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Effects of sperm mitochondrial activity on motility, parameter of human fertility

Alaa Ahmed[1], Thaer M. Farhan[2]

[1]High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al Nahrain University, Baghdad, Iraq.

[2]College of Medicine, Al-Nahrain University, Baghdad, Iraq

alaa.ahmed@ierit.nahrainuniv.edu.iq

ABSTRACT

Mitochondria play a significant key in sperm metabolism as well as power generation. The mitochondria nucleolus is essential for several sperm activities. such as sperm capping, hyperactivation, and acrosome reactions, and oocyte fusion, mitochondrial membrane potential can be used to study sperm mitochondrial activity, the mitochondrial integrity of sperm is evaluated by examination of mitochondrial membrane potential MMP, which may be achieved by using fluorescent dyes like JC-1 probe is composed of 5,5,6,6-tetrachloro-1,1,3,3-tcarbocyanine iodide. To study the correlation between mitochondrial membrane potential Red MMP and sperm motility .

This study included 42 male samples who were separated into two groups. (21 normozoospermic, 21 asthenozoospermia) through the period of attendance at the infertility clinic at High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, and Um Al-Banin Center for Management and in vitro fertilization the collected semen was obtained and seminal fluid analysis was assisted according to (WHO,2021). fluorescent staining of the sample prepared is done by using an MMP Assay Kit (with JC-1) was performed. A positive significant correlation between sperm motility percentage and red MMP intensity ($p < 0.001$). Mitochondrial activity plays an essential function in sperm motility and male fertility

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KEYWORD Male infertility, Sperm Motility, Mitochondrial Membrane Potential, MMP Assay Kit (with JC-1).

1. Introduction

Infertility is defined by the World Health Organization as the failure to conceive after at least a year of unprotected intercourse (WHO 2010 [1]).

According to research, male factors account for 20–40% and female factors for 30–55% of all instances of infertility, respectively. Both male and female factors are responsible for 35% of cases, and only 5–10% of infertility cases have an unclear etiology (Leisegang, 2019[2]). Sperm motility is a necessary condition for Male virility. Research and interest in sperm motility began in 1919, when Lillie Frank Rattray, an American zoologist and author of the book "Problems of Fertilization," discussed the energy metabolism of spermatozoa for the first time. "Once they are fully differentiated, spermatozoa are presumably incapable of acquiring food outside of the gonad; definitely, there is no chance for substance restoration in the event of external insemination." Since then, multiple studies have focused on the cell's "power plant," the mitochondrion, highlighting the importance of this organelle in cellular homeostasis and sperm motility. (Barbagallo et al.,2020 [3]). Increased sperm motility is generally considered to be an important aspect of typical male fertility. Males with immotile or poorly motile sperm are typically sterile unless assisted reproductive technology is used (Turner, 2005[4]). Perhaps the best sperm activity is motility (Darszon et al., 2006[5]). After ejaculation, food molecules in seminal plasma and the environment of the female reproductive canal provide energy to mammalian sperm. Similar to other animal cells, the majority of this energy is transformed into adenosine triphosphate (ATP), and other high-energy molecules and utilized for biological function. In sperm, adenosine triphosphate is needed for a number of processes, such as motility, fusion reactions during the acrosome reaction, and transporting

molecules through membranes in the face of concentration gradients. Adenosine triphosphate is also used in a number of metabolic processes, such as regulatory signaling pathways as a result of which cyclic adenosine monophosphate is formed (CAMP), and phosphorylation of protein kinases (Visconti, 2012 [6]). The primary portion of the tail's fibrous sheath contains the majority of the enzymes required for glycolysis. The mitochondrial gyres in the middle piece, however, are where oxidative phosphorylation takes place. Despite this, spermatozoa from the most well-researched mammals, like humans and lab rats, rely on glycolysis for the generation of adenosine triphosphate (Storey, 2008[7]).

CAMP and calcium are the two primary second messenger chemicals involved in the beginning and control of sperm motility (Luconi and Baldi, 2003[8]). Sperm mitochondria have been linked to sperm motility and fertilization, and several techniques to assess their activity have been developed. A variety of criteria may be utilized to investigate sperm mitochondria. This includes mitochondrial activity, MMP levels, and mitochondrial calcium levels; nevertheless, the processes involved in ATP generation and the functional dynamics of sperm mitochondria are not entirely known., despite the significance of sperm mitochondria to the ability to fertilize. (Losano et al., 2018[9]); (Barbagallo et al.,2020[3]). The fluorescent dye JC-1 has been widely used to assess sperm quality, although under specific experimental circumstances, significant issues have been found, Tetramethyl rhodamine methyl ester perchlorate (TMRM), a different fluorescent dye, is used to measure sperm MMP. They discovered that the accuracy of MMP changes detected by TMRM is similar to that of the commonly used JC-1 staining approach. (Uribe et al., 2017[10]) ;(Barbagallo et al.,2020[3]).

2. Patients and Methods:

This study included 42 male samples who were separated into two groups. (21 normomozoospermia, 21 asthenozoospermia) through the period of attendance at the infertility clinic at High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, and Um Al-Banin Center for Management and in vitro Fertilization the collected semen was obtained and seminal fluid analysis was assisted according to (WHO,2021[11]). MMP Assay Kit (with JC-1) was performed to evaluate sperms motility, active sperms (red)MMP, and inactive sperms(green)MMP.

- **MMP Assay Kit (with JC-1)**

The Mitochondrial Membrane Potential Assay Kit (with JC-1) is established to detect early apoptotic cells by measuring changes in mitochondrial membrane potential with JC-1 as a fluorescent probe. As a positive control reagent, this kit includes carbonyl cyanide m-chlorophenylhydrazone (CCCP)[E-CK-A301C] to cause a reduction in mitochondrial membrane potential. Fluorescent dye formation in mitochondria may be observed and measured using flow cytometry, fluorescent microscopy, confocal microscopy, and a fluorescence screen analyzer (Perry et al., 2011[12]). Similarly, the application of fluorescent ratio identification allows researchers to compare measurements of membrane permeability while simultaneously measuring the degree of mitochondrial depression happening in a diseased situation (e.g., cellular stress, apoptosis, etc.) (Sivandzade et. al., 2019[13]).

- **Principle of detection**

The reduction in the potential of the mitochondrial membrane (MMP) is a mark of the beginning phases of apoptotic. JC-1 is a common fluorescent probe for measuring the membrane potential of mitochondria (MMP). When the membrane permeability has a large potential in

normal cells, JC-1 aggregates the mitochondrial matrix to create a polymer that can produce red fluorescence. The change of JC-1 fluorescence color from red to green may be utilized to identify a reduction in cell membrane potential (MMP), and the transition of JC-1 fluorescence color can be used as an early detection signal of cell apoptosis.

3. Results

Correlation between patient's sperms motility percentage and red fluorescence mitochondrial membrane potentials was a positive significant correlation between patient's sperms motility percentage and patient's red fluorescence mitochondrial membrane potentials (r=0.406; p=0.008) as presented in table 1 and figure 1. The Comparison of mitochondrial membrane potential red fluorescence (Red MMP) according to sperms motility There were also higher and significant red MMP values (p=0.004) in patients with normal sperms motility (10.22 ± 1.54 versus 4.70 ± 0.91 in patients' group with low sperms motility), as demonstrated in Table 2 and Figure2.

(Table 1): Correlation between patient's sperm motility% and Red MMP

	Pearson's correlation coefficient (r)	***p-value***
Correlation between patient's Sperms motility percentage and Red MMP	0.406	0.008 S

S: Significant ($p \leq 0.05$); Red MMP: Mitochondrial membrane potential red fluorescence

Table (2): Comparison of red MMP values according to sperms motility

Parameter Mean ± SD	Normal sperms motility n.=21	Low sperms motility n.=21	p-value
Red MMP	10.22 ± 1.54	4.70 ± 0.91	0.004 Ŧ S

Red MMP: Mitochondrial membrane potential red fluorescence; S: Significant ($p \leq 0.05$); Ŧ: Independent sample t-test

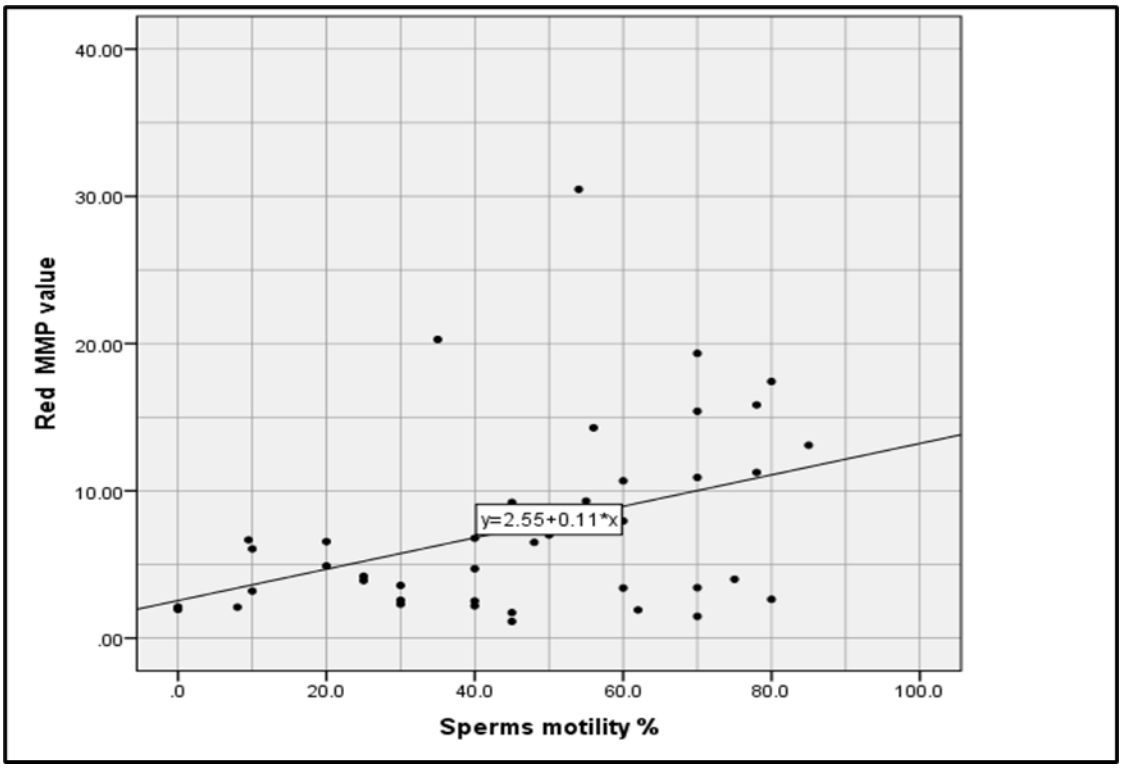
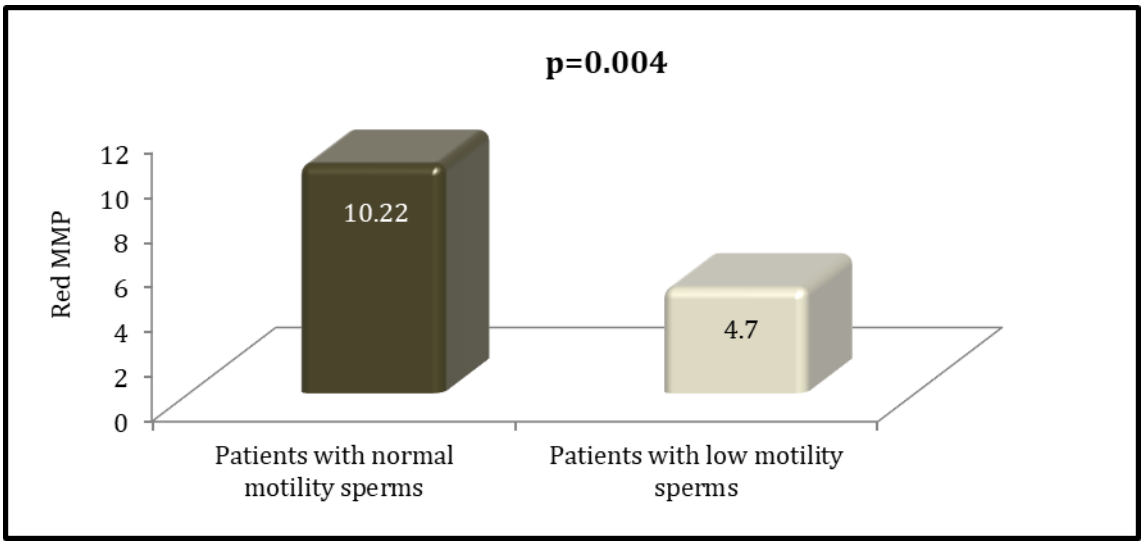


Figure (1): Correlation between patient's sperms motility % and Red MMP



Figure(2): Comparison of red MMP values according to Sperms motility

4. Discussion

Sperm motility is one of the crucial factors needed to achieve a meeting between male and female gamete naturally in order to get fertilization process. Sperm after ejaculation is energized by the ATP, and another high-energy molecule present in semen via glycolysis and oxidative phosphorylation that take place in the mitochondria of sperm (Storey 2008[7]). According to the current study data that correlate sperm motility and sperm mitochondrial membrane potential (MMP) as shown in Figure 1. Table1. Sperm function and malfunction have been linked to mitochondrial dysfunction. Excessive reactive oxygen species (ROS) generation may be caused by mitochondrial malfunction and lead to sperm failure. The researchers believed that analysing sperm metabolic condition and mitochondrial activity status might give new information to supplement standard sperm examination. The findings of the current study are consistent with some other previous studies that emphasize the role of mitochondrial function in

producing ATP for sperm function in humans and mice. (Moscatelli., et al 2017[14]) (Briggiler., et al 2021[15]).

5 . Conclusions

Mitochondrial membrane potential MMP is crucial, for sperm metabolism of energy and important index. Mitochondria are the major regulator of sperm motility.

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Author Contribution

Alaa Ahmed performed the study; Thaer M. Farhan supervised the work .

Conflict of Interest

The authors declare no conflict of interest .

Ethical Clearance

The study was approved by the Ethical Approval Committee.

Financial Disclosure

There is no financial disclosure.

Ethical Clearance

The study was approved by the Ethical Approval Committee

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