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Mitochondrial DNA as A Marker of *In-Vitro* Fertilization Outcome in Polycystic Ovarian Syndrome Patients.

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ABSTRACT

Polycystic Ovarian Syndrome (PCOS) is the most common reproductive disorder that affects 6-10% of all women in reproductive age. This syndrome is characterized by clinical or biochemical hyperandrogenism, oligo-anovulation, and polycystic ovaries, with concurrent hormonal and metabolic alterations that alter the follicle maturation and reduce oocyte quality. In Vitro Fertilization (IVF) has been used as an intervention to improve pregnancy rates. IVF pregnancy outcomes after embryo transfer could remain low due to poor oocyte quality. Embryo selection is mainly based on morphological criteria. However, good morphology of the embryo does not necessarily indicate good functional status, some of these embryos might be genetically abnormal.
The change in mitochondrial DNA (mtDNA) copy number is related to the occurrence, severity of PCOS, and oocyte maturation. Mature oocytes usually contain at least 100,000 mtDNA copies, which are necessary for normal development. The number of mtDNA remains stable, and the maintenance of mtDNA copy numbers at an appropriate level is crucial for maintaining mitochondrial function and cell growth. This review summarizes the recent findings for mtDNA as a marker for IVF embryo viability and selection for implantation and predicting pregnancy outcomes.

Keywords: Mitochondrial DNA, oocyte quality, embryo viability, pregnancy outcomes.

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Polycystic ovary syndrome:

PCOS is an ovarian disorder characterized by hyperandrogenism, ovulatory dysfunction, and polycystic ovaries [1]. It may be the most common female endocrinopathy in the developed world. However, it most likely represents a heterogeneous disorder and one whose pathophysiology and etiology are debated. PCOS affects young women with oligo-ovulation (which can lead to oligomenorrhea), infertility, acne, and hirsutism. It also has notable metabolic sequelae, including an elevated risk of diabetes and cardiovascular risk factors [2].

Clinical diagnosis:

The criteria for polycystic ovarian morphology proposed by the Rotterdam consensus group includes the presence of 12 or more follicles measuring between 2 and 9 mm in diameter and/or an increased ovarian volume of greater than 10 cm^3. This presentation in one ovary sufficiently defines the polycystic ovary [3]**.** Each criterion considers PCOS a diagnosis of exclusion, and other diagnoses, such as congenital adrenal hyperplasia, non-classic adrenal hyperplasia, Cushing syndrome, androgen-secreting tumor, idiopathic hyperandrogenism, idiopathic hirsutism, hyperprolactinemia, and thyroid disorders must be excluded. Because 20%–30% of otherwise normal women have evidence of multiple cysts on their ovaries, the presence of polycystic ovaries alone was not considered sufficient by any group [4].

Pathophysiology:

Many hypotheses and several mechanisms have been identified as participating in the pathophysiology of PCOS.The thickness of follicular shells that make them resistant to rupture, ovarian hyperandrogenism, Luteinizing Hormone (LH) hypersecretion, hyperinsulinemia, and impairment of follicular maturation due to excess production of anti-mullerian hormone, a paracrine factor that physiologically blocks the follicular maturation. But the real physiopathology of this syndrome remains unclear yet [5].

There are different pathways that may be involved in its etiology such as ovarian or extra ovarian hyperandrogenism that increases LH/ Follicle-Stimulating Hormone (FSH) ratio which increases estrogen levels and decreases serum progesterone level. This results in reproductive disorders (non-ovulation and menstrual irregularities) and metabolic abnormalities (glucose intolerance and insulin resistance, obesity, cardiovascular disease, and type 2 diabetes) [6].

Hyperandrogenism can occur via several pathways that cause ovarian disruption and irregular menstrual cycle. The first pathway depends on the hypothalamic-pituitary axis and the increase of LH secretion. Elevation of the release of Gonadotropin-Releasing Hormone (GnRH) and subsequent LH secretion is the most important pathophysiological feature of PCOS [7]. The most important reason for GnRH secretion elevation is the dysfunction of gonadotropin inhibitory hormone due to reduced expression of arginine phenylalanine related peptide-3 [8]**.**

The second cause of hyperandrogenism is related to metabolic abnormalities such as obesity, insulin resistance, and hyperinsulinemia. Obesity is associated with dysregulated production of adipokines as Adiponectin, which is an insulin-sensitizing and anti-inflammatory agent. Also, it is associated with chronic inflammatory state and elevated levels of IL-6 and TNF-α [9]. Obesity increases insulin resistance, and the result is increased hyperinsulinism further aggravates hyperandrogenism. Excess insulin increases the sensitivity of the ovary to LH, by interfering with the process of homologous desensitization to LH in normal ovulation cycle [10]. Hyperinsulinemia can either act on the Insulin Growth Factor-1 (IGF-1) receptor, which is related to the insulin receptor or amplifies the free IGF-1 level by inhibiting the hepatic synthesis of IGF-1-binding proteins. In the same way, hyperinsulinemia inhibits the Sex Hormone-Binding Globulin (SHBG) synthesis, leading to an increase of free testosterone [5]. IGF-1 also potentiates the action of LH on its thecal receptor, thus stimulating steroidogenesis [10].

PCOS and infertility:

One of the major causes of female infertility is anovulation and only 7–18% of PCOS patients are ovulatory. PCOS is a complex syndrome including clinical, endocrine, and metabolic disorders which may lead to infertility [11]. Insulin resistance, hyperandrogenism, and obesity play a significant role in the

pathophysiologic process of PCOS. Insulin resistance and hyperinsulinemia are associated with ovarian secretion disorder increasing the androgen production by theca cells that lead to hyperandrogenism [12]. Hyperandrogenism leads to chronic oligo-/ anovulation and menstrual disturbances [13].Disturbance in factors involved in normal follicular development, follicular growth becomes arrested as follicles reach a diameter of 4–8 mm. Because a dominant follicle does not develop, ovulation does not ensue [14].

Assisted reproductive technology:

Women with PCOS tend to require ovulation induction or Assisted Reproductive Technology (ART) to become pregnant due to oligo-ovulation or anovulation, this infertility treatment often results in an evaluated rate of multiple births [15]. There are various fertility treatment options for the induction of ovulation in PCOS women including clomiphene citrate, metformin, gonadotropins, inositols, and laparoscopic drilling [16].

IVF is considered a third-line treatment for anovulatory PCOS women who fail to conceive despite ovulation induction or because there are additional fertility factors, for example, tubal damage or male subfertility [17]. Although infertile women with PCOS may typically present with increasing oocytes retrieved in IVF cycles, most oocytes have poor quality[18]. Several stimulation protocols have been published for the treatment of patients with PCOS undergoing IVF, including clomiphene Citrate associated with Human Menopausal Gonadotrophins (HMG), HMG alone, recombinant FSH (recFSH) alone, GnRH agonist associated with HMG or recFSH, and GnRH antagonist associated with HMG or recFSH [16].Multiple pregnancy and ovarian hyperstimulation syndrome are serious risks in patients undergoing ART. Multiple pregnancy rate can be decreased by a strict policy of elective single embryo transfer[19].Procedures of IVF involve monitoring and stimulating a woman's ovulatory process to remove ovum or ova from the woman's ovaries and to let the sperms fertilize those eggs in an artificial fluid medium in a laboratory. The fertilized egg or zygote is cultured for 2–6 days in an artificial growth medium and then, transferred to the mother's uterus [20].

Non-invasive evaluation of embryo viability is typically based on morphologic and morphometric grading systems which are affected by differences in individual judgment[21]. Embryo morphology does not reflect functional status, as 30–50% of morphologically good embryos might be chromosomally abnormal [22]. Analysis of metabolic parameters in embryo culture media has been used as non-invasive embryo selection methods, such as glucose, lactate, pyruvate, amino acids, and oxygen consumption[23].

Mitochondrial DNA:

Mitochondria produce energy in the form of ATP via Oxidative Phosphorylation (OXPHOS) [24], therefore the number of mitochondria in a cell is related to the energy requirements of the cell and can vary depending on many factors such as the environment and redox balance of the cell [25]. Mitochondrial dysfunction can affect key cellular functions, result in a variety of diseases [26], and altered mtDNA levels have been reported in a wide range of human diseases[27].

mtDNA comprises 0.1–2% of the total DNA in most mammalian cells. There are several unique features of the mtDNA: Human mtDNA is a double-stranded circular DNA molecule of 16,569 bp, weighting 107 Daltons and encodes thirteen polypeptides, all of which are core components of Complexes I, III, IV, and V of the respiratory chain [28]. These thirteen proteins are transcribed and translated by dedicated machinery in the mitochondrial matrix, with human mtDNA also encoding 22 tRNAs (one tRNA per amino acid, with two each for serine and leucine) and two ribosomal RNAs for this purpose [29]. The Electron Transport Chain (ETC) comprises ~ 90 individual protein subunits, encoded by both nuclear DNA and mtDNA. Assembly of a functional ETC requires coordinated regulation and expression of these components by the two separate genomes. Beyond the 13 ETC proteins encoded in mtDNA, the remainder of the human mitochondrial proteome is encoded in and expressed from the nuclear genome. The import of nuclear-encoded proteins through membrane-embedded protein translocases into the mitochondrial matrix requires a membrane potential between the intermembrane space and the matrix [30].

PCOS and mtDNA abnormalities:

As with many tissues the reproductive organs have specific metabolic and energy requirements during development and an adult function. The mature oocyte is the largest cell in the human body and has a

correspondingly high mitochondrial content. The numbers of mitochondria and their function are tightly linked to the development of the oocyte [31].

The number of mtDNA remains stable, and the maintenance of mtDNA copy numbers at an appropriate level is crucial for maintaining mitochondrial function and cell growth [32]. Changes in mtDNA copy numbers reflect mitochondrial disorders caused by environmental oxidants and gene-environment interactions and are risk factors of several diseases. The change in mtDNA copy number is related to the occurrence and development of PCOS. For example, PCOS women have lower mtDNA copy numbers than those without PCOS. The mtDNA copy number of patients with PCOS is negatively correlated with insulin resistance level, waist circumference, and triglyceride levels and positively associated with SHBG levels. The decrease in SHBG not only increases the biological activity of androgen but is also closely related to insulin resistance and metabolic syndrome [33].

Promoting Glucose Transporter (GLUT) is essential for intracellular glucose transport. Glucose restriction is reportedly associated with GLUT1 deficiency, which leads to decreased mitochondrial function, such as decreased mitochondrial membrane potential and activation of mitochondrial-dependent apoptosis. Therefore, decreased expression of GLUTs and related mitochondrial dysfunction may be associated with insulin in PCOS [34].

Alterations in mtDNA copy number may be associated with the development of PCOS, and the extent of mtDNA copy alteration is associated with the severity of PCOS [35]. mtDNA plays a fundamental role in the increase in Reactive Oxygen Species (ROS), and decreased mtDNA content in peripheral leukocytes is associated with the development of type 2 diabetes, which is the late-stage complication of PCOS [36].

Oxidative stress is one of the pathogenic mechanisms underlying numerous diseases, including type 2 diabetes [37]. Prolonged increased ROS production, induced by insulin resistance and hyperglycemia, in turn, impairs protective mechanisms such as the endothelial production of nitric oxide and promotes pathogenic signaling pathways, including mitochondrial dysfunction [38]. mtDNA content was found to decrease in skeletal muscle and peripheral blood leukocytes of patients with diabetes [39].

Increased level of ROS could decrease ATP content and impair mtDNA copy number. A low level of ROS increases mtDNA copy number [40]. Oxidative stress promotes nuclease activity against mtDNA, and this leads to the accumulation of mtDNA fragments [41]. A high level of ROS could inhibit the enzymeactivity that was involved in mtDNA replication. Thus, the oocytes with a low mtDNA copy number demonstrated less developmental potential [42].

Mitochondria are controlled by dual genes, andmutations in these genes may lead to respiratory chaindysfunction and subsequently result in reduced ATPproduction and excessive ROS production. Due to the unique structural properties of mitochondrial genes, the frequency of DNA mutations is much higher than that of nuclear DNA [43]. Such mutations have been proven tobe associated with metabolic abnormalities, such as hypomagnesemia,hypertension, and hypercholesterolemia. Detailedmechanisms are not yet clear, but theseabnormalities are related to PCOS[44]. Most of the abnormally expressed genes involved in the pathogenesis of PCOS are listed in **Table 1.**

Table 1: Mitochondrial genome abnormalities related to PCOS

Gene	Abnormality	Reference
DRP1	Downregulated	[51]
PINK1	Downregulated	
PGC-1α	Down/upregulated	[34, 50, 51, 53, 54]
NRF1	Down/upregulated	[34, 50, 51]
TFAM	Down/upregulated	[34, 50]
ND1	Downregulated	[50]
ND3	Downregulated	
mtCopII	Downregulated	
mtCopIV	Downregulated	

As-b	Downregulated	
NIX	Upregulated	[53]
RHEB	Upregulated	
NDUFA3	Downregulated	[54]
SDHD	Downregulated	
UCRC	Downregulated	
COX7C	Downregulated	
ATP5H	Downregulated	
UCP2	Downregulated	
A3302G in $tRNA^{leu(UUR)}$	Mutation	[48]
C3275A in $tRNA^{leu(UUR)}$	Mutation	
C7492T in $tRNA^{ser(UCN)}$	Mutation	
T4363C in $tRNA^{Gln}$	Mutation	[47, 48]
T4395C in $tRNA^{Gln}$	Mutation	
A7543G in $tRNA^{Asp}$	Mutation	
A8343G in $tRNA^{Lys}$	Mutation	
T10454C in $tRNA^{Arg}$	Mutation	
A14693G in $tRNA^{Glu}$	Mutation	
$tRNA^{Cys}$	Mutation	[47]
12S RNA	Mutation	
16S RNA	Mutation	
D-loop	Mutation	
12S RNA	Hypermethylated	[49]
16S RNA	Hypermethylated	
ND4	Hypermethylated	
D310 in D-loop	SNPs	[46]
A189G in D-loop	SNPs	

mtDNA Displacement loop (D-loop)plays an important role inmtDNA replication and transcription because it containsthe starting point and promoter for the replication of theheavy and light chains of mtDNA. Mutations in thisregion will result in mitochondrial dysfunction by alteringmtDNA replication and transcription[45].A study sequencedthe mitochondrial D-loop in 118 patients withPCOS and 114 South Indian controls and showed that significant association of D310 (P=0.042) and A189G (P=0.018) SNPs with PCOS. The qRT-PCR analysis revealed a significantly diminished mtDNA copy number in PCOS patients compared to controls (P=0.038). Furthermore, mtDNA copy number was significantly lower in PCOS cases carrying D310 and 189G alleles when compared to non-carriers (P=0.001 and 0.006 respectively)[46].Zhou et al. found16 bases changed in the D-Loop gene, 7 variants in the 12S rRNA gene, and 3 variants in 16S rRNA in PCOS patients but were absent in the control group, suggested that variants in tRNA genes may be associated with PCOS [47]. Another study identified nine mt-tRNA mutations that may be associated with PCOS-IR: mt-$tRNA^{Leu(UUR)}$ A3302G and C3275A, mt-$tRNA^{Gln}$ T4363C and T4395C, mt-$tRNA^{Ser(UCN)}$ C7492T, mt-$tRNA^{Asp}$ A7543G, mt-$tRNA^{Lys}$ A8343G, mt-$tRNA^{Arg}$ T10454C, and mt $tRNA^{Glu}$ A14693G, which are located in evolutionarily conserved nucleotides [48]. Jia et al. isolated gilt oocytes from normal and polycystic ovaries and assessed the quality of mitochondria and the methylation of related genes in oocytes. They found 12S rRNA, 16S rRNA, and ND4 coding sequences were hypermethylated in PCO oocytes, which is by suppressed expression of mtDNA-encoded genes and impaired mitochondrial function[49].Several key genes including TFAM, PGC-α, and NRF1, and mtDNA-encoded genes, such as Nicotinamide Adenine Dinucleotide (NADH) dehydrogenase subunit 1, NADH dehydrogenase subunit 3, mitochondrial respiratory chain complex II, mitochondrial respiratory chain complex IV, and ATP synthase subunit b associated with the biogenesis of mitochondrial and the rate of mitochondrial oxygen consumption and the production of ATPwere found to have significantly reduced expression levels in dihydrotestosterone treated rat islets [50]. Zhang, et al. demonstrated that hyperandrogenism and insulin resistance induce mitochondria-mediated damage and a resulting imbalance between oxidative and antioxidative stress responses in the gravid uterus as the expression of Drp1, Pgc1a, Nrf1, and Pink1 mRNAs was decreased, but MFN1, OPA1, TFAM, PARKIN, RHEB, and ATP13a mRNA expression was not altered in PCOS like rats and the normal expression of these genes in the gravid uterus maintains normal fetal development [51]. PGC- 1α can bind and coactivate the transcriptional function of NRF-1 on the TFAM promoter and regulate the replication and transcription of mtDNA [52].The mRNA levels of PGC-1α, a

regulator of mitochondrial biogenesis, was downregulated in the classic PCOS group compared with the control group, and the methylation ratio of PGC-1 a promoter was higher in the classic PCOS group (P<0.001). Autophagy that occurs in mitochondria is called mitophagy. The expression of mitochondrial mitophagic receptors NIX and RHEB were increased in CCs in women with classic PCOS compared with the control group (P<0.001) [53]. Skov et al. used quantitative real-time PCR to examine gene expression levels of one gene from each of the five respiratory complexes (I–V) and found that nucleus-encoded OXPHOS-related genes (NDUFA3, SDHD, UCRC, COX7C, and ATP5H) decreased. These findings provide evidence showing that mitochondrial oxidative metabolism is impaired and that mitochondrial functional genes are downregulated in the skeletal muscle of women with PCOS [54].

Mitochondrial function in the oocyte and early embryo:

Although there are several metabolic pathways for ATP production, 88% of ATP generated from glucose is provided by mitochondrial OXPHOS, with the remainder coming from glycolysis and the tricarboxylic acid cycle [55]. Severe deficiency of cellular ATP content often leads to cell apoptosis [56]. All of the complex processes the oocyte goes through before ovulation and fertilization require energy, which is derived mainly from ATP production via OXPHOS [57]. Early embryo development and implantation potential have been correlated with mitochondrial function and activity [58]. Furthermore, higher ATP content from human oocytes and embryos has been correlated with better reproductive results among infertile patients [59]. The mitochondria of maturing oocytes and pre-implantation embryos undergo qualitative structural transformations and changes in cytoplasmic distribution. These structural changes result in a highly active oxidative metabolism and ATP production, impacting oocyte quality, and embryo development [60]. Oocyte energy production relies essentially on pyruvate as a substrate, leading to ATP generation through OXPHOS within the ETC. Glycolysis in oocytes is limited by low phosphofructokinase expression. In contrast, cumulus cells have good glycolytic capacity; they confer metabolic support to oocytes by providing pyruvate via gap junctions [61]. Pyruvate is the primary substrate that derives from the cumulus cells to the oocyte to generate ATP. Other amino acids and intermediate metabolites are potentially involved [62].

Embryo quality and mtDNA abnormalities:

Low ATP levels in oocytes are associated with reduced embryo viability and implantation potential. Therefore, embryo viability has been suggested to be related directly to oocyte mitochondrial activity and the ability to produce energy [59]. mtDNA copy number has been proposed as a surrogate measure of mitochondrial function. Oocyte mtDNA increases until the stage that immediately precedes fertilization, which coincides with ovulation. In healthy embryos, the accumulated mtDNA is divided equally among all cells during embryogenesis. Thus, the mtDNA copy number per cell progressively diminishes, whereas the cellular requirements for ATP increase. This may be particularly relevant, as the replication of mtDNA will only resume at the blastocyst stage [63]. During initial embryo cleavage, the mitochondrial distribution between blastomeres may be unequal. Whenever it occurs, cells receiving less mtDNA exhibit a reduced bioenergetic capacity and consequently evolve to lysis and death [31]. mtDNA content in cumulus cells is correlated with the amount detected within the oocyte for each cumulus-oocyte complex. There is an association between higher mtDNA amounts in cumulus cells and good-quality embryos [64]. Granulosa and cumulus cell mitochondrial status directly influence embryonic development, as well as the capacity for the activation, maturation, and fertilization of oocytes. mtDNA mutations or deletions have been correlated with mitochondrial dysfunction, low ATP levels, and embryonic developmental arrest [65]. Mitochondrial hyperproliferation is likely to be indicative of a compensatory mechanism for intracellular metabolic stress. Embryos that are subject to energy depletion may increase mtDNA biogenesis in an attempt to compensate for energy deficiency [66]. However, such aberrant and unregulated replication do not necessarily reflect better ATP production because these mitochondria are most likely dysfunctional [63]. Fertilized oocytes have a considerably greater amount of mtDNA, whereas oocytes from older women or from those who present with ovarian insufficiency express fewer mtDNA copies before fertilization [63].

Studies of mtDNA copy number in different samples taken from peripheral blood, oocytes, embryos, granulosa cells, cumulus cells, and embryo culture medium confirmed the relation between mtDNA content and oocytes quality, embryo viability, and pregnancy outcomes. A previous study by Murakoshi, et al. showed significantly lower mtDNA copy numbers in unfertilized oocytes and uncleaved embryos in women who were older than 40 years of age (p <0.05) and directly correlated with cytoplasmic volume and earlier cleavage

speed of the blastomeres [67]. Another study showed that mtDNA content is critical to fertilization outcomes and serves as an important marker of oocyte quality. The mean mtDNA copy number for the fertilized oocytes was 250,454, whereas for the unfertilized group it was 163,698, P<.002 [68]. López, et al. found significant oocyte mtDNA depletion in HIV-infected infertile women when compared with uninfected controls (32% mtDNA decrease, P<0.05)[69].A study analyzed mtDNA content of 113 unfertilized oocytes obtained from 43 patientsshowed that mtDNA copy number was significantly lower in cohorts suffering from fertilization failure compared to cohorts with a normal rate of fertilization[70]. May-Panloup, et al. found that mtDNA copy number was significantly lower in oocytes from women with ovarian insufficiency compared to control (100 000±99 000, P<0.0001) [71]. Wai, et al. genetically manipulated mtDNA copy numbers in the mouse by deleting one copy of TFAM, an essential component of the mitochondrial nucleoid to investigate the effects of mtDNA copy numbers on fertility and found that the critical threshold for mtDNA set at approximately 50 000 copies in the mature oocyte. Subthreshold embryos can progress normally through cleavage but spontaneously abort after implantation[72]. Cumulus cells mtDNA content for good was significantly higher than poor-quality embryos, p < 0.0001[73]. Desquiret-Dumas, et al. reported that there was a significant link between the content of mtDNA in cumulus granulosa cellssurrounding an oocyte and the embryo quality, with significantly higher mtDNA copy numbers being associated with good quality embryos compared with fair or poor-quality embryos[64]. Also, a low mtDNA copy number detected in blood cells may be a peripheral indicator of a low mtDNA content in the oocytes and thus a lower fertilization potential. mtDNA copy number assessment in peripheral blood of 104 subfertile women and 208 controls showed a significant decrease of mtDNA copy number in peripheral blood in subfertile, p < 0.001[74]. Lee, et al. found that the mtDNA copy number was lower, P<0.01 in women with PCOS [35]. Measurement of mtDNA copy number in embryo culture can be used as a non-invasive marker of embryo quality. Embryos that successfully developed into blastocysts exhibited a significantly higher mtDNA/gDNA ratio in the culture medium compared with those that arrest, P = 0.0251[75].

Reduced mtDNA/gDNA ratio may reflect poor mitochondrial antioxidant activity that may lead to cell apoptosis. Oxidative stress is recognized to play a central role in the pathophysiology of PCOS [76]. Increased level of follicular fluid ROS and decreased level of antioxidant enzymes like superoxide dismutase in PCOS women result in polyunsaturated lipids peroxidation and degradation with the formation of malonaldehyde that impair oocyte quality and embryo development [77]. ROS considered as a byproduct of the OXPHOS process and high level of ROS in the cell activate ROS- detoxifying enzymes through the activation of Antioxidant Response Elements (ARE) gene promotors. Cells with low mitochondrial activity, OXPHOS did not induce ARE gene expression and failed to generate this anti-oxidant response [78].

Bioinformatic study:

Data set (GSE92324) analysis was performed to get the relation between oxidative stress and mitochondrial activity and the success of IVF. Differentially expressed genes were detected having corrected P-value≤.05 (Bonferroni correction). By analysis, the expression of genes involved in oxidative stress response in the endometrium biopsy of IVF failed patients and control found that SOD2, GPX1, GPX4, and NFE2 were significantly downregulated while TXNRD2 was significantly upregulated. Also, the expression of nuclear gene encoding mitochondrial proteins showed that MTFP1, CMPK2, SLC25A21, ME3, MRPS2, TUBA4A, and HKDC1 was significantly downregulated whileGLS2 was significantly upregulated **Error! Reference source not found.**.

Table 2: Dysregulated genes involved in oxidative stress response and nuclear genes encoding mitochondrial proteins with the observed fold change (fold change >2.0) in implantation failurepatients compared to control (GSE92324)

Downregulated genes			Upregulated genes		
Oxidative stress response					
Gene symbol	Fold change	q-value	Gene symbol	Fold change	q-value
SOD2	-4.8815	0.0434*	TXNDC8	15.474867	0.3599
GPX1	-2.9487861	0.0117*	TXNRD2	15.452599	0.0005***
GPX4	-18.448886	< 0.0001***	NOS1	5.3854858	0.3154

NFE2	-13.10492	0.0009***	GSTA5	2.5900003	0.0266*
Nuclear genes encoding mitochondrial proteins					
MTFP1	-2.2187	0.0434*	HEL2	3.41179	0.4082
ATP5G2	-2.94951	0.0745	GLS2	5.338367	0.0464*
SLC25A1	-5.993631	0.0592	MRPL45	3.735369	0.9626
MRPL47	-3.60461	0.2359	PDF	2.44924	0.7623
CMPK2	-3.271369	0.0266*	MTFMT	2.894407	0.5051
SLC25A21	-2.10453	0.0360*	PLEC	6.156112	0.4667
SLC25A25	-4.473588	0.4328	ATM	7.179194	0.3385
ME3	-5.678033	0.0009***			
MRPS2	-3.07506	0.0004***			
TUBA4A	-2.060851	0.0328*			
HK3	-4.409137	0.0534			
HKDC1	-2.199963	0.0002***			
BCL2L12	-2.620016	0.2414			
BCL2L10	-5.807605	0.0105*			
BCL2L11	-2.586387	0.6135			

*Significant q value

MTFP1 gene involved in mitochondrial division probably by regulating membrane fission. Loss-of-function induces the release of cytochrome c, which activates the caspase cascade and leads to apoptosis [79]. Also, Enzyme deficiency in the pathway of deoxyribonucleotide synthesis in mitochondria can cause mtDNA depletion syndromes. CMPK2 encoding human mitochondrial UMP-CMP kinase that participates in dUTP and dCTP synthesis in mitochondria [80], which explain the decreased mtDNA/gDNA ratio in the clinically negative pregnancy outcome group.

Decreased MRPS2, which encodes a mitoribosomal protein cause a multiple-OXPHOS-complex dysfunction [81]. TUBA4A gene encodes tubulin that binds with a high affinity to mitochondrial membranes for ensuring intracellular transport and DNA segregation in cell division. Change in tubulin may affect ATP/ADP and other respiratory substrates fluxes lead to a reduction of OXPHOS and the promotion of apoptosis [82]. Hexokinase domain containing 1 is the fifth isoform of hexokinase that encoded by the HKDC1 gene. It has been suggested to play a more major role in glucose metabolism during pregnancy[83]. The product hexokinase-catalyzed reaction, glucose-6-phosphate, was found to potentiate ATP release from mitochondria [84]. Early embryo development and implantation potential have been correlated with mitochondrial function and activity. Furthermore, higher ATP content from human oocytes and embryos has been correlated with better reproductive results among infertile patients [85].

Under the stressed condition, GLS2 gene expression upregulated to enhance mitochondrial respiration and ATP generation and, furthermore increase GSH level and decreased ROS in cells [86]. SLC25A21 gene encoding oxodicarboxylate transport that function as mitochondrial carriers that transport C5–C7 oxodicarboxylates across the inner mitochondrial membrane. SLC25A21 defect was maternally inherited and can cause defective oxoadipate dehydrogenase complex [87]. ME3 gene encodes a mitochondrial NADP(+)-dependent isoform is an important enzyme that catalyzes the oxidative decarboxylation of malate to yield CO2 and pyruvate with the concomitant production of NAD(P)H [88].The expression of thirty nuclear genes encoding mitochondrial proteins and genes involved in oxidative stress responsebetween implantation failure patients and controls were shown using heat map **Figure 1.**

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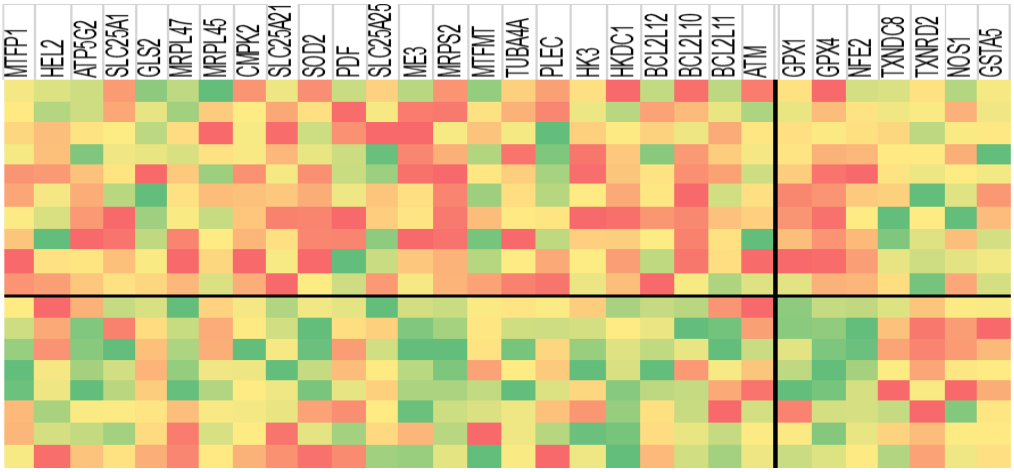
Figure 1: Heat map of nuclear genes encoding mitochondrial proteins (in the left side) and genes involved in oxidative stress response (in right side) expression between implantation failure patients and controls. Each row represents a sample and each column represents a gene. High relative expression is indicated in green and low relative expression in red.



CONCLUSION

The above review supports that mtDNA content is linked to embryo quality. Thus, the quantification of mtDNA copy numbers during the IVF procedure may allow the selection from a given cohort of oocytes those that are more likely to lead to good quality embryos. It would be particularly interesting, from an ethical point of view, to choose the oocytes with the greatest potential for fertilization, thus limiting the production of supernumerary embryos.

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