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Correlation of Sperm Mitochondrial DNA 7345 and 7599 bp Deletions with Male Infertility in Jordanian Population

دور نقصان الجينوم لميتوكوندريا الحيوان المنوي في عقم الرجال: دراسة للمجتمع الأردني

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Dedication

I dedicate this thesis to my father, who has always been helping me throughout my whole life

To my mother for her patience and prayers

To my brothers and sisters for their support

To every student who wants to be special at somewhere in this whole world

I dedicate this thesis

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List of Abbreviations

Abbreviation	Description
ATP	Adenosine Triphosphate
BMI	Body Mass Index
Cytb	Cytochrome b
COX	Cytochrome Oxidase
E_2	Estradiol
FSH	Follicle Stimulating Hormone
HPG	Hypothalamic-Pituitary-Gonadal
IVF	*In-vitro* Fertilization
LH	Luteinizing Hormone
mtDNA	Mitochondrial Deoxyribonucleic Acid
ND	NADH Dehydrogenase
NADH	Nicotinamide Adenine Dinucleotide Hydride
OAT	Oligoasthenoteratozoospermia
OXPHOS	Oxidative Phosphorylation
PCR	Polymerase Chain Reaction
ROS	Reactive Oxygen Species
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
SHBG	Sex Hormone Binding Globulin
tRNA	Transfer Ribonucleic Acid
TBE	Tris-Borate-EDTA
WHO	World Health Organization

Abstract

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Infertility is known as the inability of the couple to conceive a child after a year of regular coition without using of contraception. Approximately, 17% of couples are suffering from infertility and the male factor accounts for about the half of the cases. Reduced sperm motility (Asthenozoospermia) which defined as the proportion of motile sperm below 40% of total motility, is considered as a major cause of male infertility. The human mitochondrial deoxyribonucleic acid (mtDNA) plays an essential role in the mitochondrial respiration and oxidative phosphorylation (OXPHOS) process that equipping sperm with required energy for their motility. Deletion mutations in sperm mitochondrial DNA (mtDNA) affect the functions of some OXPHOS proteins and as a consequence, sperm motility will be affected. The purpose of this study was to investigate the correlation between 7599 and 7345-bp sperm mitochondrial DNA deletions and asthenozoospermic infertility in the Jordanian population. Semen specimens from infertile and healthy males were collected from the Royal Jordanian Medical Services *In-vitro* fertilization (IVF) units at King Hussein Medical Center and Prince Rashid Ben Al-Hasan Military Hospital. Then the DNA was extracted from

sperm specimens followed by mtDNA amplification. Polymerase chain reaction (PCR) was conducted, and then DNA sequencing was performed for the PCR products. This study indicated that there is a strong correlation between the presence of 7599-bp mtDNA deletion and the reduction in sperm motility (P-value < 0.05). On the other hand, no significant correlation was detected between the presence of 7345-bp mtDNA deletion and having a reduction in sperm motility. In conclusion, screening for deletions in sperm mtDNA can be used as pre-diagnosic molecular mareker for male infertility, which may help in guiding the patients to attend special *In-vitro* fertilization (IVF) techniques like the Intra-cytoplasmic sperm injection (ICSI) or using cryopreservation for sperm, thus, increase the chance of having successful pregnancy in the future. Further investigations are required to study other mtDNA mutations for their asscіation with male infertility.

Keywords: Male infertility, Sperm motility, Asthenozoospermia, Mitochondrial DNA, mtDNA deletions.

Chapter One

1. Introduction

Infertility can be described as the inability of a couple to conceive a child after one year of regular coition without using of contraception (Brugo-Olmedo *et al.*, 2001). It may be attributable to male or female factors (Demain *et al.*, 2017). Approximately, 17% of couples are suffering from this condition (Hull *et al.*, 1985). Among all infertility cases, it tends to result due to male factor in about a half of the cases (Kumar and Singh, 2015).

Male infertility can be investigated by doing the routine seminal fluid analysis as it has been outlined by the World Health Organization guidelines (WHO, 2010). The evaluation of the seminal fluid is generally based on the assessment of its volume, sperm count, liquefaction time, sperm motility and morphology (Wang and Swerdloff, 2014).

Sperm motility is significantly important in the fertilization process. It makes the sperm able to migrate from vagina towards Fallopian tube and penetrate the ovum (Ortega *et al.*, 2011). Reduction in the motility of sperm is called asthenozoospermia which is a major cause of male infertility that characterized by decreasing in sperm motility below 40% (Baker and Clarke, 1987). The rapid forward progressive movement of sperm is relying on mitochondria; which supply the sperm with the required energy through production of adenosine triphosphate (ATP) via oxidative phosphorylation (OXPHOS) process (Amaral *et al.*, 2013).

The human mitochondrial deoxyribonucleic acid (mtDNA) is a 16569 bp circular double-stranded DNA molecule that encodes for 13 polypeptides, two ribosomal ribonucleic acid (rRNAs) and 22 transfer ribonucleic acid (tRNAs), that play an

essential role in the mitochondrial respiration and oxidative phosphorylation (Kao *et al.*, 1998). As well, mtDNA has a large number of direct repeats of different sizes ranging between 4 to 17 base pair (bp) (Ambulkar *et al.*, 2016). Human mtDNA, if compared to the nuclear DNA, has different and unique properties. It replicates rapidly and lacks efficient proofreading, so, the rate of mutations will be higher than the nuclear DNA up to 17 times (Pandey *et al.*, 2012). Moreover, it lacks efficient DNA repair mechanism (Kao *et al.*, 1995). Lacking DNA repair mechanism makes the mtDNA more exposed to the attack of free radicals or by reactive oxygen species (ROS) (Aitken and Clarkson, 1988). Furthermore, increased rate of nucleotide deletions and mutations in sperm mtDNA affects sperm development and as a consequence will affect flagellar movement.

The most common and widespread mtDNA deletion is the 4977-bp deletion, which tends to affect 40% of individuals with mitochondrial dysfunctions (Shamsi *et al.*, 2008). Some studies in different populations reported a correlation between the 4977-bp mtDNA deletion and asthenozoospermia condition (Kao *et al.*, 1995), whereas in some other populations, there was no correlation between the 4977-bp deletion and the abnormalities in count, morphology or motility of spermatozoa (John *et al.*, 2001). However, the results of studying the role of this deletion in asthenozoospermia are still contradictory.

Moreover, there are other mtDNA deletions that showed an association with poor sperm motility; for instance, 7599 and 7345-bp deletions have been studied by Kao *et al.* (1998), they reported a significant correlation between these mtDNA deletions and poor sperm motility. However, Talebi *et al.* (2018) reported that the 7599-bp mtDNA deletion was associated with asthenozoospermia, and no correlation was detected between the 7345-bp mtDNA deletion and reduced sperm motility (Talebi *et al.*, 2018)

Asthenozoospermia is considered as a main cause of male infertility (Marmor and Grob-Menendez, 1991). Thus, screening of human mitochondrial DNA for mutations can help in the molecular diagnosis of male infertility. Therefore, the association of 7599 and 7345-bp human mitochondrial DNA deletions will be studied to investigate the impact of these deletions on male infertility in the Jordanian population.

1.2 Rationale

Male infertility is considered as a serious condition affecting many couples in Jordan. Sperm motility is a fundamental factor for a successful fertilization process, thus the reduced sperm motility (Asthenozoospermia) is a major cause of impaired male fertility. Asthenozoospermia may occur as a consequence of mitochondrial DNA deletions, so the screening of mitochondrial DNA for deletions can help in the early diagnosis of male infertility and promote the identification of the etiologies behind the reduced sperm motility.

1.3 Objectives

1. To evaluate the incidence of the 7599 and 7345-bp mtDNA deletions among asthenozoospermic infertile Jordanians.

2- To investigate the correlation between the 7599 and 7345-bp sperm mitochondrial DNA deletions and asthenozoospermia-related infertility.

3- To evaluate the use of this correlation as a molecular marker for male infertility in the Jordanian population.

Chapter Two

2. Literature review

2.1 Spermatogenesis

Spermatogenesis is a multi-step process of germ cells division and differentiation that takes place in the seminiferous tubules in the testes which comes up with the producing of mature male gametes (spermatozoa) (Johnson, 1995). This process is divided into three main steps (Figure 1): mitosis division for duplication of spermatogonia, followed by meiosis, which begins when the type B spermatogonia enter the prophase of the first meiotic division, the chromosome number is reduced to haploid from diploid and results in the production of new cells called primary spermatocytes which divide to produce secondary spermatocytes that continue with the second meiotic division to end up with round spermatids formation. The last step, spermiogenesis, includes a transformation of the round spermatid to form the complex structure of the mature spermatozoa (De Kretser *et al.*, 1998).

The only somatic cells that are found in the seminiferous tubules called Sertoli cells, which maintain the testicular function and formation, as well spermatogenesis process control (Griswold, 1998). Spermatogenesis process is under hormonal control, which is the main rule of Sertoli cells in this process. They provide the indispensable factors for the successful spermatogenesis by the presence of follicle stimulating hormone (FSH) and testosterone hormone receptors which are the essential hormones for spermatogenesis regulation (Sofikitis *et al.*, 2008).

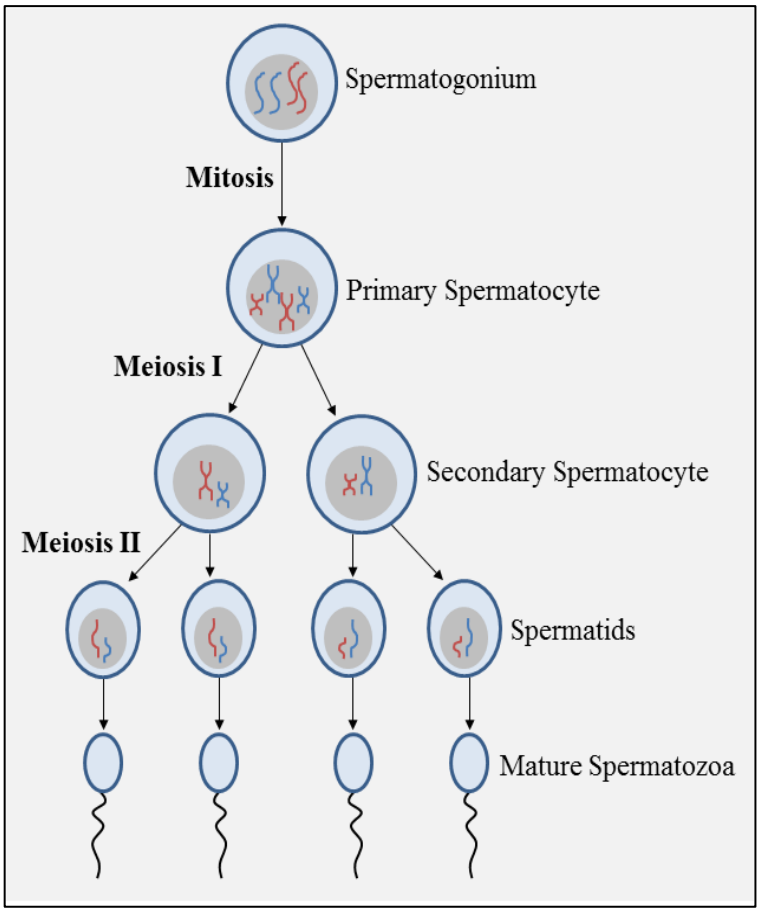

Figure 1: Stages of spermatogenesis process. It divides into three major steps; mitosis followed by the first meiotic division followed by a second one to generate the spermatids that will be differentiated into mature sperm. Adapted from (Alberts *et al.*, 2002).

The presence of a group of cells called leydig cells adjacent to the seminiferous tubules contributes in the spermatogenesis process by providing Sertoli cells with testosterone (Neaves, 1975).

2.2 Structure of the mature human spermatozoon

The mature human spermatozoon has a unique and well-defined structure that consists of three main parts; the head, tail and a small part located in the upper portion of the tail called the middle piece (Figure 2). The head is oval in shape of a length ranges from 4 to 5 µm and an average width of 3 to 3.3 µm. The middle piece is of 3.5 to 4 µm length, and l µm width. Whereas the tail length is ranged from 40-60 µm (Duijn, 1952).

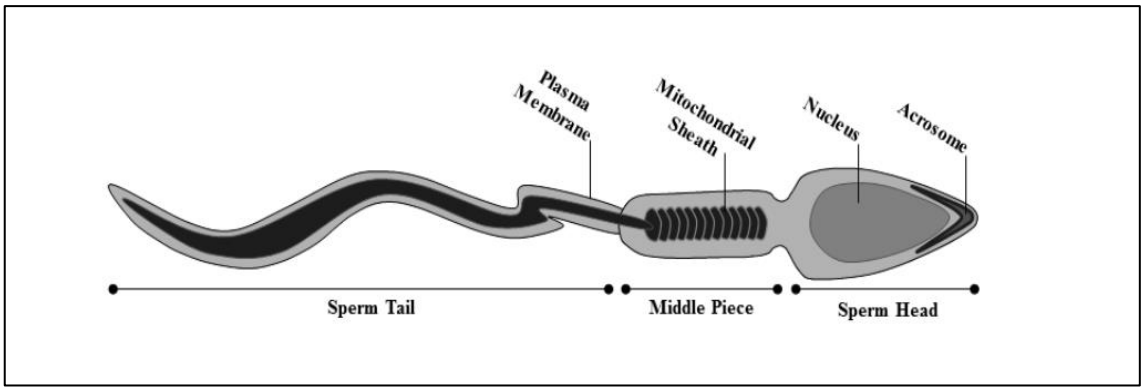

Figure 2: Structure of the mature spermatozoon. Adapted from (Borg *et al.*, 2009).

The head of the spermatozoa consists of a haploid nucleus contains a highly condensed genome, and surrounded by a cap-like structure called the acrosome; the connection point between the spermatozoon and the ovum during the fertilization process by the secretion of hydrolytic enzymes, mainly hyaluronidase and acrosin which help in the breakdown of the outer membrane of the ovum (zona pellucida) (Chavarria *et al.*, 1997).

The middle piece is the energy supplier for the sperm tail owing to presence of the mitochondria that are well-coordinated to make a compressed sheath around longitudinal fibers of the spermatozoon's tail, it is believed that they are responsible for the motility of the sperm (Fawcett, 1975). The normal movement of the sperm has to be progressive and directional, so the sperm tail generates about 10 beats per second generating a wavy movement from the end to the top of the tail and driving the sperm head forward (Wallach *et al.*, 1980). This pattern of movement requires quite large amounts of energy which provided by the mitochondria as the form of adenosine triphosphate (ATP) via oxidative phosphorylation process (Gibbons, 1983).

In the middle piece there are about 50-75 mitochondria and a single copy of mitochondrial deoxyribonucleic acid (mtDNA) for each mitochondrion (Hecht *et al.*, 1984).

2.3 Sperm abnormalities

According to the WHO guidelines, the lower reference values for seminal fluid assessment including a seminal fluid volume of 1.5ml/ejaculate, sperm count of 15 million/ml (Normal count: 15-200 million/ml), total motility of 40% and about 5% of sperm with normal morphology (WHO, 2010).

Infertility due to male factor may be associated with anomaly in either sperm count which referred as oligozoospermia, motility which is asthenozoospermia or may be a result of abnormal sperm morphology which known as teratozoospermia. The presence of these abnormalities in sperm is clinically known as oligoasthenoteratozoospermia (OAT) which is responsible for the majority of male infertility cases (Kobayashi *et al.*, 2012).

The majority of oligoasthenoteratozoospermia (OAT) cases result due to idiopathic testicular dysfunction (Hirsh, 2003). There is a hormonal imbalance associated with idiopathic OAT, mainly testosterone and FSH which are principle sex hormones in spermatogenesis process regulation. Decreasing of serum testosterone as well as elevated serum FSH levels was observed in patients with idiopathic OAT (Cavallini, 2006).

Reduction in sperm count down to the lower reference value (15 million/ml) following WHO criteria which termed as oligozoospermia and is considered a challenge in

conceiving (WHO, 2010). Most commonly, it is a result of reproductive idiopathic spermatogenic disorders or may result due to systemic disorders (McLachlan, 2013).

Likewise, reducing sperm motility, or asthenozoospermia, is considered one of the most common causes of reduced male fertility, the absence of motility (complete immotile spermatozoa) was reported in 1 of 5000 infertile males (Ortega *et al.*, 2011). The fertilization efficiency depends on the movement style of spermatozoa; if the sperm move in circular way it cannot swim towards the ovum, while straight forward moving sperm can reach the ovum in the fertilization process and so on (Wallach *et al.*, 1980).

Asthenozoospermia was observed in males with defects in seminiferous tubules, thus reduced spermatogenesis capacity. Also, abnormalities in the middle piece of the spermatozoa middle piece; length of the middle piece, abnormal arrangement of mitochondria or even the absence of mitochondria is associated with asthenozoospermia (Pedersen *et al.*, 1971).

Sperm motility is classified into three major types: progressive motility (straight forward or large circular movement), non-progressive motility (small circular or the tail barely withstand moving the head) and immotile (complete absence of motility) (WHO, 2010). Studies reported that there is a significant association between sperm good motility and successful pregnancy rate in comparison with those compared to those correlated with poor motility (Bostofte *et al.*, 1983).

On the other hand, sperm may come with abnormalities in head, neck and middle piece or tail (Figure 3). As well, spermatozoa may have all morphological defects together (Kruger *et al.*, 1986). Previous studies indicated that the reduction in the normal morphological sperm percentage is significantly associated with the decline in the fertilization rate (Kruger *et al.*, 1993).

The head of sperm is considered abnormal if it was small or large, tapered, pear-shaped, rounded, amorphous or if the head contains vacuoles. Likewise, if there was any erroneous insertions of the middle piece inside the head or if it was thick, sharply bent or thin or cytoplasmic droplets may present so there is a defect in the middle piece. If the tail was duplicated, short or broken or come with smooth hairpin bends or coiled and have an irregular width it considered abnormal (Baker and Clarke, 1987).

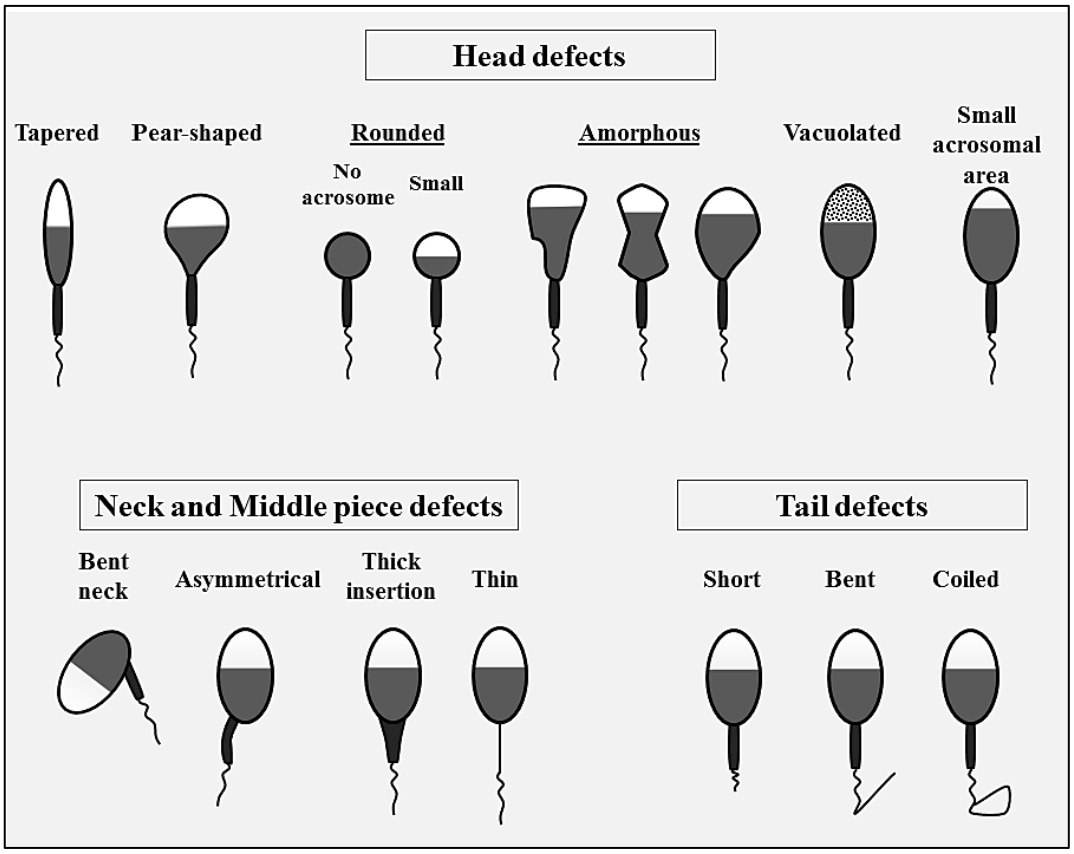

Figure 3: Schematic drawings of the major defects of human spermatozoon. Adapted from (Kruger *et al.*, 1993).

2.4 Impact of drugs on male fertility

Some drugs may affect the fertility rate in males. There are some mechanisms through which drugs may impair male fertility: direct gonado toxic effect on spermatogenesis through germ cell destruction or by altering Sertoli cells function. Commutation of the

hypothalamic-pituitary-gonadal (HPG) axis, hormonal treatments, anabolic steroids and psychotropic medications as well may interact with the feedback mechanisms of the HPG axis, which may lead to affect the levels of testosterone or gonadotropins. Moreover, erectile impairment may associate with drugs that interfere with neurological or vascular-mediated events needed for an ordinary erectile process (Thompson, 1993).

On the other hand, some commonly used antibiotics were studied to investigate their impact on male fertility. Gentamicin, spiramycin and penicillin G-potassium were used; all of them showed an association with halting the spermatogenesis process and each one was affected the process at different stage (Timmermans, 1974).

Furthermore, some chemotherapeutics that used in cancer treatment showed an association with the reduced fertility rate of the male patients (Nudell *et al.*, 2002). Patients with lymphoma for example and exposed to some chemotherapeutic agents for treatment at the different stages of the cancer their fertility was remarkably reduced, following chemotherapy, cases of oligozoospermia were observed, germ cells or Sertoli cells damage become susceptible to damage too. Also, abnormal levels of hormones; reduced testosterone level and elevation in luteinizing hormone (LH) and FSH levels were detected following treatment (Tal *et al.*, 2000). Thus, sperm cryopreservation which is a clinical procedure to conserve sperm cell for a long time is considered as the method of choice for male patients who diagnosed with cancer; this method takes place prior to treatment (Botchan *et al.*, 2013).

2.5 Effect of obesity on male fertility

Obesity is defined as a medical condition associated with increased weigh, body fat or elevation of the body mass index (BMI) which is the measure for the body weight in relation to height. There is a significant correlation between obesity and increased BMI

with the health status of the person including fertility, furthermore, the prevalence of obesity is reaching epidemic levels in many regions worldwide (Hammoud *et al.*, 2006). The ideal BMI upon the most studies is between 20 and 24.9 (Nuttall, 2015).

Alterations in spermatogenesis process may result due to obesity. Reduced sperm count and decreased quality of the seminal fluid were associated with elevated BMI. Moreover, reproductive hormones levels showed an association with BMI; testosterone and sex hormone-binding globulin (SHBG) levels were remarkably decreased in males with high BMI, whereas elevated levels of free androgen index and estradiol (E_2) associated with increasing BMI (Jensen *et al.*, 2004).

2.6 Effect of age on male fertility

It was suggested that the reproductive functions will progressively decrease with advanced paternal age for different causes. That decline may include decreasing in the quality of seminal fluid, reduction in the sperm fertilization potential, genetic defects in sperm or elevated risk of abnormalities in the offspring (Crosnoe and Kim, 2013).

Among a variety of studies on the impact of male age on the fertility status, by comparing the subjected males of ages between 30 and 50 year-old, reducing in the volume of semen from 3% to 22%, reducing in the percentage of morphologically normal sperm from 4% to 18% and reducing in sperm motility between 3% to 37% (Kidd *et al.*, 2001).

Moreover, several previous studies reported a relationship between increased age and the incidence of DNA damage of sperm cells, which may be due to increased DNA double strand breaks and decreased apoptosis rate during spermatogenesis. DNA double strand breaks were detected using neutral micro-gel electrophoresis (comet) assay

(Singh *et al.*, 2003), which is a sensitive fluorescence microscopic method allows to detect DNA double strand breaks in an individual cell and estimate its distribution in cell populations (Wojewódzka *et al.*, 2002).

On the other hand, it was proved that the presence of higher frequencies of some abnormalities such as aneuploidy and single point mutations as the paternal age increased (Kühnert and Nieschlag, 2004). Germ cells in males divide continuously. In estimation, before puberty, 30 spermatogonial stem cell divisions occur, when they begin their meiotic divisions. Thereafter, 23 mitotic divisions happen in each year, so, by increasing of the age the chromosome replications will increase, thus, elderly males may have an elevated risk of having errors in DNA copies (Crow, 2000).

Another study of the kinetics of the spermatozoa for males with ages between 22 and 80 years, the quantitative analysis of sperm movement mentioned that by increasing of male age, few motile sperm produced and they move in a curvy path instead of forward way, as a result, distance that sperm travelled per unit time was decreased, and thus reduced fertilization potential (Sloter *et al.*, 2006).

2.7 Impact of oxidative stress on male fertility

Oxidative stress is defined as inability of the body to detoxify and/or neutralize the effect of the reactive oxygen species (ROS) which are free radicals and highly reactive oxidizing agents; superoxide anions (O_2•), peroxyl (ROO•), hydrogen peroxide (H_2O_2), and hydroxyl (OH•) radicals (Aitken *et al.*, 1992).

ROS are major source of DNA damage, and then the accumulation of oxidative stress on the cells will produce sperm cells with defects in their function due to DNA damage which might be a cofactor behind the occurrence of male infertility (Bisht *et al.*, 2017).

The oxidative stress resulted by ROS affects DNA integrity due disruption of proteins and lipids of the sperm cell plasma membrane (Aitken, 2013).

It was shown that the oxidative stress affects sperm function by reducing their motility. That phenomenon was observed by John Macleod in 1943. He found that, by incubating human spermatozoa in high oxygen tension environment they lost their motility, that mechanism could be frustrated by catalase which is an enzyme has the ability to decompose H_2O_2 (MacLeod, 1943).

Sperm motility is achieved by ATP generated from mitochondria via oxidative phosphorylation. This mechanism begins with a redox reaction between oxygen and hydrogen where generates ROS are generated as byproducts. Elevated levels of ROS affect sperm in two ways: Firstly, oxidation of lipids of plasma membrane of spermatozoa affecting their motility and accordingly, their capacity to penetrate the outer membrane of the ovum. Secondly, damaging DNA and ribonucleic acid (RNA) of spermatozoa which affecting the proportion of the embryo from the paternal genome (Valko *et al.*, 2007).

Moreover, previous studies reported an impact of cigarette smoking on male fertility through their role in the inducing of oxidative stress. Cigarettes contain many harmful chemical substances and carcinogens that tend to generate an oxidative stress and therefore affecting male fertility. It was found that 8-Oxo-2'-deoxyguanosine (8-oxo-dG) which is a major form of DNA oxidation and its concentration reflects the intensity of the oxidative stress, cigarette smokers have an elevated level of 8-oxo-dG in sperm DNA which lead to induce sperm DNA damage and thus affecting male fertility (Shen *et al.*, 1997). In addition, a relationship was found between alcohol consumption and

decreased testosterone level and semen volume, and consequently male fertility will be affected (Bisht *et al.*, 2017).

Therefore, to reduce and inactivate the effect of ROS, an antioxidant scavenging systems will take place here in addition to many antioxidant therapies and a blend of agents, such as vitamins C and E, also selenium and zinc which are commonly used as treatments of male infertility (Keskes-Ammar *et al.*, 2003).

2.8 The human mitochondrial DNA (mtDNA)

Mitochondrial DNA (mtDNA) was discovered by electron microscopy in the middle of 1960s by Margit and Sylvan Nass, by applying special staining procedures they found that within mitochondria there are fibrous structures contain nucleic acid which is a second genome and they reported that these structures as mitochondrial DNA (Nass and Nass, 1963). The whole sequence of the human mtDNA was first identified in 1980 by (Anderson *et al.*, 1981).

Human mitochondrial deoxyribonucleic acid (mtDNA) is a circular and double-stranded (Guanine-rich heavy strand and Cytosine-rich light strand) DNA molecule of 16569-bp size (Figure 4). It is composed of thirty seven genes; thirteen genes coding for proteins which are the building blocks for oxidative phosphorylation (OXPHOS) process, also two genes coding for rRNAs (16S and 12S) and another twenty two genes coding for tRNAs (Kao *et al.*, 1998).

Oxidative phosphorylation (OXPHOS) is an essential process to supply cells with required energy requisite for metabolism in the form of ATP; this process is achieved by mitochondria. All thirteen polypeptides encoded by mtDNA are components of

mitochondrial electron transport chain at the site of oxidative phosphorylation which occurs in the inner membrane of the mitochondria (Smeitink *et al.*, 2001).

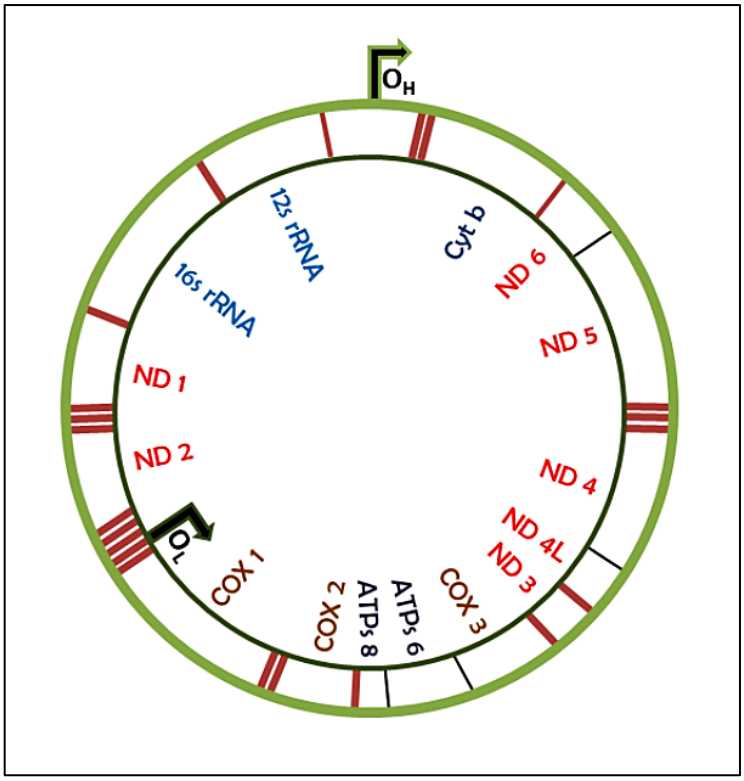

Figure 4: The human mitochondrial DNA (mtDNA). The genome consists of 37 genes coding for 13 polypeptides, 22 tRNA, and 2 rRNA (16S and 12S). The starting points of replication on the heavy strand (O_H) and the light strand (O_L) are indicated. Adapted from (Phillips *et al.*, 2014)

There are five complexes composed of subunits that construct OXPHOS; seven subunits of complex I or Nicotinamide Adenine Dinucleotide (NADH) dehydrogenase; ND-1, ND-2, ND-3, ND-4, ND-4L, ND-5, and ND-6, which is the first protein in the OXPHOS process, four subunits of complex II (Succinate dehydrogenase) which is the only complex that encoded by nuclear DNA, Cytochrome b (Cytb) subunit which is a part of complex III (Cytochrome c reductase), three subunits of cytochrome oxidase (COX) which are related to complex IV and finally two subunits of complex V (ATP

synthase 6 and 8) which is the final enzyme in the OXPHOS process (Bornstein *et al.*, 1998).

The human mtDNA has unique properties when compared to nuclear DNA. It lacks introns. Moreover, the replication rate is much higher than nuclear DNA, without proofreading or repair systems, therefore, the rate of mutations is higher than the nuclear DNA (Pandey *et al.*, 2012).

2.9 Mitochondrial DNA deletions and male infertility

It was reported that the motility of spermatozoa is substantially dependent on the mitochondrial OXPHOS process; by experiments, suppression of the OXPHOS activity, via inhibition of its mtDNA coded complexes using specific chemicals, the motility of sperm was blocked and therefore male fertility will be decline (Ruiz-Pesini *et al.*, 2000).

Several mtDNA deletions were documented to be highly correlated to male fertility where poor sperm motilities were evident. Of these, 4977-bp deletion is the most former and is the most common (Guo *et al.*, 2017). Kao *et al.* (1995) reported that there is a significant association between the existence of 4977-bp deletion and males with asthenozoospermia (Kao *et al.*, 1995).

Moreover, the 4977-bp deletion is responsible for losing of seven structural genes from mitochondrial genome, these genes are: ND-5, ND-4, ND-4L, ND-3, also cytochrome Oxidase COX III, ATPase 6 and ATPase 8, 5 tRNA genes are deleted too (Figure 5) (Kao *et al.*, 1998). The site of the common deletion (4977-bp) is between 8469 and 13447-bp, and there is one direct repeats of 13 bp (5'-ACCTCCCTCACCA-3') at both sides of the deletion (Chen *et al.*, 2011).

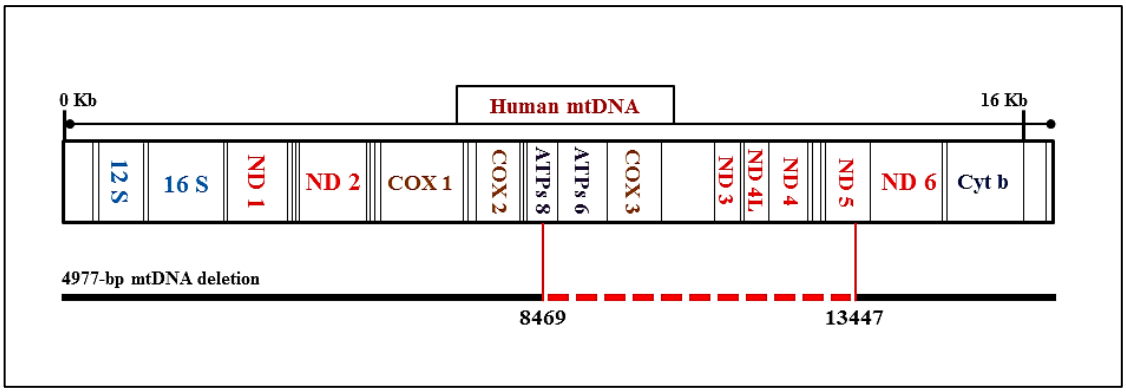

Figure 5: The 4977-bp (common deletion) within the whole mtDNA. Deleted genes are indicated between the vertical red lines. Adapted from (Kao *et al.*, 1998).

The mtDNA 4977-bp deletion was studied in patients with asthenozoospermia compared to those who have spermatozoa with normal high motility, by application of various Percoll gradients in order to separate spermatozoa in respect to their motility percentage and using of polymerase chain reactions to investigate the presence of that mtDNA deletion, as a result, it was reported that the occurrence of 4977-bp deletion was proportionally associated with spermatozoa of reduced motility percentage compared to normal motile spermatozoa (Kao *et al.*, 1995).

The mitochondria were studied as energy production organelles and its role in the motility of spermatozoa was investigated. A previous research in Italy was done to study the relationship between mtDNA mutations and reduced male fertility. They classified patients into groups according their sperm motility and in order to link sperm motility with their mitochondrial functionality, they used a special technique that reflects the respiration functionality of the spermatozoa. As a result, they found that the functional oxygen-consuming organelles were solely found in the motile spermatozoa. Moreover, by the analysis of immotile and motile sperms, the results showed that the

immotile sperms have mitochondrial mutations, thus, presence of mtDNA deletions is significantly associated with reduced male fertility (Carra *et al.*, 2004).

The overall content of sperm mtDNA has been studied by Kao *et al.* (2004), patients with reduced sperm motility and males who have normal motile sperm were examined for their movement properties using a special computer-assisted seminal fluid analyzer. Then, the content of mtDNA in spermatozoa of both groups was determined by PCR and the mass of mtDNA was analyzed as well. The results indicated reducing in sperm mtDNA content in asthenozoospermic individuals compared to males who have normal motile sperm, which as a consequence, associated with impaired male fertility potential (Kao *et al.*, 2004).

However, a previous study reported that the presence of 4977-bp mtDNA deletion in spermatozoa does not reflect reduced male fertility. However although, presence of mtDNA deletions is responsible to induce testicular dysfunction, normozoospermic males were reported to have considerable levels of the 4977-bp mtDNA deletion, so the assessment of the existence of mtDNA deletions in spermatozoa is inadequate for the characterizing of male infertility (Cummins *et al.*, 1998).

Furthermore, another two mtDNA deletions; 7599 and 7345-bp deletions were investigated and their role in male infertility was studied. The mtDNA 7599-bp deletion is located between 8642 and 16243-bp and characterized by the presence of 7 nucleotides direct repeats (5'-CATCAAC-3') at its both sides. Wherease, 7345-bp mtDNA deletion lies between 9009 and 16354-bp (Kao *et al.*, 1998). The molecular approaches have discovered the association between 7599 and 7345 bp mtDNA deletion with some mitochondrial genes functionality; ATPase8 (lost with 7599-bp only), ATPase 6, Cytochrome Oxidase (COX) III, Cytochrome b, NADH Dehydrogenase

(ND) 3, 4, 4L, 5, and 6. Also, those deletions include losing of 8 tRNA genes (Figure 6), all of these genes play a significant role in the construction of mature sperm and responsible for the progressive flagellar movement after ejaculation (Talebi *et al.*, 2018).

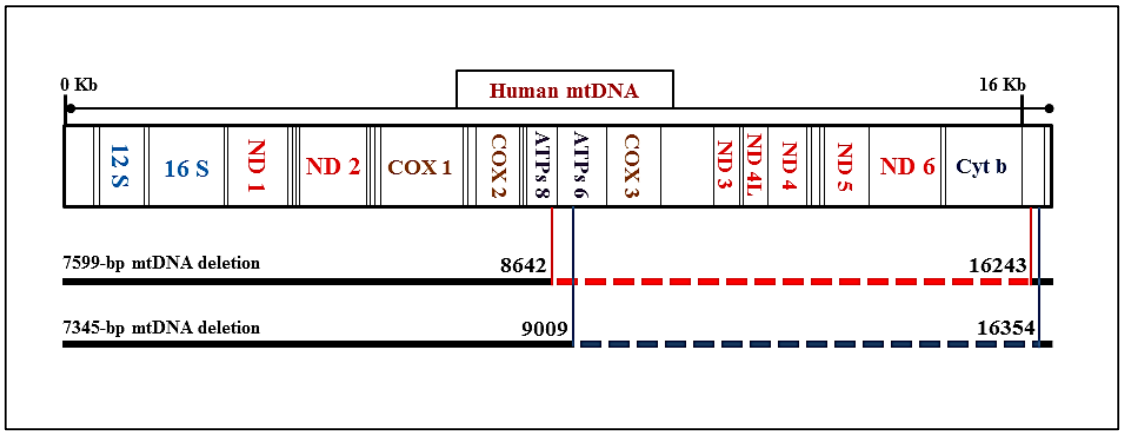

Figure 6: Locations and deleted genes within 7599 and 7345-bp mtDNA deletions. The sites of both deletions and the lost mtDNA genes appear; 7599-bp mtDNA deletion is shown between the two red lines, whereas 7345-bp mtDNA deletion is indicated within the blue lines. Adapted from (Kao *et al.*, 1998).

The impact of 7599 and 7345-bp mtDNA deletions were studies in several populations, one of the first studies was done by Kao *et al.* (1998), they investigated the role of these deletions in male infertility. They found that the prevalence of deletions in mtDNA was significantly elevated in patients with asthenozoospermia compared to males who have normal motile sperm (Kao *et al.*, 1998).

Moreover, another study on the impact of the presence of 7599-bp mtDNA deletion on sperm motility and male fertility was done by Hosseinzadeh Colagar and Karimi (2014). They proved that the existence of that large scale mtDNA deletion was markedly associated with reduced sperm motility because it is responsible for losing of some mitochondrial genes that are responsible for mitochondrial respiration which provides

sperm with required energy for their motility and consequently, affecting male fertility (Hosseinzadeh Colagar and Karimi, 2014).

Also, the role of the presence of another mtDNA deletion of 7436-bp in reduced sperm motility was investigated in 2016 in India. Semen specimens of patients with asthenozoospermia and males who have normal motile sperm were studied, sperm specimens were fractionated according to their motility percentage, and then by application of PCR technique to check for the presence of the deletion, they confirmed that the presence of 7436-bp mtDNA deletion was linked with reduced sperm motility (Ambulkar *et al.*, 2016).

The correlation of mtDNA 7599 and 7345-bp deletions with diminished male fertility were studied recently in Iranian population. Sperm samples from infertile males and fertile males were collected, then the patients samples were classified into four groups according to their seminal and sperm characteristics, and then spermatozoa were separated using osmotic shock procedure (elimination of contaminants) then the presence of deletions was detected using PCR. As a result, they found that 7599-bp mtDNA deletion was present in patients with asthenoteratozoospermia and oligoasthenoteratozoospermia (OAT), which led them to a conclusion that this deletion could be considered as molecular diagnostic parameter to investigate diminished sperm quality. In contrast, the 7345-bp deletion did not show a correlation with sperm motility (Talebi *et al.*, 2018).

Furthermore, the association of the existence of 7491-bp mtDNA deletion in spermatozoa of asthenozoospermic males with reduced male fertility was studied by John *et al.* (2001). They included an additional step following the separation of spermatozoa using different Percoll gradients; magnetic beads coated with special

antibody were used in order to get rid of leukocytes which considered as contaminants of sperm specimens. As a result, they suggested that there was no association between these mtDNA deletions and sperm count, morphology or motility (John *et al.*, 2001).

Chapter Three

3. Materials and Methods

3.1. Specimens collection and semen analysis

Semen specimens from 121 patients diagnosed with asthenozoospermia of ages between 18 and 40 years were collected and divided into six groups according to the motility percentage of sperm motility as the following: group one includes patients with 0% sperm motility (completely immotile), group two includes patients with 1% to 5% sperm motility, group three involves patients with 6% to 10% sperm motility, group four includes patients with 11% to 15% sperm motility, group five comprises patients with 16% to 20% sperm motility, finally, group six includes patients with 21% to 35% sperm motility.

By taking into consideration, patients who drink alcohol and who smoke cigarettes in addition to patients who have varicocele and whom are aged more than 40 years were excluded from the study (Ambulkar *et al.*, 2016).

Semen specimens from 79 men with normozoospermia (high percentage of sperm motility) were collected as a control. Similarly, samples were also subcategorized according to the percentage of sperm motility into three groups as follow: group one includes samples with percentage of 50% to 55% sperm motility, group two involves samples with 56% to 60% sperm motility and finally, group three includes samples with 61% to 75% sperm motility (Bahrehmand Namaghi and Vaziri, 2017).

Semen specimens were collected from the Royal Jordanian Medical Services *In-vitro* fertilization (IVF) units at King Hussein Medical Center and Prince Rashid Ben Al-

Hasan Military Hospital, where infertile patients provide their seminal fluid for evaluation. Seminal fluid specimens from all subjects were obtained by masturbation after three to five days of sexual restraint. Semen specimens were collected in a special sterile, wide-mouth and non-toxic container and placed in the lab. Semen specimens were incubated at 37°C to allow liquefaction to occur for 30 min. Then, semen specimens were evaluated by a senior clinical embryologist following the guidelines of WHO laboratory manual for the examination and processing of human seminal fluid (WHO, 2010).

Semen specimens were fractionated using Percoll media (45% and 90% gradients) at 1000×g for 20 minutes. After that the pellets were collected and washed two times with sperm washing medium to get rid of percoll remnants, then spermatozoa were collected by centrifugation using microcentrifuge at 670×g for 10 minutes. Finally, the pellet that contains spermatozoa was kept at −20°C until DNA extraction (Ieremiadou and Rodakis, 2009).

3.2 Sperm mtDNA amplification

The total DNA was extracted using QIAamp DNA Mini Kit (QIAGEN) following the manufacturer instructions. After that, the mitochondrial DNA of spermatozoa was amplified using REPLI-g Mitochondrial DNA Kit (QIAGEN), following the manufacturer recommendations. Then, the density ratio for amplified mitochondrial DNA from all specimens was measured, and specimens that have an optimal density ratio (260/280) close to or equal 1.8 were chosen and then stored at − 20°C.

3.3 Polymerase Chain Reaction (PCR)

3.3.1 Amplified mtDNA confirmation

Polymerase chain reaction (PCR) to confirm the occurrence of the mtDNA in the extracted genome reaction was prepared in 30 µl total volume containing 15 µl of 2X master mix (Promega, GoTaq), 1 µl of each primer, 3 µl of diluted amplified mtDNA and 10 µl of nuclease-free water. The PCR was performed using specific primer pair (F1-R1) (Table 1), and the fragment size of the amplicon is expected to be 414-bp (Table 2). PCR was carried out using a thermal cycler (BIOER, XP) upon the following program: the initial denaturation step was done at 95^{o}C for 2 min followed by 40 cycles of: denaturation at 95^{o}C for 30 seconds, 55^{o}C for 30 seconds which is the annealing step and extension at 72^{o}C for 1 min. Finally, the reaction was ended with the final extension step at 72^{o}C for 5 min.

The resulted PCR products were resolved by electrophoresis using 1% agarose gel at 100V for 45 min, with the use of 1X Tris-Borate-EDTA (TBE) buffer then visualized by UV. A 50-bp DNA marker was used.

Table 1: Oligonucleotides primers used for mtDNA confirmation and PCR amplification of 7599 and 7345-bp deletions (Kao *et al.*, 1998)

Primer	Primer Sequence
F1	5'-AACATACCCATGGCCAACCT-3'
F2	5'-TGAACCTACGAGTACACCGA-3'
F4	5'-GCCCGTATTTACCCTATAGC-3'
F5	5'-ACGAAAATCTGTTCGCTTCA-3'
R1	5'-GGCTACTGCTCGCAGTGCGC-3'
R3	5'-CTTTGGAGTTGCAGTTGATG-3'
R4	5'-TGCGGGATATTGATTTCACG-3'

3.3.2 Detection of 7599 and 7345-bp mtDNA deletions

In order to detect the existence of 7599 and 7345-bp deletions in the mtDNA samples, PCR reaction was performed using (F4-R3 and F2-R3) and (F5-R4) primers pairs respectively (Table 1), and the length of the PCR products is predicted to be (406 and 756-bp) and 555-bp respectively (Table 2). PCR amplifications to detect both deletions were done and each one was 30 µl as total volume containing 15 µl of 2X master mix (Promega, GoTaq), 1µl of each one of the specific primers, 3 µl of diluted amplified mtDNA and 10 µl of nuclease-free water. PCR conditions for detection of 7599-bp deletion were started with initial denaturation step at 95^{o}C for 2 min followed by 35 cycles of: denaturation at 95^{o}C for 30 seconds, annealing at 55^{o}C for 30 seconds and extension at 72^{o}C for 1 min. Then, the final extension step was performed at 72^{o}C for 5 min.

On the other hand, PCR conditions to detect 7345-bp deletion were started with initial denaturation step at 95^{o}C for 2 min followed by 40 cycles of: denaturation at 95^{o}C for 30 seconds, 60^{o}C for 30 seconds for annealing followed by extension step at 72^{o}C for 1 min. Finally, a final extension step was performed at 72^{o}C for 5 min.

The resulted PCR products was resolved by electrophoresis using 1% agarose gel at 100V for 45 min, with the use of 1X Tris-Borate-EDTA (TBE) buffer then visualized by UV. DNA marker of 50-bp was used.

Table2: Primer pairs used for mtDNA confirmation and for detection of 7599 and 7345-bp mtDNA deletions. Adapted from (Kao *et al.*, 1998)

Primer pair	Amplified Position	Length of PCR product in deleted mtDNA (bp)
(F1-R1) $_{mtDNA}$	3304–3717	**414**
(F5-R4) $_{7345\text{-}bp}$	8531–16,430	**555**
(F2-R3) $_{7599\text{-}bp}$	7901–16,255	**756**
(F4-R3) $_{7599\text{-}bp}$	8251–16,255	**406**

3.4. Statistical analysis

SPSS software was used for data analysis. Descriptive frequency analysis was calculated for 7599 and 7345-bp mtDNA deletions among study population. Spearman's correlation analysis was conducted to evaluate the relationship between the presence of the corresponding deletions and the reduction in the sperm motility. Furthermore, binary logistic regression analysis (Odds ratio, 95% confidence intervals and p-value) was performed. The p-value was considered significant if it was ≤ 0.05. Graphs were drawn using GraphPad Prism 7.00 software.

Chapter Four

Results

The mean age of the patients group was 34.4 year-old, whereas, the mean age was 32.7 year-old for the control group.

As shown in table 3, we have reported that 77 patients (63.6%) were having the 7599-bp deletion in their sperm mtDNA in comparison to only 27 (34.2%) subjects among control group (p-value= 0.001). In regard of the 7345-bp mtDNA deletion, 41 patients (33.9%) were having the deletion in comparison to 30 subjects (38.0%) among control group (p-value= 0.329).

Table 3: Frequencies of 7599 and 7345-bp mtDNA deletions among control and study subjects

mtDNA deletion	Patients		Control		P-value
	No.	%	No.	%	
7599-bp	77	63.6	27	34.2	**0.001**
7345-bp	41	33.9	30	38.0	**0.329**

In addition, control and study subjects were subcategorized based on the percentage of sperm motility. The incidences of the 7599-bp mtDNA deletion among control and study subjects are shown in table 4 and table 5 respectively.

Table 4: Frequencies of positive samples for 7599-bp mtDNA deletion among patients group in accordance to motility

Motility Category	Number of Patients	Positive Samples	Percentage	P-value
0%	20	14	70%	
1-5 %	20	15	75%	
6-10%	20	13	65%	
11-15 %	20	11	55%	**0.440**
16-20 %	20	14	70%	
21-35 %	21	10	47.6%	
Total	**121 patients**	**77**	**63.6%**	

Table 5: Frequencies of positive samples for 7599-bp mtDNA deletion among control group in accordance to motility

Motility Category	Number of Controls	Positive Samples	Percentage	P-value
50-55%	25	13	52%	
56-60 %	27	10	37%	**0.017**
61-75%	27	4	14.8%	
Total	**79 Controls**	**27**	**34.2%**	

Similarly, the incidences of the 7345-bp mtDNA deletion among control and study subjects are shown in table 6 and table 7 respectively.

Table 6: Frequencies of positive samples for 7345-bp mtDNA deletion among patients group in accordance to motility

Motility Category	Number of Patients	Positive Samples	Percentage	P-value
0%	20	9	45%	
1-5 %	20	7	35%	
6-10%	20	4	20%	
11-15 %	20	4	20%	**0.009**
16-20 %	20	13	65%	
21-35 %	21	4	19%	
Total	**121 patients**	**41**	**33.9%**	

Table 7: Frequencies of positive samples for 7345-bp mtDNA deletion among controls group in accordance to motility

Motility Category	Number of Controls	Positive Samples	Percentage	P-value
50-55%	25	12	48%	**0.111**
56-60 %	27	12	44.4%	
61-75%	27	6	22.2%	
Total	**79 Controls**	**30**	**38%**	

The frequency of 7599-bp mtDNA deletion among patients and controls groups according to the motility percentage is indicated in figure 7, and the percentage of the 7599-bp deletion is indicated in figure 8. The prevalence of the 7599-bp mtDNA deletion was notably high in asthenozoospermic patients.

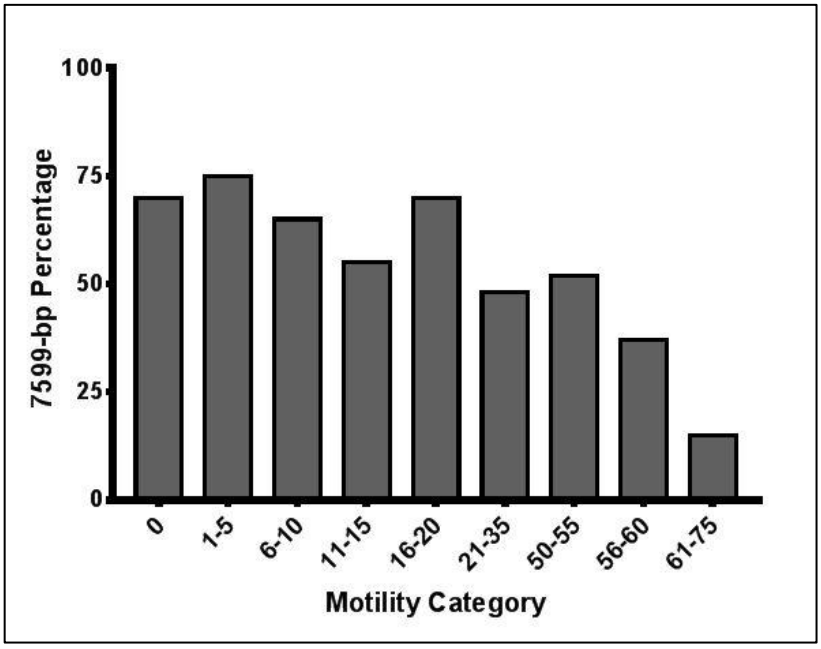

Figure 7: The frequency of 7599-bp mtDNA deletion among patients and control groups.

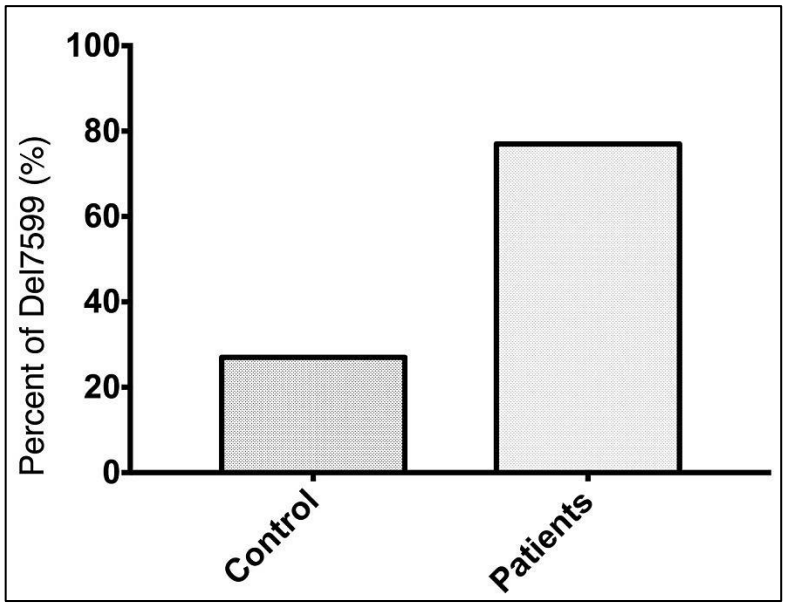

Figure 8: Percentage of the 7599-bp mtDNA deletion among control and patients groups.

On the other hand, the frequency of the 7345-bp mtDNA deletion among patients and controls groups according to the motility percentage is indicated in figure 9, and the percentage of the 7345-bp deletion is indicated in figure 10. The prevalence of the deletion was not associated with reduced sperm motility.

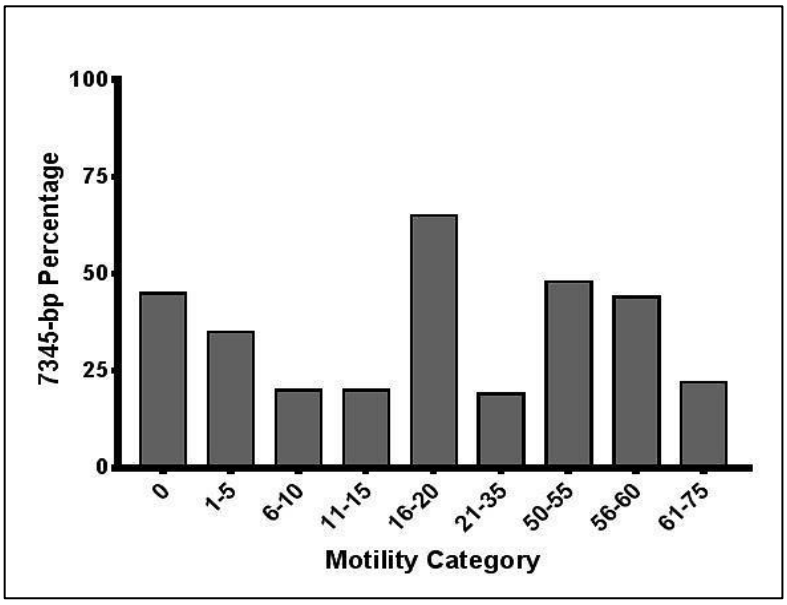

Figure 9: The frequency of 7345-bp mtDNA deletion among patients and control groups.

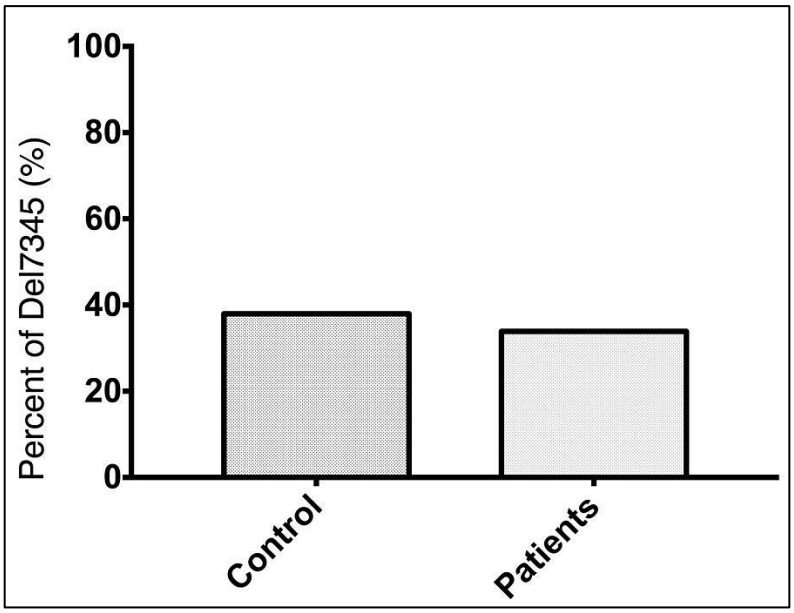

Figure 10: Percentage of the 7345-bp mtDNA deletion among control and patients groups.

The PCR product of mtDNA for confirmation of the mtDNA and detection of the 7599 and 7345-bp deletions were checked by agarose gel electrophoresis as shown in figure 9.

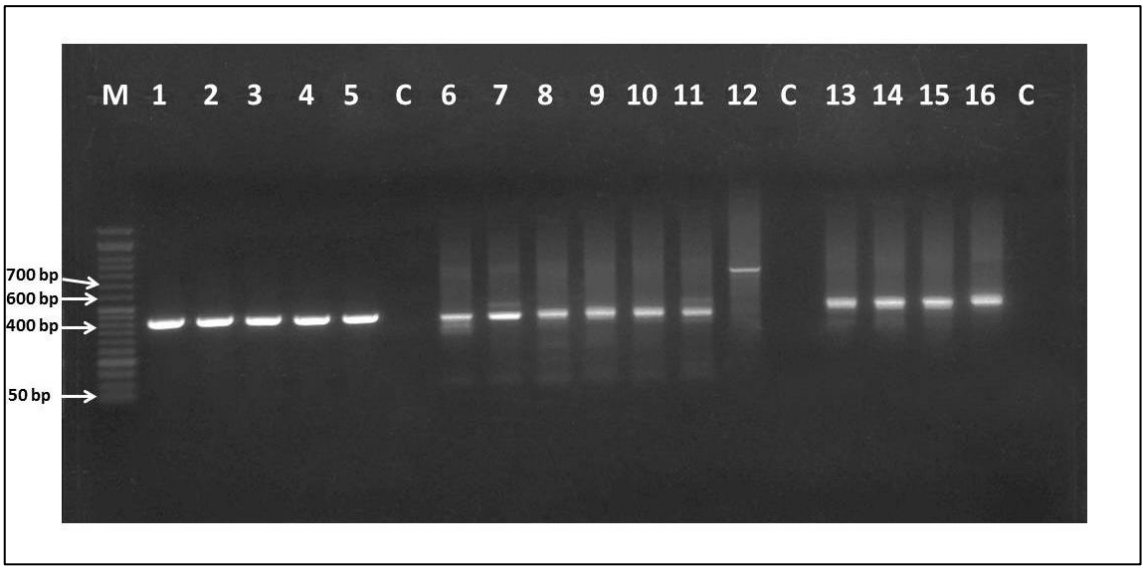

Figure 11: Agarose gel electrophoresis for mtDNA PCR products. Samples from 1-5 for mtDNA confirmation using $(F1\text{-}R1)_{mtDNA}$ primers (bands sizes of 414 bp). Samples from 6-11: bands of 406 bp size for confirmation of 7599-bp deletion using $(F4\text{-}R3)_{7599\text{-}bp}$ primers. Sample 12 indicates a band of 756 bp for the confirmation of 7599-bp deletion using $(F2\text{-}R3)_{7599\text{-}bp}$ primers. The last samples from 13-16 indicate the bands of 555 bp for the confirmation of the 7345-bp deletion using $(F5\text{-}R4)_{7345\text{-}bp}$ primers. M and C indicated for DNA marker and negative controls respectively.

Our findings about 7599 and 7345-bp mtDNA deletions were also confirmed by sequencing for the products obtained by PCR as shown in figure 12 and figure 13 respectively.

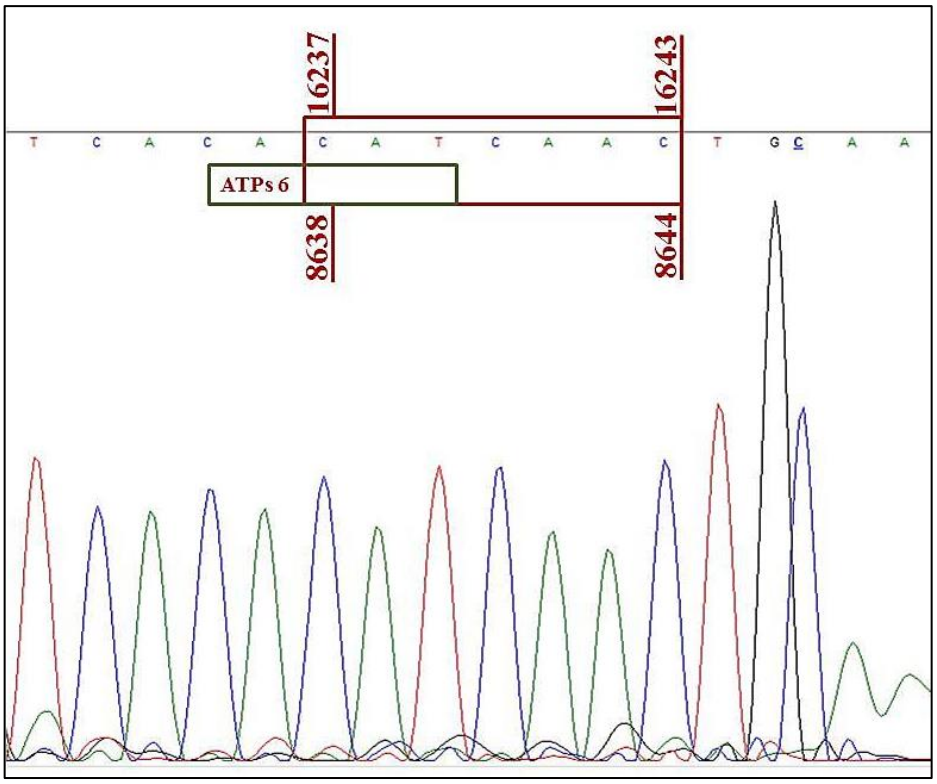

Figure 12: Sequencing result for sperm mtDNA PCR product for 7599-bp mtDNA deletion. The nucleotides sequence inside the red box indicated the seven nucleotides direct repeat at both sides of the deletion as indicated by the numbers above and below the box. ATPase 6 gene is included within deletion.

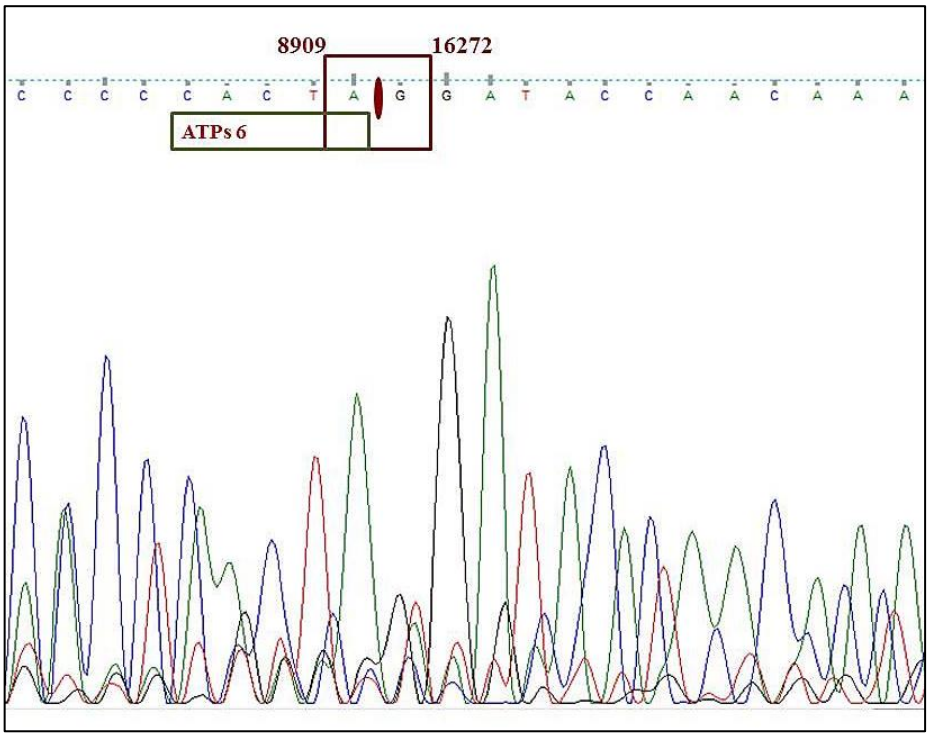

Figure 13: Sequencing result for sperm mtDNA PCR product for 7345-bp mtDNA deletion. The red arrow indicates the site of the deletion breakpoint. ATPase 6 is included within deletion.

This study indicated that there is a strong correlation between the presence of 7599-bp mtDNA deletion and the reduction in the motility of spermatozoa. The odds ratio was 4.097 with (2.149 and 7.811) 95% confidence intervals. Also the p-value was less than 0.05. Also, upon results obtained by Spearman's correlations analysis, we found a correlation between the 7599-bp mtDNA deletion and reduced sperm motility; the value was 0.340 with p-value less than 0.05.

On the other hand, there was no significant correlation between the occurrence of 7345-bp mtDNA deletion and having a reduction in sperm motility. The odds ratio was 0.530 and the 95% confidence intervals were (0.272-1.035), whereas the p-value was 0.063.

In addition to that, the 7599-bp deletion showed more significant correlation with motility percentage when controlling for the 7345-bp deletion (R^2=0.351 and the p-value < 0.001).

Chapter Five

Discussion

Reduced sperm motility (asthenozoospermia) is considered as a major cause of male infertility (Baker and Clarke, 1987). The energy provided to spermatozoa in the form of ATP is produced via OXPHOS mechanism of the mitochondria which located in the middle piece of the spermatozoon (Gibbons, 1983). It was found that there is a copy of the mtDNA in each mitochondrion (Hecht *et al.*, 1984)

The mtDNA differs from nuclear DNA by the fact that it lacks efficient proofreading and repairing mechanisms, so the rate of mutation is much higher than the nuclear DNA (Aitken and Clarkson, 1988). Moreover, the mtDNA is susceptible to be attacked by ROS that are generated from the normal mitochondrial respiration process, and as a consequence the mtDNA may be damaged due to elevated oxidative stress which lead to affect sperm functions that includes their motility (Aitken, 2013). The OXPHOS process is under controlling of nuclear DNA and mtDNA, the mtDNA plays a critical role in this process; it is responsible for encoding four of the five major complexes of OXPHOS (Bornstein *et al.*, 1998).

Several mtDNA deletions were documented to be highly correlated to asthenozoospermic male infertility. Among these, the 7599 and 7345-bp deletions were studied.

In the current study, we found a highly significant correlation between the presence of 7599-bp mtDNA deletion and the reduction in sperm motility. On the other hand, no significant correlation was detected between the presence of 7345-bp mtDNA deletion and asthenozoospermia.

Our results are consistent with a recently published study in Iran by Talebi *et al.* (2018). They found a significant association between the incidences of the 7599-bp mtDNA deletion and asthenozoospermia, also it was found in the patients of oligoasthenoteratozoospermia and the occurrence of this deletion associated with the loss of ATPase 6 gene in addition to the other OXPHOS genes of the mtDNA, which as a consequence affects the sperm motility. In contrast, they did not find any significant association between the prevalence of the 7345-bp deletion and having a reduction in sperm motility (Talebi *et al.*, 2018).

Also, it was proved that the existence of the large scale mtDNA deletions such as 7599-bp and 7491-bp was markedly associated with reduced sperm motility because it is responsible for losing of some mitochondrial genes and then affecting male fertility. They also proposed that screening of mtDNA for such deletion may provide a clear evidences behind the etiologies of male infertility (Hosseinzadeh Colagar and Karimi, 2014).

Moreover, the role of the 7599 and 7345-bp mtDNA deletions in male infertility was investigated by Kao *et al.* (1998). They found a significant association between the incidence of the 7599-bp deletion in the sperm mtDNA and the reduction in the sperm motility (Kao *et al.*, 1998), the results of our study are consistent with their findings.

In regard to the 7345-bp mtDNA deletion, Kao *et al.* (1998) reported a significant association between the incidence of the 7345-bp deletion and asthenozoospermia; the results of our study are not consistent with that. In comparison with our findings, the discrepancy in the results may be due to small study subjects which were 36 patients compared to 121 that were included in this study. They also reported that these deletions are responsible for the loss of some genes that take places in the OXPHOS process.

Such a loss will affects the activity of the mitochondria in energy production and therfore frustrates sperm motility which as a consequence elevates the proportion of male infertility (Kao *et al.*, 1998).

On the other hand, the results of our study are not consistent with the results of a research study that was done by John *et al.* (2001). The correlation of the existence of 7491-bp mtDNA deletion in spermatozoa was investigated in 33 semen samples. They proposed that the using magnetic beads coated with special antibody for the separation of sperm may help in the elimination of leukocytes which considered as contaminants of sperm specimens. As a result, they suggested that there was no association between these mtDNA deletions and sperm count, morphology or motility (John *et al.*, 2001)

According to the results of our study, the significant difference in the incidence of 7345-bp mtDNA deletion among patients group but not among control subjects does not necessarily means that this deletion is having any clinical contribution to sperm motility considering the fact partial correlation analysis has revealed that 7599-bp mtDNA deletion is significantly correlated to motility percentage, while 7345-bp mtDNA deletion is not significantly correlated to motility percentage among study subjects. In support of that the 7599-bp deletion has been shown to be even more significantly correlated to motility percentage when controlling for the 7345-bp deletion.

The inconsistency between results may be due to variations in the methodology, especially the sperm separation step. Moreover, the prevalence of the deletions in sperm mtDNA among control groups, may be due to the effect of the environmental factors such as elevated scrotal temprature, the expusure to pesticides, radiation and cigaritte smoking which lead to the accumulation of the oxidative stress and therefore increase the chance of DNA breaks and damage. Also, using of some medications and antibiotics

that were reported to have an effect on the male fertility. Furthermore, the number of subjects in several studies was low and the variation between populations may be a factor behind the contradictory results.

Chapter Six

Conclusion

The current study aimed to investigate the correlation of sperm mtDNA 7345 and 7599-bp deletions with male infertility in the Jordanian population, we reported is a highly significant correlation between the presence of 7599-bp mtDNA deletion and asthenozoospermia. On the other hand, no significant correlation was reported between the presence of 7345-bp mtDNA deletion and asthenozoospermia. Our findings can help in the early diagnosis of asthenozoospermic male infertility and can be used as molecular markers for male infertility.

Recommendations

Although patients who have deletions in their sperm mtDNA have a lower chance of conceiving children, they can sidestep money and time consuming in using medications of sperm motility improvement by guiding them directly to attend special *In-vitro* fertilization (IVF) techniques like Intra-cytoplasmic sperm injection (ICSI); sperm motility is not required in this technique because the sperm can be directly injected into the ovum, or using cryopreservation for sperm, thus increase the chance of having successful birth in the future. Further investigations are required to study other mtDNA mutations for their assciation with male infertility.

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Appendix

استبانة

نموذج موافقة على المشاركة في اجراء بحث علمي

يهدف هذا البحث الى الكشف عن وجود علاقة بين حدوث طفرة في الحمض النووي للميتوكندريا وانخفاض نسبة الحركة المسببة للعقم الذكوري في الأردن.

سوف يتم التعامل مع المعلومات بسرية تامة واحترام خصوصية المريض ، علما بانه يحق له الانسحاب من الدراسة متى ما شاء.لذلك يرجى تحلي الدقة عند الاجابه على الاسئلة لما يعود من فائدة على نتائج البحث.

الاسم :

أوافق على المشاركة في البحث المذكور اعلاه وعلى الاجابة عن الاسئلة بشكل صحيح

التوقيع:

رقم العينة المتسلسل :

التاريخ: / /

الرقم الطبي:

رقم الهاتف:

العمر:

الوزن:

الطول:

طبيعة العمل:

فترة الامتناع عن الجماع :

هل انت متزوج ؟

كم هي فترة الزواج؟

هل اتبعت وسائل تمنع الحمل؟

هل لديك اطفال؟

هل حصل الحمل بشكل طبيعي ام باستخدام وسائل المساعدة على الانجاب؟

كم عمر اصغر طفل لديك؟

هل يعاني احد افراد العائلة من تاخرالانجاب؟

هل انت مدخن؟

هل تخضع لحمية غذائية عن طريق اخصائي تغذية؟

هل قمت باجراء الفحوصات المخبرية التالية؟ ادخل النتائج المتوفرة بجانب كل فحص:

FSH	**MIU/ML**	**Testosterone**	**ng/dl**
B 12	**pg/ml**	**VIT D**	**ng/ml**
Prolactin	**ng/dl**	**Estradiol (E2)**	**pg/ml**

Arabic Abstract

ملخص

الطلافحه، علي مروان. دور نقصان الجينوم لميتوكوندريا الحيوان المنوي في عقم الرجال: دراسة للمجتمع الأردني. رسالة ماجستير، جامعة اليرموك، 2018.

المشرف الرئيس: د. مظهر الزعبي

المشرف المشارك: د. خالد البطاينة

يُعرف العقم بأنه فشل حدوث الحمل بعد مرور سنة من الزواج دون استخدام وسائل منع الحمل. ويعاني ما يقارب 17% من الأزواج من هذه الحالة، ويُعزى النصف من حالات العقم إلى العامل الذكوري؛ إذ تعد حركة الحيوانات المنوية عامل مهم جداً في عملية الإخصاب؛ وذلك لأنها تزيد من قدرة الحيوان المنوي على الوصول إلى المهبل والانغراس في البويضة في موقع الإخصاب، ويُعد انخفاض حركة الحيوانات المنوية والتي تُعرف بأنها نقصان عدد الحيوانات المنوية المتحركة إلى أقل من 40% من مجموع حركة الحيوانات المنوية أحد الأسباب الرئيسية للعقم عند الذكور.

يلعب الحمض النووي للميتوكوندريا دوراً أساسياً في عملية التنفس الخلوي التي تزود الحيوانات المنوية بالطاقة اللازمة لحركتها ويؤثر حدوث طفرات الحذف في الحمض النووي لميتوكوندريا الحيوان المنوي على وظائف بعض البروتينات الهامة في عملية التنفس الخلوي وبالتالي على حركة الحيوانات المنوية.

يهدف هذا البحث إلى دراسة احتمالية وجود علاقة بين حدوث طفرات حذف (7599-bp و 7345-bp) في الحمض النووي لميتوكوندريا الحيوان المنوي مع العقم الذكوري المرتبط بنقصان حركة الحيوانات المنوية لدى الرجال الأردنيين.

تم جمع عينات السائل المنوي من الرجال الذين يعانون من ضعف الخصوبة المرتبط بضعف الحركة في الحيوانات المنوية من وحدات الإخصاب التابعة للخدمات الطبية الملكية الأردنية في مدينة الحسين الطبية ومستشفى الأمير راشد بن الحسن العسكري، متبوعًا بمضاعفة الحمض النووي لميتوكوندريا الحيوان المنوي ثم تم إجراء تفاعل البلمرة المتسلسل ثم إجراء تسلسل لنواتج ذلك التفاعل.

وقد أشارت هذه الدراسة، إلى وجود علاقة قوية بين أحد طفرات الحذف تلك (7599-bp) وانخفاض حركة الحيوانات المنوية (P-value < 0.05). ومن جهة أخرى، لم يُلاحظ وجود أي علاقة بين حدوث طفرة الحذف الأخرى (7345-bp) وانخفاض حركة الحيوانات المنوية.

ووفقاً لهذه النتائج، فإنه يمكن للكشف عن حدوث طفرات الحذف في الحمض النووي لميتوكوندريا الحيوان المنوي أن يساهم في التشخيص المبكر للعقم الذكوري والذي بدوره يوجه المرضى لاستخدام وسائل المساعدة على الإنجاب مثل الحقن المجهري للبويضة بالحيوان المنوي أو باستخدام تقنية التجميد لحفظ الحيوانات المنوية وبالتالي زيادة فرصة نجاح حدوث الحمل في المستقبل.

تجدر الإشارة إلى أن هناك حاجة إلى مزيد من الدراسات حول هذا الموضوع للكشف عن أنواع أخرى للطفرات في الحمض النووي لميتوكوندريا الحيوان المنوي وعلاقتها بالعقم الذكوري.