the Proteobacteria species, and continues to survive as endosymbionts in eukaryotic cells (1). Mitochondria is a double-membrane organelle found in most eukaryotic cells that is primarily responsible for cellular energy production. In addition to providing energy to cells, mitochondria are involved in several other tasks, including signal transduction, cell differentiation, death, and cell cycle control and growth (2).

In somatic tissues, mitophagy is a part of the cellular cycle mechanism responsible for destroying damaged mitochondria. Any deviation from this process can lead to mitochondrial dysfunction and the accumulation of defective organelles, which plays a major role in the aging process (3, 4).

Mitochondria, unlike other organelles of animal cells, tend to rely on their DNA. Hence, their work depends not only on mitochondrial DNA (mtDNA) itself, but also on proteins transcribed from nuclear DNA (5).

One of this organelle's unique properties is that a spe-

cific genome is conserved and has many copies. Most prokaryotic ancestral genes may have been lost during endosymbiont evolution or transferred to the eukaryotic host nuclear genome (4).

mtDNA is a small genome inherited from the mother. A mature oocyte contains more than 150,000 copies of mtDNA, while sperm only contain about 100 copies. Mitochondrial oxidative phosphorylation is suggested to be an essential determinant of oocyte quality and sperm motility (6).

There are two chains of human mitochondrial DNA - heavy and light. Heavy chains have extensive amounts of guanine and encode 12 subunits of the oxidative phosphorylation process and two ribosomal RNAs (12s and 16s tRNA). On the other hand, the light strand encodes one subunit and eight tRNAs. As a result, mtDNA generally contributes to oxidative phosphorylation, encoding two rRNAs, 22 tRNAs, and 13 protein subunits (7).

Conservation of the mitochondrial genome is presumed to be due to the noticeably hydrophobic nature of these 13 proteins encoded by this genome (8). Transportation of these hydrophobic proteins between the cytoplasm and

Received: 21/March/2021, Accepted: 16/October/2021

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Royan Institute  
International Journal of Fertility and Sterility  
Vol 16, No 2, April-June 2022, Pages: 70-75

the mitochondrial matrix via the internal and outer membranes is considered problematic. Although mtDNA is noticeably polymorphic, it appears to have an adequately conserved gene content and structure as evidenced by the results of animal studies to date, which have shown similar sets of mitochondrial genes (9, 10).

The mitochondria pleiotropic elucidates the main position of these organelles in the aging process, and in numerous genetic and neurodegenerative illnesses, cancer, weight problems, and diabetes (11). There is growing evidence that mitochondria additionally contribute to fertility, and mitochondrial defects may be at least partly responsible for male and female infertility. Mitochondrial defects seem to affect, not just the gametes, but also the fertilisation process and early embryonic development (12).

Various mitochondrial DNA and, consequently, mitochondrial functional defects can decrease fertility of the male and female gametes, deteriorate foetal health, and lead to foetal loss (13, 14). Although some mtDNA defects do not directly affect fertility, they can be transmitted to the foetus through various diseases. Mitochondrial DNA defects in oocytes are transmitted directly to the foetus; thus, they can be transmitted more effectively than sperm mitochondrial defects. Unlike oocytes, the mitochondrial genome in sperm has an indirect effect on fertility (15). Therefore, in this narrative review study, we intend to evaluate and report the impact of the mitochondrial genome on pregnancy and infant health.

In this narrative review, we selected a set of related articles from 2000 to 2020 obtained after a search result of the terms “Fertility, Infertility, Miscarriage, Mitochondria, Sperm, mtDNA, Oocyte”, and other synonymous keywords in the PubMed, Science Direct, SID, Google Scholar, and Scopus databases. A variety of studies from different categories were included in this paper. Then, a subset of documents was evaluated and selected based on their titles and abstracts. After the search and evaluation phases, the papers were carefully studied, and the causes of mtDNA defects and their associated disease characteristics were registered and categorized. The data obtained were recapitulated, then organized and presented in this study. The Ethics Committee of Kurdistan University of Medical Sciences, Sanandaj, Iran approved this study (IR.MUK.REC.1399.184).

### **The role of the mitochondrial genome on sperm properties**

First, we examined the effects of the mitochondrial genome on sperm fertility. Energy production along with apoptosis are two primary roles of mitochondria in spermatogenesis. In addition, mitochondria are involved in several effective processes in spermatogenesis and fertility. The sperm mitochondria are located on the margins of the tail microtubules in order to provide the necessary energy for motility. The mitochondrial volume is related to the flagella's period and frequency (16). The effects of mitochondria in germ cell proliferation, mitotic regulation, and cell destruction via apoptosis are well-known (17).

Sperm DNA integrity is one of the vital determinants of fertilisation and sperm fertility; both endogenous and exogenous factors can damage and endanger foetal health. Clinical studies suggest that men with idiopathic infertility appear to have moderate to severe sperm DNA damage. The most crucial source of this DNA damage appears to be oxidative stress, which is caused by free radicals that are primarily produced in the mitochondria. Due to the high level of unsaturated fatty acids in the membrane and the small volume of cytoplasmic space, sperm cells are more susceptible to oxidative attacks than other cells. Structural defects in the mitochondria, often due to mitochondrial DNA changes, facilitate the release of free radicals into the sperm cytosol, and result in nuclear damage and infertility (13, 18).

Sperm parameters that include motility, capacity, acrosomal reaction, and oocyte interaction depend on mitochondrial regulation of reactive oxygen species (ROS) levels. If the mitochondria fail to supply sperm with motor energy, the sperm physically loses their fertility (2).

Studies show that the proportion of mtDNA deletion in sperms with poor motility and reduced fertility is significantly higher than in those with high motility and good fertility. Male infertility can be defined as low sperm motility (asthenozoospermia) and/or low sperm counts (oligospermia). Some evidence shows that there are mitochondrial genome (mtDNA) mutations in patients with fertility problems; therefore, it is assumed that mitochondrial respiratory chain defects might contribute to male infertility. In addition, the evidence suggests that the frequency of three major 4977, 7345, and 7599 base pair mitochondrial genome deletions in azoospermic and oligospermic patients are higher than in fertile men (19).

Mitophagy is the basis of paternal mitochondrial elimination shortly after fertilisation (20). The paternal mitochondria lose mitochondrial membrane potential ( $\Delta\Psi_m$ ) after entering the oocyte, which stimulates mitochondrial destruction (20-23).

The mitochondrial genome is processed and repaired by identical enzymes and mechanisms present in the nucleus. However, mitochondria lack some of these nuclear enzymes; therefore, the mitochondrial DNA repair system appears to be weaker than the nucleus (24). Along with ROS generation and the lack of histone-protective proteins, this poor repair system may be responsible for the high rate of mutations in mtDNA (25, 26).

mtDNA mutations are involved in mitochondrial and age-related diseases. Pathogenic mtDNA mutations are always present in fragments of mtDNA copies. One study has examined sterility in male mice from mutations in DNA. The results showed that a reduction in the copy number of mtDNA was associated with mitochondrial damage to spermatocytes and spermatids in the testes, which resulted in infertility. In contrast, increasing the copy number of mtDNA increases fertility, and it is common for morphology and spermatocyte proteome tests to be performed. Thus, increasing the copy number of mtD-

NA can positively improve the malignant disease caused by mtDNA mutations, which significantly influences the development of future therapies to treat mitochondrial dysfunction (27).

The large 8.7 k-b fragment of mtDNA contains complex III (cytochrome B), complex IV (cytochrome c oxidase, COXIII), complex V genes, and ATPase 6 and 8 synthase genes. A study that assessed the 8.7k-b fragment by long-range PCR showed several deletions in this fragment. The results of this study indicated that the 8.7 k-b fragment deletion frequency in infertile groups was higher than in the control group. In addition, a comparison of different types of infertility showed that the deletion rate was higher in oligoasthenoteratozoospermia (OAT) patients. As a result, they concluded that a significant relationship existed between deletion of the 8.7 k-b fragment and male infertility, which was particularly more effective in OAT subgroups (28).

Examination of sperm mitochondrial parameters is performed to determine semen quality. According to Table 1, these parameters include mitochondrial membrane potential (MMP), mtDNA copy number (mtDNAcn), mtDNA integrity, and apoptotic indexes. These mitochondrial biomarkers are strongly influenced by the individual's lifestyle and predict the quality of semen in the population. The relationship between these mitochondrial biomarkers and semen characteristics is shown in Table 1.

Studies have shown that current drinkers have higher MMPs, and mtDNAcn increases with age (29).

**Table 1:** Mitochondrial biomarkers and semen characteristics

	Semen volume	Semen concentration	Total sperm count	Sperm motility
MMP	Direct	Direct	Direct	Direct
mtDNAcn	–	Indirect	Indirect	Indirect
mtDNA integrity	–	Direct	Direct	Direct

MMP; Matrix metalloproteinase and mtDNAcn; Mitochondrial DNA copy number.

### The role of the mitochondrial genome in oocyte properties

In all mammals, the mitochondria of metaphase II oocytes are tiny organs surrounded by numerous cristae near the dense electron matrix (12). This phenomenon shows weak energy activity and low ATP production (30). Mitochondria then appear to spread to the ooplasm at this stage (31). The internal structure of mitochondria does not change after fertilisation, but these organs undergo nuclear rearrangement in response to energy requirements. Nuclear localisation in blastomeres appears to have been preserved during the first division of embryonic cells (32, 33). Inadequate mitochondrial redistribution may lead to poor oocyte fertilisation and embryonic development (31).

After fertilisation, the oocyte needs an extensive amount of energy to supply important events such as spindle formation, chromatid separation, and cell division. Before

the blastocyst is implanted, the developing zygote is mainly dependent on mitochondria for its energy demand. The number of mitochondria tends to decrease with each cell division, and the foetus receives its mitochondria only from the oocyte. Mitochondrial DNA mutations diversify the efficiency of the oxidative phosphorylation pathway and thus the production of cellular energy. Mild destructive mutations may significantly reduce sperm cell function due to the high energy demand and low mitochondrial numbers, but have little effect on somatic cells or oocytes (34).

Mitochondria of the oocytes of infertile women have numerous DNA deletions and nucleotide changes that may hinder their function. A combination of the fewer mtDNA copies and increased number of mutations and deletions in mtDNA can lead to inadequate mitochondrial activity for foetal growth and prompt pregnancy failure (35). The mtDNA copies in patients who suffer from fertilisation failure are significantly lower than those with regular fertilisation. For unknown reasons, the number of mitochondrial oocyte copies in preterm infants was significantly lower compared to patients with *in vitro* fertilization (IVF) failure due to severe sperm abnormalities. Small amounts of mtDNA may be due to cytoplasmic oocyte maturation (36). Compared to younger women, more mtDNA deletions have been observed in oocytes of older women (37). Previous reports on the relationship between oocyte quality and mitochondrial content suggest that low mtDNA content can prevent fertilisation (38).

Studies on mtDNA segregation in pedigrees indicate that in the event of a point mutation, there can be a thorough change in the set of mutations in offspring compared to parents in a single generation (39-41), considering the large number of mitochondrial genome copies in oocytes, assuming that very few mtDNAs can fill the oocyte and, thus the organism. This idea led to the bottleneck theory (42). According to this theory, there can be a significant reduction in mitochondrial counts before they are greatly amplified during ovulation.

Primitive gametes recruit less than ten copies of the original mitochondrial genome to fit the organism. Because the number of mitochondria per cell is low, cells with the best mitochondrial profile can be selected and those with mitochondrial defects can be eliminated. In most cases, this mechanism disrupts the mutant mitochondrial genome and homogenizes the mtDNA population. In order to maintain mitochondrial integrity across generations, deleterious mutations, such as deletions, are usually eliminated and not passed from the mother to the ovaries. This mechanism also significantly reduces the rate of maternal transition point mutations or can inadvertently amplify them (40).

Thus, the bottleneck theory explains how mtDNA is renewed and purified from descendant to descendant through a "narrow neck". Following a sharp decrease in mtDNA content on bottleneck removal, germ cells increase the selected range of mitochondria by clonal selec-



tion and allow individuals to have a homogeneous group of mtDNAs (43).

mtDNA is highly polymorphic in single nucleotide polymorphism (SNPs) haplogroups and subhaplogroups, which reflects species migration and phylogenetic evolution (44). An increasing number of reports suggest that various mtDNA haplogroups that contribute to various mitochondrial respiratory chain activities may be associated with specific human diseases. The effect of specific haplogroups on mtDNA on human fertility has been shown, and a link has been found between different haplogroups and sperm quality. For example, high intake of haplogroup T is associated with a decrease in sperm motility and patients with complex I and IV respiratory chain dysfunction (45). Similarly, high presentation of haplogroup U is associated with reduced sperm motility and vitality (46). The consequences of mtDNA polymorphisms at the molecular level can prompt biochemical abnormalities in specific cells if associated with environmental factors. The possibility of mtDNA haplogroups that affect oocytes and embryonic well-being is an ongoing area of research. Animal studies suggest that specific polymorphisms in the mtDNA control region can affect the proportion of high developmental ability oocytes retrieved by the ovum pick-up (OPU) procedure and the rate of transferable nuclear transfer embryos (47). In addition, the results of animal studies showed that haplogroups could affect the fertility rate. In this study, further analysis through pairwise polymorphism comparison, which resulted in two significant haplogroups, showed that the blastocyst formation rate also varied significantly between these two groups. These results suggest that foetal growth ability might be affected by mitochondrial haplogroups; however, this hypothesis is still being tested at a large scale (48).

Mitochondria undergo constant destruction and renewal in cells, as a quality control mechanism to ensure efficacy. Mitochondrial destruction involves a selective form of autophagy (referred to as mitophagy) through which the entire organelle, including its mtDNA molecules, is destroyed. Significantly, this intrinsic pathway is associated with mitochondrial dynamics. On the one hand, the fusion of mitochondria is made possible by mixing the defective mitochondrial content with functional content. On the other hand, mitochondrial fission causes the dysfunctional organelles of the mitochondrial network to be deleted by preventing their further fusion and completion, prompting their destruction through mitophagy.

The rate of mitochondrial demolition specifies whether oocyte oncotic cell death (after significant demolition) or apoptotic cell death (after milder demolition) or early foetal growth continues before foetal cell death (49, 50). This variation in type or cell death time is likely due to a decrease in ATP levels. Oncosis, a process that does not require ATP, occurs in oocytes that are greatly reduced by ATP, while apoptosis (a process that requires ATP) (51) occurs in oocytes or early embryos that are damaged but

can still be preserved. The low ATP levels in these observations can be easily explained by impaired mitochondrial ATP production.

Interestingly, injection of approximately 5000 mitochondria into the oocytes in rats with high ovarian atresia resulted in reduce the severity of apoptosis to a significant 70% in the control oocytes (without medication mitochondrial or no buffer injection only) compared to 36% injected eggs with mitochondria. Oocyte mitochondrial DNA defects can be associated with various diseases (52). Diseases of mitochondrial DNA comprise a series of diseases that are either inherited or caused by mutations. People with mitochondrial DNA diseases may have a combination of healthy and mutated mitochondrial DNA in their cells, called heteroplasmy, which could affect the severity of their disease. Despite the effect of sperm on the quality and the result of fertilisation, the foetus acquires the mitochondrial diseases from oocytes; sometimes, mitochondrial replacement therapy is the only way to prevent transmission of these diseases (53).

Previous reports have shown that the average mtDNA copy number did not significantly differ in dystrophic ovary patients compared to their control groups, but it was dramatically lower in the oocytes of women with ovarian failure ( $P < 0.0001$ ). The two ends of the mtDNA copy number spectrum were distinct, and there was no interference between patients with ovarian failure and those with typical characteristics. These results showed that a decrease in mtDNA content was associated with the poor oocyte quality observed in ovarian failure.

These findings are indicative of abnormal mitochondrial biosynthesis and possibly cytoplasmic immaturity during oocyte development. In case of ovarian dystrophy, impaired oocyte maturation may not be associated with mitochondrial biosynthesis. These changes in egg maturation may explain the clinical outcomes observed in these two syndromes. A diffuse deficiency of the oocyte group indicates ovarian dystrophy, but a subset of mature oocytes may still develop into quality embryos after fertilisation. This vastly different from ovarian incompetence, which is characterized by poor quality oocytes and embryos. According to recent reports, a decrease in mtDNA in immature oocytes associated with ovarian failure may be due to a mutation in a specific mtDNA polymerase (POLG1), which is responsible for the onset of menopause (54, 55). Since POLG1 and TFAM play a crucial effect in mtDNA replication and there are strong links between ovarian failure and early menopause, it has been hypothesized that insufficient replication capacity of mtDNA due to POLG1 mutations may lead to the same mtDNA removal that we studied (56, 57). Contributions to the study of the reduced expressions of the mitochondrial genes *ND2*, *COI*, *COII*, *ATPase 6*, *COIII*, *ND3*, *ND6*, and *Cyt b* in infertile human oocytes support the idea that mitochondrial quality is closely related to the viability and growth of oocytes. Foetal growth is highly dependent on ATP content (58).

Although paternal mitochondria are not directly inherited, they do affect the results of fertilisation and fertility. In some cases, damage to mitochondrial DNA can also damage the mitochondrial structure and lead to free radical release. Because of the characteristics related to sperm structure, these free radicals mainly affect the sperm and cause infertility (59). Deleting mtDNA copies in sperm leads to reductions in fertility, asthenozoospermia and oligospermia, and respiratory chain disorders; these deletions increase with age. A decrease in the number of mtDNA can distort both normal differentiation and growth of spermatocytes and spermatids in the testes. In contrast, by increasing the number of mtDNA copies, fertility can be improved, which could act as a basis for developing treatments for various types of infertility. Mitochondrial parameters such as MMP, mtDNAcn, mtDNA integrity, and apoptotic parameters for determining the quality of semen can be used to determine the causes of infertility.

## Conclusion

Most mitochondrial genetic defects are inherited from the mother through the oocyte; thus, the maternal mitochondrial genome is critical. The foetus needs an extensive amount of energy for the post-fertilisation events. Still, harmful mutations in mtDNA impair the oxidative phosphorylation pathway and cellular energy production, which lead to miscarriage. Like sperm, the number of mtDNA copies in the oocyte is directly related to cell fertility, such that infertile cells have substantially fewer copies than fertile cells. As a result, low mitochondrial DNA content due to insufficient mitochondrial biogenesis or cytoplasmic maturation may adversely affect oocyte fertility. Low mtDNA counts constitute a significant cause of infertility in older women.

## Acknowledgements

The authors of this manuscript would like to express their gratitude to the Vice-Chancellor of Research and Technology of Kurdistan University of Medical Science. There is no financial support and conflict of interest in this study.

## Authors' Contributions

A.A.; Contributed to the concept, design, and draft of the manuscript. R.R.D., B.K.; Design, performed the literature search and screening, data extraction, manuscript draft, and obtained Ethical Committee approval. M.H.; Drafted the manuscript. All authors read and approved the final version of the manuscript.

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