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Mitochondrial Genetics and its Role in Female Infertility: Molecular Insights"

Dr. Aseel Mahmood Khadim
M.B.Ch.B., C.A.C.M.S. \ (Obstetrician and Gynecologist) Iraqi Ministry of Health, Anbar Health Directorate, Ramadi Teaching Hospital for Maternity and Children, Anbar, Iraq, aseelmhmd1981@gmail.com

Dr. Estabraq Ali Al-Waily
M.B.Ch.B., C.A.B.O.G., D.O.G. \ (Obstetrician and Gynecologist), Fellowship of the Arab Board in Obstetrics and Gynecology, Subspecialty Fellowship in Gynecologic Oncology, Iraqi Board of Medical Specialization Iraqi Ministry of Health, Medical City Directorate, Baghdad Teaching Hospital, Baghdad, Iraq, estabraqalwaily@gmail.com

Dr. Hadeel Azawe Ali
M.B.Ch.B., C.A.B.O.G., D.O.G. \ (Obstetrician and Gynecologist), Iraqi Ministry of Health, Al-Russafa Health Directorate, Al-Alaweha Teaching Hospital, Baghdad, Iraq

Abstract: Mitochondria is the power plants of the cell, of infertility caused by poor quality and aging of oocytes. A factor that is often ignored but that is involved in the decrease in the quality of oocytes are mitochondrial DNA abnormalities, where it is responsible for the production of more than 90% of the adenosine triphosphate (ATP) necessary for cellular function by oxidative phosphorylation. These abnormalities affect the energy production of mitochondria, the dynamic balance of the mitochondrial network, and the pathogenesis of mtDNA diseases in the offspring. The purpose of the current study, we evaluated clinical findings of infertile women in comparison with non-sterile women and analysis the impact of mitochondrial genetics on patients.

A total of 90 participants had enrolled in demographic and clinical outcomes in this cross–sectional study. The current study was divided into clinical data, including mitochondrial, copy number, and pathogenic mutations DNA of 90 cases, where the 60 participants were infertile women, while 30 cases were healthy women. It is also assessed mitochondrial membrane potential in the women.

Our findings showed that the H group of mtDNA got 30.0% of infertile women and 36.7% of healthy women. According to mitochondrial copy number, we found these items of nuclear DNA had different estimations, where infertile women had 125.4 ± 35.2 of peripheral blood and 88.7 ± 28.5 of endometrial tissue, while the healthy women group had 158.9 ± 42.1 of peripheral blood and 121.3 ± 31.8 of endometrial tissue. This study was also domonstrated that primary infertility patients have indicated 33.3% of low mtDNA, while secondary infertility patients have 44.4% of low mtDNA. Furthermore, these findings have shown a low mitochondrial membrane potential of 36.7% for the infertile women group, but 13.3% for the healthy women group.

Our study concludes that there is a positive correlation between each of mitochondria and genetic vulnerability, which have a significantly impact in causing of infertility in women.

Key words: Mitochondria; Female; Infertility; Pathogenesis Of Mtdna; And Mtdna Copy Number.

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Introduction

Mitochondria, known as the ”power plants" of cells for the functions they perform, are cellular organelles that have two bilayer membranes formed by phospholipids. The inner membrane is folded inwards, forming what are known as ridges, in order to have a much larger surface area that guarantees the efficiency of the biological reactions that take place in the mitochondria itself **{1, 2}**. The most important and best delineated function of mitochondria is the generation of adenosine triphosphate (ATP) through oxidative phosphorylation (OXPHOS) thanks to the electron transport chain (ETC) **{3}**.

Also, the energy that is released by the electrons flowing through ETC is used to create a proton gradient across the inner membrane of the mitochondria, so that when the protons enter the intermembrane space, thanks to complexes I, III, and IV, ATP is generated from adenosine diphosphate (ADP) **{4, 5}.** This process releases reactive endogenous oxygen species (ROS) produced by the premature leakage of electrons that become toxic when they exceed normal physiological levels, damaging cells and possibly contributing to aging **{6, 7, 8}**.

Moreover, the functioning of OXPHOS produces most of the endogenous reactive oxygen species (ROS), about 90%, which are involved in many cellular regulatory pathways, but can become toxic when accumulated, where crucial role in energy production and mitochondria played an essential role in the biosynthesis of organic compounds, apoptosis, calcium homeostasis and thermogenesis, as well as in cell signaling pathways and gene expression **{9, 10}**.

It has been estimated that mature oocytes have more than 150 thousand copies of mtDNA. Although mature oocytes with less than 4000 mtDNA copies can be fertilized and normally develop to the blastocyst stage, the threshold of 40 – 50 thousand mtDNA copies is needed for the post-implantation development of mature oocytes **{11}.**

Most cleavage stage embryos with a low mtDNA copy number are unable to complete post-implantation development **{12}.** A negative correlation between maternal age and mtDNA copy number in human oocytes has been reported. Poor oocyte quality in ovarian failure has also been correlated with a low Mitoscore **{13}.**

3. Patients & Methods

3.1. Study Design

A cross-sectional study was conducted on 90 women who provided full written informed consent. Data were collected and analyzed using SPSS version 24.0. All data were collected from women at the fertility center in Thi Qar, Iraq, during a 12-month follow-up period between February 2024 and February 2025, where this study aimed to record and evaluate the outcomes of women who experienced infertility and to analyze the impact of mitochondrial genes on the participants.

According to inclusion and exclusion criteria, the study included only women aged 28 to 36 years with infertility, women who completed all required examinations and administrative procedures, women with both primary and secondary infertility, and some obese women, while following criteria were excluded, women younger than 28 or older than 36 years, women who did not consent to participate, women with incomplete or missing files, and women with an autoimmune disease.

3.2. Sampling Collection of Participants

To achieve the study's objective, the 90 registered patients were divided into two groups to evaluate mitochondrial genes and their role in women. The first group consisted of 60 women diagnosed with infertility, including 42 with primary infertility and 18 with secondary infertility, while second group, the control group, comprised 30 healthy women who were not considered infertile or to have no reproductive disorders, where demographic data had enrolled of including age, body mass index, and type of infertility, that it detected extraction data of DNA/RNA, which biopsies could from all women participating in our study mid-secretory menstrual cycles in EDTA-coated containers using a

commercial silica column-based kit then total RNA extracted from the women's endometrial tissue and processed with DNase I enzyme to remove contamination of the isolated genomic DNA.

4. Results

Based on demographic features, **Table 1** was figured out to almost both groups were closed in age and BMI, 34.6 ± 4.2 and 26.2 ± 4.3, respectively, where only 60 cases of the infertile women group, including primary infertile, had an out of 70% and secondary infertile had 30% in the total infertile women group.

Table 1:- Basics and demographic parameters of 90 participants enrolled in this study.

Characteristic	Infertile women (n=60)	Healthy women (n=30)	Total (N=90)
Age (Years), Mean ± SD	35.3 ± 4.3	32.8 ± 3.9	34.6 ± 4.2
BMI (kg/m²), Mean ± SD	27.2 ± 4.4	25.2 ± 3.3	26.2 ± 4.3
Primary Infertility, n (%)	42 (70.0%)	N/A	42 (46.7%)
Secondary Infertility, n (%)	18 (30.0%)	N/A	18 (20.0%)

Also, we categorized mitochondrial DNA haplogroups in **Table 2**, where the most cases of mtDNA Haplogroup were H to U, which got 31 out of 60 interfile women group, while H to U got just 18 out of 30 healthy women group. Furthermore, our findings noticed the existence of pathogenic mtDNA mutations into patients, and it showed that the presence of any pathogenic mutation had 25% in interfile women, which is higher compared to compare with healthy women of 10%.

Table 2: Classification of mitochondrial DNA haplogroups in the patients.

mtDNA Haplogroup	Infertile women (n=60)	Healthy women (n=30)	p-value
H	20 (33.33%)	12 (40.0%)	0.5
U	11 (18.33%)	6 (20.0%)	0.7
J	9 (15.0%)	3 (10.0%)	0.4
T	7 (11.67%)	5 (16.67%)	0.3
K	5 (8.33%)	2 (6.67%)	0.8
Other Haplogroups	8 (13.33%)	2 (6.67%)	0.6

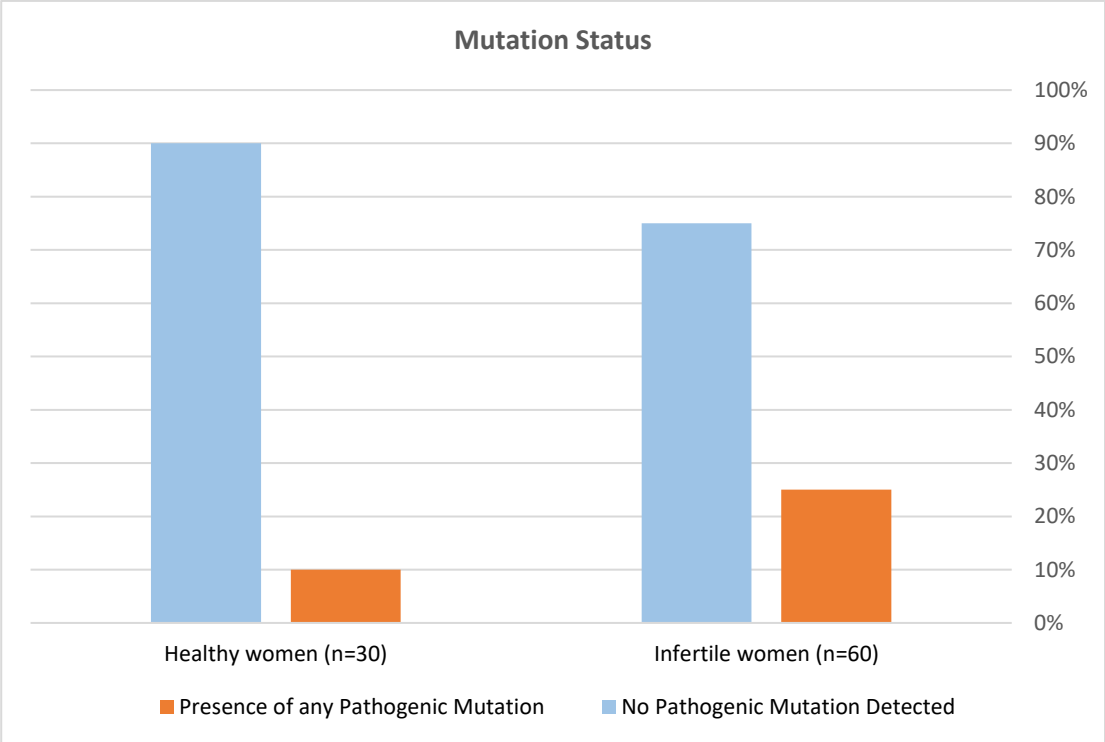

Figure 1: Enroll data of pathogenic mtDNA mutations for 90 patients.

Furthermore, clinical outcomes mitochondrial copy number in **Table 3** had found out in association with nuclear DNA, but we demonstrated that peripheral blood was 125.2 ± 34.8 in interfile women and 157.8 ± 43.5 in healthy women, as well as endometrial tissue found 89.3 ± 27.4 of interfile women and 120.6 ± 33.5 related to healthy women.

Table 3: Determining the copy number of mitochondrial DNA related to nuclear DNA.

Sample Type	Infertile women (n=60)	Healthy women (n=30)	p-value
Peripheral Blood	125.2 ± 34.8	157.8 ± 43.5	< 0.003
Endometrial Tissue	89.3 ± 27.4	120.6 ± 33.5	< 0.002

According to gene expression in **Table 4**, our study indicated to decline of genetic expression into infertile women, which ranged from 0.68 to 0.82, in comparison with healthy women, who had presented high gene expression ranging from 0.96 to 1.09. The decrease in gene expression highlighted the deterioration in the oxidative phosphorylation system, which is considered the main driver of energy production in infertile women. In addition, this study evidenced mtDNA copy number within interfile women in **Table 5**, where 26 cases of primary interfile women had low mtDNA CN <100, but only eight secondary interfile women had low mtDNA CN <100 of the total 60 women. According to **Table 6**, discovered pathogenic mutations, where total cases have mutations involved with 15 interfile women, while only 3 of healthy women, where m.3243A>G was the most common mutation, who prevalence into six out of 60 interfile women, while two out of 30 health women.

Table 4:- Identification levels of gene expression related to mitochondrial genes.

Mitochondrial Gene	Infertile women (n=60)	Healthy women (n=30)	p-value
MT-ND1	0.74 ± 0.23	1.03 ± 0.17	< 0.01
MT-ND4	0.82 ± 0.24	1.09 ± 0.21	< 0.01
MT-CO1	0.68 ± 0.20	0.96 ± 0.15	< 0.01
MT-CO3	0.73 ± 0.22	0.99 ± 0.19	< 0.01
MT-ATP6	0.79 ± 0.25	1.05 ± 0.23	< 0.01

Table 5:- Distribution of mtDNA copy number on the infertile women.

Infertility Type	Low mtDNA CN (<100)	Normal/High mtDNA CN (≥100)	Total
Primary Infertility	26 (65%)	14 (35%)	40 (100%)
Secondary Infertility	8 (40%)	12 (60%)	20 (100%)

Table 6: Determining of discovered pathogenic mutations.

Specific Mutation	Gene	Infertile women (n=60)	Healthy women (n=30)
m.3243A>G	MT-TL1	6 (8.3%)	2 (6.7%)
m.8344A>G	MT-TK	5 (6.7%)	0 (0.0%)
m.8993T>G	MT-ATP6	2 (5.0%)	1 (3.3%)
Other rare mutations	Various	2 (3.3%)	0 (0.0%)
Total with mutations		15 (25%)	3 (10%)

Table 7: Pearson's r correlation in the analysis of the association between mitochondrial parameters and hormonal levels.

Mitochondrial Parameter	FSH	LH	AMH
mtDNA Copy Number	- 0.46	- 0.34	+ 0.53
MT-CO1 Expression	- 0.44	- 0.27	+ 0.49
MT-ATP6 Expression	- 0.39	- 0.26	+ 0.46

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Moreover, our study evaluated mitochondrial membrane potential related to women into both groups in **Figure 2**, which low flow cytometry got 38.33% and normal or high got 61.67% in the interfile women group, while low flow cytometry got 16.67% and normal or high got 83.33% in the healthy women group.

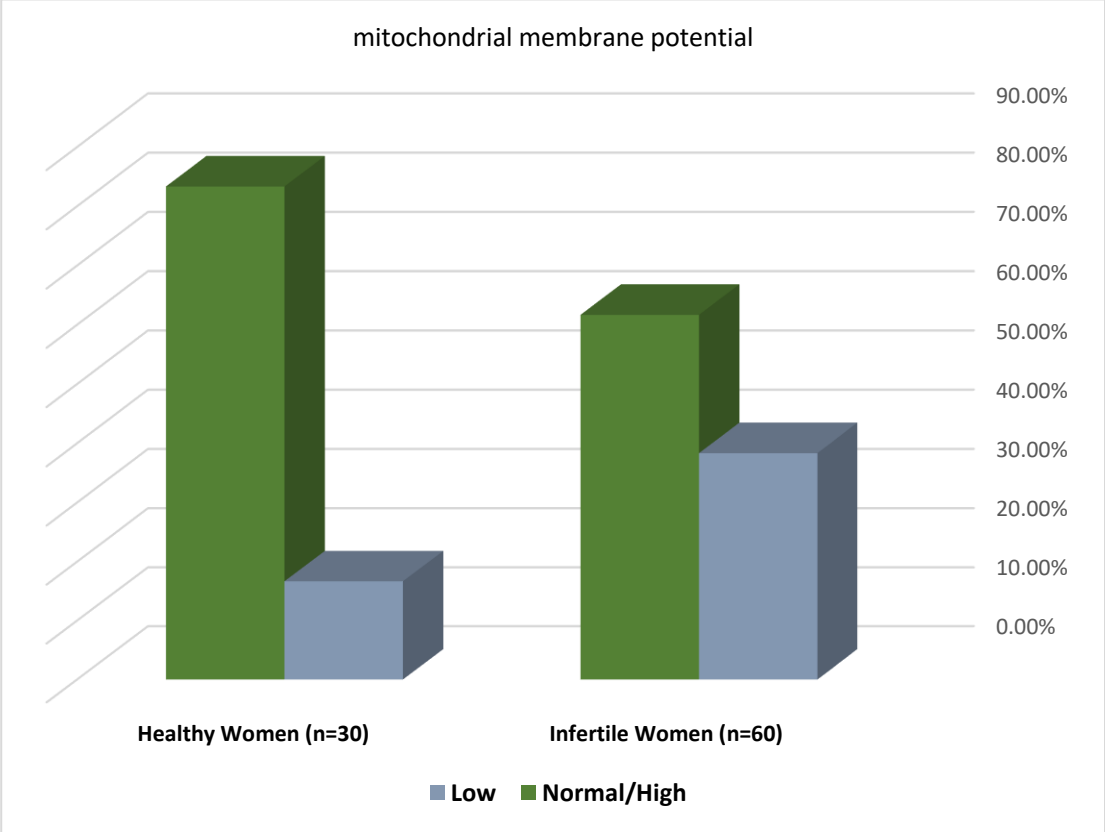

Figure 2: Evaluation of clinical outcomes of mitochondrial membrane potential in the women of both groups.

5. Discussion

Mitochondria are the cause of some cases of infertility. One of the critical factors related to low oocyte quality is the aging of oocytes. The clearest option is usually to opt for donated oocytes or embryos; however, MRT could provide an efficient and complete replacement of the entire cytoplasmic component as well as healthy mitochondria **{14}.**

Haplogroups and health conditions, especially neurodegenerative, endocrine, along with cardiovascular problems, have been extensively researched. Because haplogroup JT is less common among women with poor ovarian reserve characteristics, such AMH as well as antral follicle count, it has been discovered as a possible predictor in favorable ovarian aging in the discipline of reproductive medicine **{15, 16}.**

The results of ovarian stimulation are correlated with mtDNA genotypes. The greater number of (MII) oocytes recovered were favorably correlated with Haplogroup K, but oocyte yield was negatively correlated with global non-synonymous variations in the protein-coding area **{17}.** The existence of non-synonymous homoplasmic variations in the protein-coding area may be detrimental to the oocyte maturation process and associated with a reduced oocyte yield, based to these findings **{18},** mutations found were unique to occur throughout other haplogroups, as well as can be relevant for research into many causes of decreased ovarian response in some families, where considered collectively alongside patients' data imply that the outcomes of reproductive treatments may be influenced by mitochondrial genetic variables **{19}.**

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Some studies found the usefulness and impact of the Mitoscore on the results of ART., it carried out this study with the aim of determining if the mitochondrial score greater than two is associated with a lower clinical pregnancy rate in patients undergoing IVF/ICSI with PGT-A. A total of 410 embryos from our center that met the selection criteria were evaluated. The median Mitoscore was 1.1 (0.73-1.53) **{20, 21},** which a study conducted in Canada of the Mitoscore predicts pregnancy success as well as pregnancy outcomes had better with lower Mitoscore values (<25) with a significantly improved pregnancy rate by 86.8% **{22, 23, 24},** which the upper values on the DNA regression have excessively high and higher than the corresponding coordinates in the rRNA and non-synonymous model, despite the fact that it adjusts effectively to the known incidences of both DNA as well as non-synonymous protein-coding variants within the general population (1.7% for DNA and 15.6% for DNA and non-synonymous). **{25}**

According to previous reports, it has been seen that the addition of Mitoscore to embryo selection criteria protocols can increase the pregnancy rate from 64.4% to 85.2%, which results could imply a significant increase in clinical pregnancy outcomes **{26}.** Another study with 270 embryos compared the Mitoscore and implantation rates in embryos biopsied on day 3 and day This study concluded that a higher amount of DNAmt, as indicated by a higher Mitoscore, is related to lower implantation rates in embryos in both groups **{27}.** Also, it documented that a Mitoscore score equal to or greater than two was associated with a greater number of aneuploid embryos, but not with the morphological quality of the embryo. The above differs from that found by Collazo et al., who documented that the Mitoscore is significantly different depending on the quality of the embryo **{28, 29}.** Also, embryos observed with lower trophectoderm quality showed a tendency to present higher Mitoscore and concluded that alterations in mitochondrial biogenesis can negatively affect the proliferation capacity of the trophoblast, impairing the subsequent differentiation of the trophoblast necessary **{30}.**

6. Conclusion

Our study shows that mitochondrial dysfunction alongside genetic defects are represented as contributing factors strongly associated with ovarian indices that result in infertility, as well as these findings demonstrates that mutations into deoxyribonucleic acid (mtDNA) significantly cause mitochondrial dysfunction, and all these genetic defects lead to a decrease in mitochondrial membrane potential, which negatively affects energy production, which it decreases in mitochondrial membrane potential is present in 38.33% of infertile women and 16.67% of healthy women.

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