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EVALUATION OF VITAMIN E, LIPID PEROXIDATION, IL-6, IL-8 AND SOME HORMONES IN SERUM AND SEMINAL PLASMA (OLIGOZOOSPERMIA AND AZOOSPERMIA) OF MEN

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ALI SAMI GHAREEB GHAREEB

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By Ali Sami Ghareeb GHAREEB

January 2024

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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Ali Sami Ghareeb GHAREEB

ABSTRACT

EVALUATION OF VITAMIN E, LIPID PEROXIDATION, IL-6, IL-8 AND SOME HORMONES IN SERUM AND SEMINAL PLASMA (OLIGOZOOSPERMIA AND AZOOSPERMIA) OF MEN

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Master of Science in Chemistry

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January 2024

Aimed this study was aimed to assess the Evaluation of vitamin E, Lipid peroxidation, IL-6, IL-8 and some hormones in serum and seminal plasma (Oligozoospermia and azoospermia) of infertile men. And research into the extent of changes that occur in immunological tests, which may lead us to identify the causes of changes in some trace elements and important hormones in the formation of sperm in men. 155 samples were studied, where group A consisted of 50 men with azoospermia aged 18 and over, group B: consisted of 50 men with azoospermia and group C: consisted of 55 men who had no problems with azoospermia (control group). They were selected, which were divided according to the number of sperm into the patient's group (Oligozoospermia and azoospermia) and healthy groups (the control group) into three groups, and the results of the study were as follows: With increasing age, problems with sperm production increase in men, and dietary diversity and lifestyle may directly affect sperm formation. Also, weight had a direct effect on men in the two groups (infertile men) when compared with the control group. The mean body mass index changed little, indicating a slight effect on sperm count. Our study confirmed that low levels of testosterone may have a direct effect on the number of sperm, but after receiving treatments, it led to high levels of testosterone, but the problem remains the same, which indicates the association of infertility (sperm disorders) with another problem that may be hormonal. The quality and number of sperm increased in men who had high or normal vitamin E levels. The immunological problems had a direct effect on spermatogenesis, as the levels of

interleukin-6 and interleukin-8 changed clearly in our study. The results demonstrate a direct relationship between a number of pro-inflammatory cytokines and semen quality. Due to its substantial association with seminal parameters and other potential inflammation markers, IL-8 may be used as a sensitive diagnostic for silent male genital tract infection. The study proved that disturbances in the metabolism of fats constitute direct problems in the process of sperm formation in men.

2024, 56 pages

Keywords: Male infertility, FSH hormone, vitamin E, IL-6, Lipid peroxidation

ÖZET

ERKEKLERDE SERUM VE SEMINAL PLAZMADA (OLIGOZOOSPERMI VE AZOSPERMI) VITAMIN E, LIPIT PEROKSIDASYONU, IL-6, IL-8 VE BAZI HORMONLARIN DEĞERLENDIRILMESI

Ali Sami Ghareeb GHAREEB
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Ocak 2024

Bu çalışmada infertil erkeklerin serum ve seminal plazmasında (Oligozoospermi ve azospermi) E vitamini, Lipid peroksidasyonu, IL-6, IL-8 ve bazı hormonların değerlendirilmesi amaçlandı. Ve erkeklerde sperm oluşumunda bazı eser elementler ve önemli hormonlardaki değişikliklerin nedenlerini belirlememize yol açabilecek immünolojik testlerde meydana gelen değişikliklerin boyutunun araştırılması. 155 örnek incelendi, A grubu 18 yaş ve üzeri azospermili 50 erkek, B grubu: azospermili 50 erkek ve Grup C: azospermi sorunu olmayan 55 erkek (kontrol grubu) oluşturuldu. Sperm sayısına göre hasta grubu (Oligozoospermi ve azospermi) ve sağlıklı grup (kontrol grubu) olmak üzere üç gruba ayrılarak seçildi ve çalışmanın sonuçları şöyle oldu: erkeklerde sperm üretimi artar ve beslenme çeşitliliği ve yaşam tarzı sperm oluşumunu doğrudan etkileyebilir. Ayrıca kilo, kontrol grubuyla karşılaştırıldığında iki gruptaki (kısır erkekler) erkekler üzerinde doğrudan bir etkiye sahipti. Ortalama vücut kitle indeksi çok az değişti, bu da sperm sayısı üzerinde hafif bir etki olduğunu gösteriyor. Çalışmamız, düşük testosteron düzeylerinin sperm sayısı üzerinde doğrudan bir etkiye sahip olabileceğini doğruladı, ancak tedavi aldıktan sonra yüksek testosteron seviyelerine yol açtı, ancak sorun aynı kaldı, bu da kısırlığın (sperm bozuklukları) ile ilişkisini gösteriyor. hormonal olabilecek başka bir problem. E vitamini düzeyi yüksek veya normal olan erkeklerde sperm kalitesi ve sayısı arttı. Çalışmamızda interlökin-6 ve interlökin-8 seviyeleri belirgin bir şekilde değiştiğinden, immünolojik problemler spermatogenezi doğrudan etkiledi. Sonuçlar, bir dizi proinflamatuar sitokin ile semen

kalitesi arasında doğrudan bir ilişki olduğunu göstermektedir. Seminal parametreler ve diğer potansiyel inflamasyon belirteçleri ile önemli ilişkisi nedeniyle IL-8, sessiz erkek genital yolu enfeksiyonu için hassas bir teşhis olarak kullanılabilir. Çalışma, yağ metabolizmasındaki bozuklukların erkeklerde sperm oluşumu sürecinde doğrudan problemler oluşturduğunu kanıtladı.

2024, 56 sayfa

Anahtar Kelimeler: Erkeklerde kısırlık, FSH h, E vitamini, IL-6, Lipit peroksidasyonu

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Ali Sami Ghareeb GHAREEB

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CONTENTS

LIST OF SYMBOLS

%	Percentage
°C	Celsius degree
cm	Cantimeter
g	Gram
mg	Milligram
min	minute
mL	Milliliter
mM	Millimolar
nm	Nanometer
U	Unit
µL	Milliliter

LIST OF ABBREVIATIONS

BMI	Body mass index
DBCP	Dibromo-3-chloropropane
EDTA	Ethylenediaminetetraacetic acid
FSH	Follicle-stimulating hormone
HDL	High density lipoprotein
IL-6	Interlukin 6
IL-8	Interlukin 8
LDL	Low density lipoprotein
LH	Luteinizing hormone
MDA	Malondialdehyde
Tc	Total cholesterol
Tg	Triglyceride
TT	Total testosterone
VitE	Vitamin E
VLDL	Very low density lipoprotein

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1. INTRODUCTION

One of the biggest societal issues facing developed countries is infertility. Generally speaking, variables connected to the male spouse account for about half of all occurrences of infertility. Male infertility treatments have been created and are currently yielding positive outcomes. Patients with nonobstructive azoospermia, in which mature sperm are absent from the testes, cannot be treated effectively. In the vast majority of cases, the reason of male infertility has not been identified, despite data suggesting that many individuals have a hereditary susceptibility to the disorder. This article examines the environmental elements thought to play a role in male infertility as well as the genes that have been conclusively linked to male infertility in humans, including our most recent discoveries (Toshinobu *et al.* 2012).

Azoospermia primarily comes in two forms: A blockage or missing link in the epididymis, vas deferens, or somewhere else along your reproductive system is what is meant by obstructive azoospermia. Even though you are making sperm, it is being prevented from leaving your body, leaving no detectable amount of sperm in your semen. Nonobstructive azoospermia: If you have this sort of azoospermia, your sperm production is weak or nonexistent as a result of problems with the testicles' structure or function or other factors (Meniru *et al.* 1997, Wosnitzer and Goldstein 2014).

As a sex steroid derived from cholesterol, testosterone has a four-ringed structure with interlocked rings. The source of all naturally occurring steroid hormones is cholesterol. Because of the ring shape, testosterone and cholesterol are greasy and do not dissolve in water but rather clump together. They do dissolve in other greasy liquids, though. As water is polar while fats and oils are not, they are referred to as nonpolar molecules. Hence, the body must alter the structure of testosterone to make it soluble in water so that it may be eliminated through urination (Vignozzi *et al.* 2012).

Interleukins are involved in the local defense system against infectious infections, but they are also thought to be pathology-mediating agents. Lately, it has been proposed

that an increase in IL-8 concentration that causes neutrophil recruitment may be crucial for the spread of viruses throughout the body. A human cytomegalovirus infection, for instance, up-regulates IL-8 gene expression and induces transendothelial neutrophil migration, suggesting that IL-8 may boost virus generation in endothelial cells through an autocrine mechanism. IL-8 levels may change under specific pathological circumstances. Little is known regarding IL-8's effects on sperm production and semen quality, as well as whether it rises in the same ejaculates as leukocyte counts and other potential indicators of subclinical infection or inflammation and may interact with other cytokines, such as IL-6. It is also unknown if the presence of potentially harmful bacteria in patients' semen who do not exhibit genital tract infection symptoms is related to elevated levels of pro-inflammatory seminal cytokines, which may reduce sperm functionality (Liu *et al.* 2004, Nanki *et al.* 2001).

1.1 The Aim of study

This study was aimed to assess the Evaluation of vitamin E, Lipid peroxidation, IL-6, IL-8 and some hormones in serum and seminal plasma (Oligozoospermia and azoospermia) of infertile men. And research into the extent of changes that occur in immunological tests, which may lead us to identify the causes of changes in some trace elements and important hormones in the formation of sperm in men.

2. LITERATURE REVIEW

The dropping birth rate is one of the most significant societal issues facing affluent nations today, even while it is often underappreciated that the number of infertile couples is increasing in many nations. While both social (i.e., social progress for women and the resulting increase in the age at which women marry) and environmental (i.e., pollution and global warming) factors are behind part of the increase in the number of patients with infertility, infertility in the male partner contributes to approximately half of all cases (Oliva *et al.* 2011).In subsequent chapters, we will first talk about the male reproductive system, and then about the spermatogenesis process and the types of sperm count disorders in men.

2.1 The Male Reproductive System

Sperm (male reproductive cells) are produced by the testes or testicles through a process known as spermatogenesis. The majority of the tissue in the testes is composed of small tubes called seminiferous tubules. Each testicle has an epididymis on the rear, which is where mature sperm are transferred and stored. The muscular tube known as the vas deferens travels from the epididymis into the pelvis before turning and entering the seminal vesicle (Huang *et al.* 2006, Kalantaridou *et al.* 2004). The majority of the fluid components of semen are produced and stored by the seminal vesicle, a tubular gland. The vesicle narrows to create the seminal duct, a straight duct that connects to the vas deferens. The seminal vesicle duct and the vas deferens join to form the ejaculatory duct. The prostate gland is entered through the ejaculatory duct, which then joins the urethra. The tube known as the urethra passes through the penis to remove semen from the vas deferens and urine from the bladder. Sperm travel from the testicles and epididymis to the vas deferens during ejaculation. The sperm is propelled forward by the vas deferens contracting (tightening). The seminal fluid advances toward the urethra as more secretions from the seminal vesicle are added. The prostate gland, which adds a milky fluid to the sperm to create semen, is where the seminal fluid flows before it reaches the urethra. The semen is finally ejaculated (released) through the urethra from the penis. A normal sperm count is regarded to be 15 million/mL or greater. Sperm

concentrations in men with low sperm counts (oligozoospermia or oligospermia) are fewer than 15 million/mL. If you have azoospermia, your ejaculate contains no detectable sperm (Fayomi *et al.* 2019, Kalantaridou *et al.* 2004).

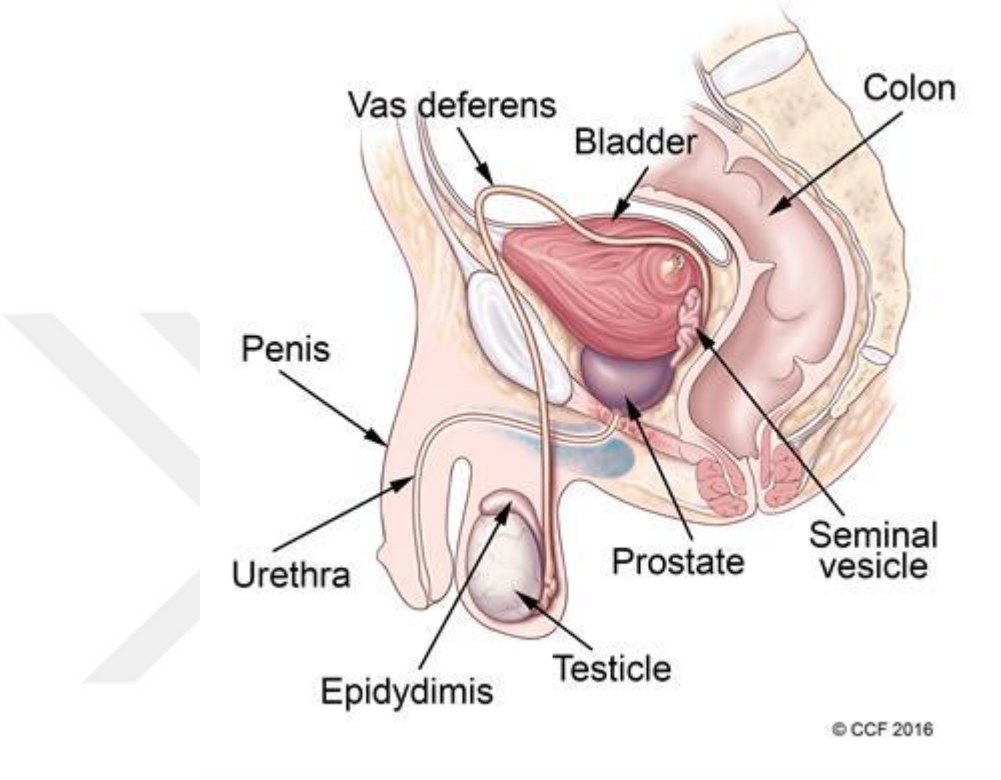


Figure 2.1 Male reproductive anatomy

The scrotum is significant because it enables the testes to be external to the body. The major purpose of the scrotum is to keep the testicles at the proper temperature so they can make sperm. The dartos muscle of the scrotum, which controls the surface area of the scrotum by contracting/wrinkling the skin, and the cremaster muscle, whose contraction draws the testes closer to the body when the outside temperature is low, work together to do this. The scrotal branches of the internal and external pudendal arteries deliver blood to the scrotum. The front portion of the scrotum receives innervation from branches of the sacral plexus, whereas the posterior portion receives innervation from the lumbar plexus (Bonde 2010, Kleisner *et al.* 2010).

2.2 Human Male Infertility

The question of whether environmental influences, such as those present at work or in a person's neighborhood, affect a man's ability to reproduce has been up for debate for a long time. The spectacular study by Whorton et al., published in 1977, had a significant impact on this discussion because it revealed that 14 of the 25 male workers involved in the production of the insecticide dibromo-3-chloropropane (DBCP) had azoospermia or oligospermia. In 1992, Carlsen et al. stated that the previous 50 years experienced a dramatic reduction in sperm count. In the same year, Brake and Krause found that Scotland's sperm counts had dropped by a mean annual rate of 2.1% since 1970, or about 25% less than they had been previous to 1959 (Carlsen *et al.* 1992, Brake and Krause 1992).

12% to 15% of sexually active couples are thought to be infertile. About 50% of the time, when factors are split down by gender, a male component can be found, either alone or in combination with a female factor. Idiopathic infertility remains a common diagnosis, with over 25% of individuals not having a diagnosable cause of infertility, despite recent technological and diagnostic improvements. Nevertheless, many observable causes of male infertility are treated or avoidable; as a result, it is crucial to have a thorough grasp of these diseases. This chapter provides a summary of the pre-testicular, testicular, and post-testicular causes of male infertility (Machen and Sandlow 2020).

A control group of healthy men chosen based on semen results has been used in many recent investigations. However, almost all of them were patients who wanted to have kids and had undergone outpatient infertility testing. Therefore, it is extremely debatable whether the male member of an infertile relationship may be called healthyeven if the semen tests were normal. Future research should include a larger cohort and a suitable selection of healthy male controls in order to clarify the connection between environmental variables and male infertility (Bartoov *et al.* 2003, Tanrikut and Schlegel 2007).

2.2.1 The human spermatogenesis

The biological process through which sperm cells are created is called spermatogenesis. It happens in a sexually reproducing organism's male gonad. The undifferentiated male germ cells go through a series of steps to become spermatozoa in this process. There are three steps that make up this process: spermatocytogenesis, spermatidogenesis, and spermiogenesis and spermiation. These phases take place in the testis' seminiferous tubules. While mature spermatozoa released into the lumen are driven by the testicular fluid to the epididymis where they are deposited, spermiation will still proceed there (cauda region). In animals, the spermatozoa will acquire full motility (hence, maturity) when they reach the female reproductive canal.Spermatogenesis in humans begins at puberty and lasts the duration of a person's life. It might take the entire process 64 days. Oogenesis in females is paired with spermatogenesis in males. The two types of gametogenesis are spermatogenesis and oogenesis (Garner and Hafez 2000,Ogawa *et al.* 2003).

2.2.1.1 Hormonal regulation

The brain's pituitary gland controls how much testosterone and sperm are produced in the testes. LH, which regulates the release of testosterone, is specifically released by the anterior pituitary. It also causes the follicle-stimulating hormone to be released (FSH). Together with testicular testosterone, these pituitary hormones control spermatogenesis. The main hormone involved in male sex, testosterone, causes the spermatogenesis-related genes to be activated (Holdcraft and Braun 2004,Divya *et al.* 2004).

2.2.1.2 Stages of spermatogenesis

There are three steps in the process of spermatogenesis: spermatocytogenesis, spermatidogenesis, and spermiogenesis and spermiation. A spermatogenic cycle is a cycle that starts at one stage and concludes at the same stage on a specific section of the

seminiferous tubule throughout the course of a specific amount of time. The number of stages in a spermatogenic cycle varies between species (Johnson *et al.* 1997).

2.2.1.2.1 Spermatocytogenesis

The initial phase of spermatogenesis is known as spermatocytogenesis. The spermatogonia in the convoluted seminiferous tubule's basal lamina repeatedly divide via mitosis, resulting in identical spermatogonia. Others enter the first meiotic division as the primary spermatocyte and proceed into the adluminal compartment of the convoluted seminiferous tubule. Primary spermatocytes and spermatogonia are both diploid (de Kretser *et al.* 1999).

2.2.1.2.2 Spermatidogenesis

Spermatidogenesis comes after spermatocytogenesis. It is therefore the middle stage of spermatogenesis. Meiosis, a form of cell division consisting of two successive divisions: the first meiotic division (meiosis I), and the second meiotic division, is the focal point of this stage (meiosis II). The seminiferous tubule is the only location in a male body where meiosis occurs in humans and other mammals. The primary spermatocyte starts meiosis I at this point, where it divides into two haploid secondary spermatocytes. Each of the secondary spermatocytes will start meiosis II right away to create four haploid, genetically distinct spermatids (Jones and Lin 1993, Huang *et al.* 2008).

2.2.1.2.3 Spermiogenesis and spermiation

After spermatogenesis, spermatogenesis occurs. It is the phase of spermatogenesis during which the spermatids develop into fully developed spermatozoa. The four steps of spermiogenesis are:Golgi phase, Cap phase, Tail phase and Maturation phase. Events include nuclear condensation, acrosome formation, mid-piece formation, and tail formation will happen to the spermatids. At this point, the spermatozoa are not entirely functioning, though. They are not yet motile, despite having developed characteristics

necessary for fertilizing an ovum (such as changing from their initial spherical shape with residual cytoplasm to one that is compact and streamlined) (Howell and Shalet 2005,Da Cunha *et al.* 1984).

After spermiogenesis, the non-motile spermatozoa will leave the seminiferous tubules to travel to the rete testis in the mediastinum testis, to the efferent ducts, and finally to the epididymis. This migration of the spermatozoa to the epididymis is called spermiation. Since the spermatozoa are not yet motile at this stage, the Sertoli cells release testicular fluid to aid them as they travel.

2.2.2 Human azoospermia

A guy has azoospermia if there are no sperm in his ejaculate. It can be brought on by obstructions in the reproductive system, hormonal abnormalities, challenges with ejaculation, or problems with testicular structure or function. Fertility can be restored and many causes are curable. It might be possible to extract live sperm for use in assisted reproductive methods for other reasons (Wosnitzer *et al.* 2007).

Azoospermia primarily comes in two forms: A blockage or missing link in the epididymis, vas deferens, or somewhere else along your reproductive system is what is meant by obstructive azoospermia. Even though you are making sperm, it is being prevented from leaving your body, leaving no detectable amount of sperm in your semen. Nonobstructive azoospermia: If you have this sort of azoospermia, your sperm production is weak or nonexistent as a result of problems with the testicles' structure or function or other factors. The earliest and primary indication for surgical sperm retrieval was irreversible obstructive azoospermia. Reviewing data from published papers makes it simple to pinpoint two sizable patient categories. Patients in the first group have congenital bilateral vas deferens absence, while those in the second group have attempted vasectomy reversals that have failed (FRV). When these patients were examined physically, different things were discovered. The vas deferens can frequently be palpated where it joins the epididymis in FRV patients, but this is not achievable in

CBAVD patients due to varying degrees of aplasia or agenesis of the vas deferens and related tissues (Meniru *et al.* 1997, Wosnitzer and Goldstein 2014).

2.3 Vitamin E- Overview

A nutrient called vitamin E is crucial for the health of your blood, brain, skin, and eyesight. Antioxidant properties are also present in vitamin E. When the body breaks down food or is exposed to radiation and tobacco smoke, free radicals are created. Antioxidants are compounds that may shield the cells from these impacts. Heart disease, cancer, and other illnesses could be caused by free radicals. If a take vitamin E for its antioxidant qualities, be aware that the supplement may not provide the same advantages as antioxidants found naturally in food. Almonds, peanuts, margarine, canola oil, and olive oil are among foods high in vitamin E. Meats, dairy products, leafy greens, and fortified cereals are additional sources of vitamin E. As an oral supplement, vitamin E is additionally offered in capsules or drops. The absence of vitamin E might result in nerve discomfort (neuropathy). For adults, a daily intake of 15 milligrams of vitamin E is advised (Azzi and Stocker 2000, Niki and Traber 2012).

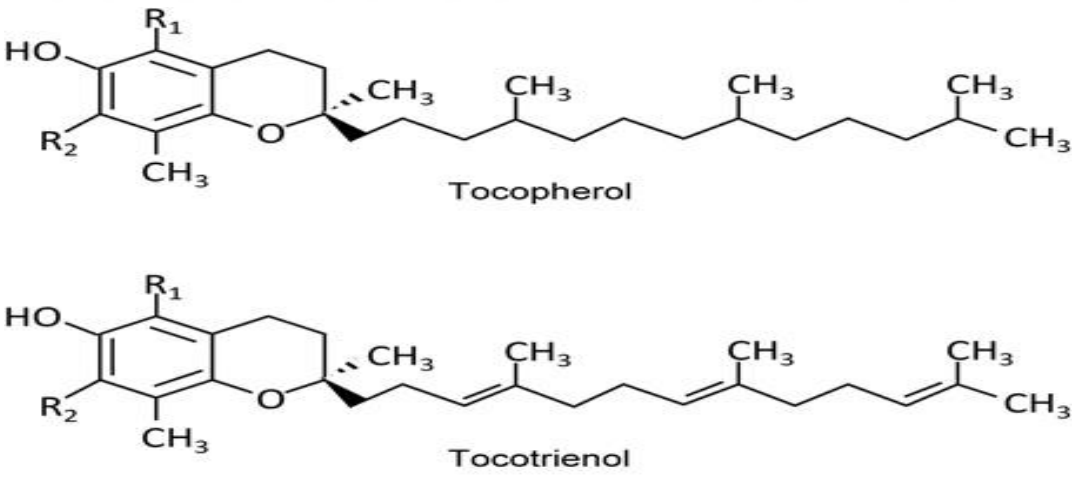
An essential vitamin, vitamin E is needed for the healthy operation of numerous organs in the body. As an antioxidant, it is also. RRR-alpha-tocopherol, a form of vitamin E that naturally occurs in foods, differs from the synthetic vitamin E found in supplements (all-rac-alpha-tocopherol). Vitamin E is used to treat vitamin E insufficiency, which is uncommon but can happen in patients with specific hereditary abnormalities and in premature children who were born very low in weight. There are other more ailments for which vitamin E is utilized, however many of these other uses lack solid scientific backing (Keen and Hassan 2016).

2.3.1 The chemical information of vitamin E

The term "vitamin E" refers to a group of naturally occurring, lipophilic chemicals, including four tocopherols and four tocotrienols, that have a chromanol ring with a side

chain at the C2 position. Vitamin E, which was first identified as a dietary component necessary for healthy reproduction, is now recognized as a key free radical scavenger in humans and shields biological components from harmful oxidative changes. The origins, purposes, and applications of vitamin E homologues' structures and physical characteristics are outlined (Niki *et al.* 1985,Brigelius-Flohé *et al.* 2002).

A chromanol ring with a side chain at the C2 position makes up the chemical structure of vitamin E, a lipid-soluble molecule obtained from plants. Eight distinct substances are referred to as vitamin E, including the four tocotrienols that correspond to each of the four tocopherols. While tocotrienols have an unsaturated isoprenyl side chain with three double bonds at C3′, C7′, and C11′, the four tocopherols have a saturated phytyl side chain. The side chains of tocotrienols have trans-configured double bonds at positions C3′ and C7′. The quantity and placement of methyl groups on the chromanol ring vary between the -, -, -, and -forms. The chromanol ring has three methyl groups at the C5, C7, and C8 sites (Figure 2.2) in tocopherol and tocotrienol, compared to two in the -, -, and -forms and one in the -forms (Schneider 2005,Matsumoto *et al.* 1995).



	α	β	γ	δ
R_1	CH_3	CH_3	H	H
R_2	CH_3	H	CH_3	H

Figure 2.2 Chemical structure of vitamin E homologues

Tocomonoenols and tocodienols, which have a single and two double bond unsaturations, respectively, have also been discovered in nature in addition to tocopherols and tocotrienols. For instance, 2,5,7,8-tetramethy1-2-(4′,8′,12′-trimethyltrideca-11′-enyl)-6-chromanol, a tocomonoenol with a single double bond at carbon 11′, was extracted from palm and rice bran oils. Since then, other groups have found tocomonoenols in plants and plant-based foods, including -tocomonoenol in palm, pumpkin seed, and sunflower oils, -tocomonoenol in kiwifruit, and -, -, and -tocomonoenol in the leaves of Kalanchoe daigremontiana and Phaseolus coccineus. Salmon tissues also contained a tocomonoenol with an unsaturation at the end of the isoprenoid chain. Additionally, it was discovered that tocodienols in palm oil had two double bonds at carbon 7′ and 11′ (Marcone 2012).

2.4 Lipid Peroxidation

Lipid peroxidation is currently thought to be one of the primary molecular mechanisms causing oxidative damage to cell structures and the hazardous process that results in cell death. First, lipid peroxidation was investigated by food scientists as a possible mechanism for the deterioration of dietary oils and fats. However, other researchers believed that lipid peroxidation was caused by toxic metabolites (such as CCl4) that disrupted intracellular membranes and caused cellular damage as well as the production of highly reactive species (Dianzani and Barrera 2008).

An oxidative chain reaction (Figure 2.3) known as lipid peroxidation occurs when one lipid molecule after another is fully or partially oxidized to create lipid peroxide (i.e., a lipid molecule containing one or more O–O bonds). Lipid peroxides break down at high temperatures to create a variety of bad-tasting and foul-smelling byproducts, including epoxides, ketones, acids, and aldehydes. Amphiphilic lipid extended bilayers with hydrophobic moieties oriented to the center and hydrophilic head groups at the two surfaces make up the majority of biological membranes. Polyunsaturated fatty acids (PUFAs), such as arachidonic and docosahexaenoic acid, are abundant in biological cell membranes, either in their pure state or when they have been integrated into triacylglycerides and phospholipids. Particularly vulnerable to oxidation are PUFAs.

Since the 1950s, there has been a great deal of research done on the relationship between lipid peroxidation and biology and human diseases due to growing worries about the possible negative effects of lipid peroxidation in cellular membranes (Halliwell *et al.* 1993, Schaich 2012).

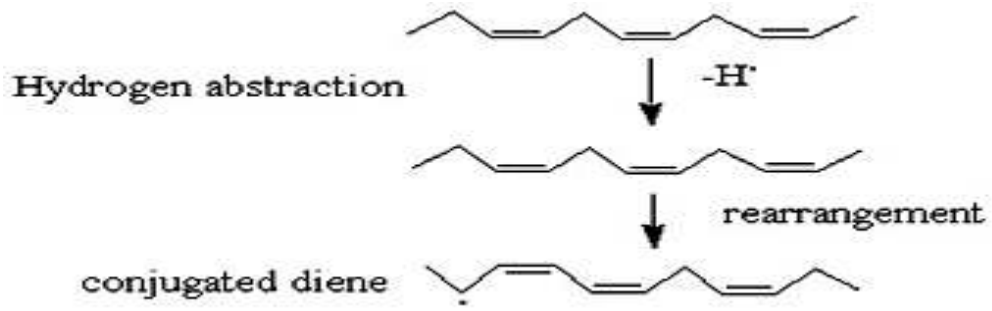


Figure 2.3 Initiation step of lipid peroxidation process

It is well known that both plants and animals experience the complex process of lipid peroxidation. It involves the development and spread of lipid radicals, the absorption of oxygen, the rearrangement of double bonds in unsaturated lipids, and the eventual destruction of membrane lipids. A number of breakdown products are produced, including alcohols, ketones, alkanes, aldehydes, and ethers (Dianzani and Barrera 2008, Repetto *et al.* 2012).

Polyunsaturated fatty acids suffer oxidative damage as a result of the chain process known as lipid peroxidation, which is started by the abstraction of hydrogen or the addition of an oxygen radical (PUFA). It is clear that the active methylene (RH) bridge represents a crucial target site since polyunsaturated fatty acids are more susceptible than saturated ones. The methylene C-H bond becomes weaker and more prone to abstraction when a double bond is present next to a methylene group. This causes a free electron to remain on the carbon, creating a radical with a carbon center that is stabilized by a chemical rearrangement of the double bonds to create a conjugated diene, which then reacts with oxygen to create a radical with a peroxyl center. A chain reaction can be started by the peroxyl radical itself by taking a hydrogen atom from another polyunsaturated fatty acid (Andreyev *et al.* 2015,Dianzani and Barrera 2008).

2.5 Interleukin-6 (IL-6)

Interleukin-6 (IL-6) is a pro-inflammatory cytokine that plays a critical role in the proliferation and differentiation of human cells. It promotes the production of numerous proteins involved in acute inflammation. IL-6, the transmembrane IL-6 receptor (mIL-6R) or soluble IL-6R (sIL-6R), and the signal-transducing component protein gp130 form a complex to mediate IL-6 signaling. As a result, there are three possible ways that IL-6 signaling might happen: by binding to the mIL-6R (traditional), the sIL-6R (trans-signaling), or the IL-6R and the gp130 on neighboring cells (trans-presentation). These routes, along with the fact that gp130 is expressed almost everywhere, cause IL-6 to have pleiotropic effects (Uciechowski and Dempke 2020). The suppression of IL-6 signaling is controlled by the presence of sIL-6R and gp130 forms in the blood as well as by the induction of suppressor molecules upon activation of the IL-6 pathways. Conversely, inflammatory and autoimmune illnesses, as well as the emergence of cancer, can be caused by an excess of IL-6 and a disruption of the IL-6 signaling pathways, indicating that IL-6 plays a crucial role in the human cytokine network. Inhibiting the cytokine itself, signaling through the IL-6 receptor, or target kinases (such JAK/STAT) linked to the signaling pathways have all been tested as potential therapeutic targets. Tocilizumab (an anti-IL-6R humanized antibody) and siltuximab (an IL-6 antagonist), among other things, have been licensed for the treatment of idiopathic multicentric Castleman's disease (iMCD), cytokine release syndrome, and rheumatoid arthritis. Although not all IL-6-associated disorders respond to IL-6 blocking, a deeper comprehension of the IL-6 pathways' underlying mechanisms may, in the near future, aid in the development of the most effective therapies for IL-6-associated diseases (Uciechowski and Dempke 2020, Mihara*et al.* 2012). Male immune infertility is caused by the seminal plasma molecules IL-6 and sICAM-1 (Shi *et al.* 2014).

2.6 Interleukin (IL)-8

Intercellular communication relies heavily on cytokines. They have crucial roles in the physiology of both women's and men's reproductive systems and are implicated in a wide range of physiological and pathological processes, most notably in the modulation

of inflammatory responses. In addition to their function in immunological regulation, there is evidence that several of these polypeptides play a direct role in the control of testicular function. They may also be effective regulators of the release of steroid hormones from the testes. The methods for controlling their action are intricate (Rees 1992, Leonard *et al.* 2019).

Both male and female reproductive tracts have a wide variety of immune response cells. These cells release lymphokines and monokines when activated, for instance by bacteria. They control immunological responses, at least in part, locally, but they also have an impact on tissues not covered by the immune system. A strong neutrophil chemotactic and activating factor is interleukin (IL)-8. It contributes to the processes of angiogenesis and adhesion and improves neutrophil attachment to endothelial cells and subendothelial matrix proteins. It binds to particular cell surface receptors in order to carry out its biological functions. Among a network of other cytokines, IL-8 may have a role in intratesticular signal transduction and may potentially have a negative impact on the characteristics of the sperm membrane. There haven't been many studies done on this cytokine's effects on reproduction (Buch *et al.* 1994).

Interleukins are involved in the local defense system against infectious infections, but they are also thought to be pathology-mediating agents. Lately, it has been proposed that an increase in IL-8 concentration that causes neutrophil recruitment may be crucial for the spread of viruses throughout the body. A human cytomegalovirus (CMV) infection, for instance, up-regulates IL-8 gene expression and induces transendothelial neutrophil migration, suggesting that IL-8 may boost virus generation in endothelial cells through an autocrine mechanism. IL-8 levels may change under specific pathological circumstances. Little is known about how IL-8 affects sperm quality and production, as well as whether it rises in the same ejaculates as leukocyte counts and other potential indicators of subclinical infection or inflammation (Grundy *et al.* 1998, Craigen*et al.* 1997).

2.7 Total Testeosterone Hormone

The hormone total testosterone, usually known as "TT," is created by the testes in men (testicles). It contributes to the formation of hair, bones, and sex organs including the penis and prostate. It also plays a role in the development of muscles and bones. It supports a man's perception of general wellbeing and sexual performance. Sperm production also requires testosterone. In accordance with Endocrine Society Recommendations, hypogonadal males who exhibit persistent symptoms of hypogonadism and low serum TT levels should be treated with exogenous total testosterone (TT) (Bhasin *et al.* 2010, Bhasin*et al.* 2006).

Men may experience symptoms such as decreased libido, erectile dysfunction, mood swings, irritability, exhaustion, or memory loss. Low serum TT levels in asymptomatic men can lead to insulin resistance, a decline in lean body mass and body strength, decreased bone mineral density, and harmful cardiovascular effects. The recommended course of treatment for males with symptomatic hypogonadism is TT replacement therapy, according to Endocrine Society recommendations. It is a tried-and-true, well-tolerated therapy for this population. TT replacement therapy has a number of known side effects, including polycythemia, liver dysfunction, poor lipid profiles, obstructive sleep apnea, and a possible increased risk of prostate cancer (Isidori *et al.* 2005, Pugh*et al.* 2004).

Not many doctors are aware of the effect that exogenous TT has on sperm production. Exogenous TT adversely affects spermatogenesis in fertile men, and most men recover when they stop taking it. 94% of the 1,549 eugonadal men who received TT treatment and were part of a meta-analysis conducted in 2006 regained sperm counts of >20 million/mL. The authors came to the conclusion that variations in individual baseline sperm counts were probably to blame for this inadequate recovery. However, it is unknown whether men who are infertile and taking TT would regain spermatogenesis. It's possible that the demographic of guys who present with infertility who are currently taking TT is different from the groups that had normal semen parameters in earlier studies. In the current study, we aimed to investigate the usage of TT in males seeking

assistance with infertility. We specifically examined the patterns and frequency of TT usage in males who presented with infertility, as well as the hormonal and semenal characteristics of these men both while using TT and after stopping. This is the first study to examine TT usage in male infertility (Liu et al. 2006,Bhasin *et al.* 2006).

2.7.1 The chemical information of testeosterone

As a sex steroid derived from cholesterol, testosterone has a four-ringed structure (Figure 2.4) with interlocked rings. The source of all naturally occurring steroid hormones is cholesterol. Because of the ring shape, testosterone and cholesterol are greasy and do not dissolve in water but rather clump together. They do dissolve in other greasy liquids, though. As water is polar while fats and oils are not, they are referred to as nonpolar molecules. Hence, the body must alter the structure of testosterone to make it soluble in water so that it may be eliminated through urination (Shiau *et al.* 2014, Williams *et al.* 1999).

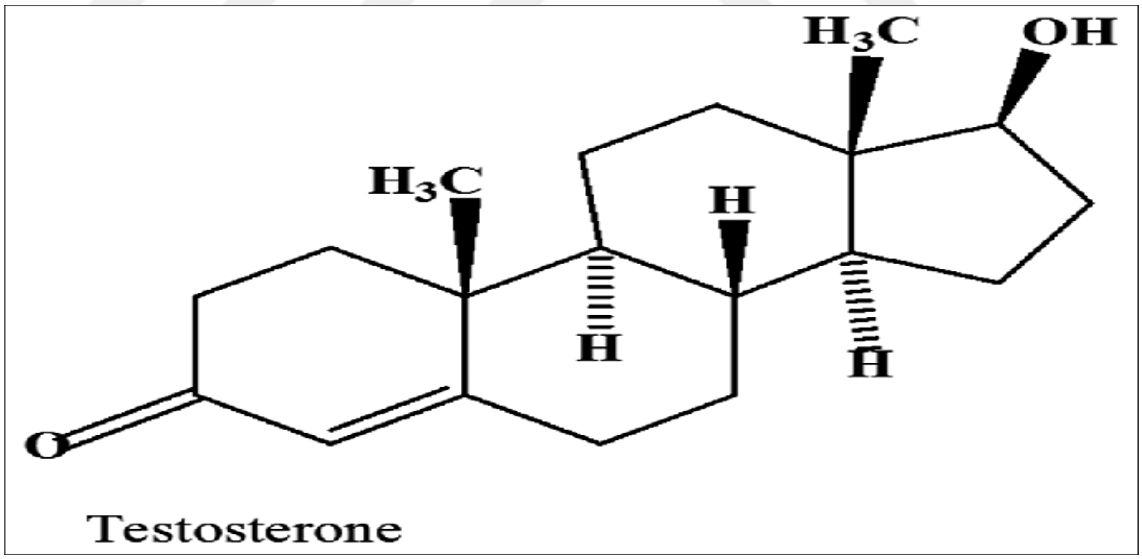


Figure 2.4 The chemical information of testeosterone

Cholesterol and testosterone both consist of four rings. A, B, C, and D are the names of the rings. The six carbons that make up rings A, B, and C give them a hexagonal form. Ring D has a pentagonal form since it contains five carbon atoms. Rings A and B are

joined together because they share a side. The sides of rings C and D are the same, thus they are also attached. The top side of ring B and one of the bottom sides of ring C are shared, forming a connection between the four rings. With this connection, the four rings form a flattened S shape, similar to two steps on a staircase (Semmens*et al.*1983, Williams *et al.* 1999).

2.7.2 Nonpolar testosterone

The ring structure of testosterone results in a molecule with a zig-zag form in three dimensions. The three-dimensional shape of testosterone resembles a three-piece folding lawn chair that has not yet been fully straightened out. As testosterone has an oily character due to its ring structure, it does not dissolve well in water. But in oily liquids, oily molecules do dissolve. This steroid can easily pass through a cell membrane due to its oily, water-averse nature. To keep the waters and salts inside the cell from mixing with the fluids and salts outside, the cell's membrane is oily (Shiau *et al*. 2014, Ding and Stallone 2001).

2.7.3 Testosterone Disposal

The body excretes excess or undesirable testosterone by depositing it in the urine. Water makes up the majority of urine, with several salts and compounds dissolved in it. Yet, the body's cells are unable to simply push testosterone into the bloodstream and then into the kidneys because testosterone is an oily molecule that does not dissolve in water. Only water-soluble compounds, like sodium chloride, respond to this kind of flushing. The body circumvents this issue by adding tiny molecules to testosterone utilizing enzymes. Like salt does when it dissolves in water, these molecules exhibit electrical charges or water-like characteristics. Because testosterone is soluble in water after being attached to these water-loving particles, the circulation flushes it out of the cell, down the kidneys, and into the urine (Elliot *et al.* 2007, Leonhardt*et al.* 2014).

3. MATERIALS AND METHODS

Male infertility is one of the most significant social issues that contemporary cultures face among all cases of infertility. Male infertility is most frequently caused by a problem with spermatogenesis. Regrettably, there is no medically curable explanation, such as hypogonadism, for men who experience inadequate spermatogenesis and become infertile. In such men, intracytoplasmic sperm injection (ICSI) or in vitro fertilization (IVF) in combination with surgical sperm extraction from the testicles offers some hope for conceiving. In this study, 155 samples were chosen to investigate infertility and our understanding of it. These samples included: 50 males with azoospermia who were 18 or older madeup Group A. Another 50 men with azoospermia made up Group B. And 55 men with polyspermia made up Group C. (control group). The group, which was split into patient (Oligozoospermia and azoospermia) and healthy (the control group) groups based on the amount of sperm, was then separated into three groups as follows:

3. Group A: Consists of 50 men with Oligozoospermia disease,
4. Group B: Consists of 50 men with azoospermia disease,
5. Group C: Consists of 55 men with normospermia (Control group).

3.1 Blood Samples

6 ml of blood samples were taken from the patients in order to conduct the tests. For the tests that need whole blood, the sample was used directly. As for the tests that need blood serum, the blood sample was separated using a centrifuge at a speed of 2000 revolutions per minute for 5 minutes. After that, the serum was used for testing.

3.2 Chemicals Used andMaterial

IL-6, IL-8, Lipid peroxidation, LH, FSH levels and some biochemical markers will be measured in patients' groups and controls group in men with infertility disease in men.

The instruments listed below will be using in accordance with the modus operandi specified by the manufacturing company and the mentioned: -

- Cobas C111
- Cobas E411
- 2- Incubator
- 3- Mini VIDAS
- 4- Spectrophotometer
- 5- ELISA
- 6- Other necessary material

The tests listed below will be carried out in accordance with the modus operandi (kit user manual) specified by the manufacturing company.

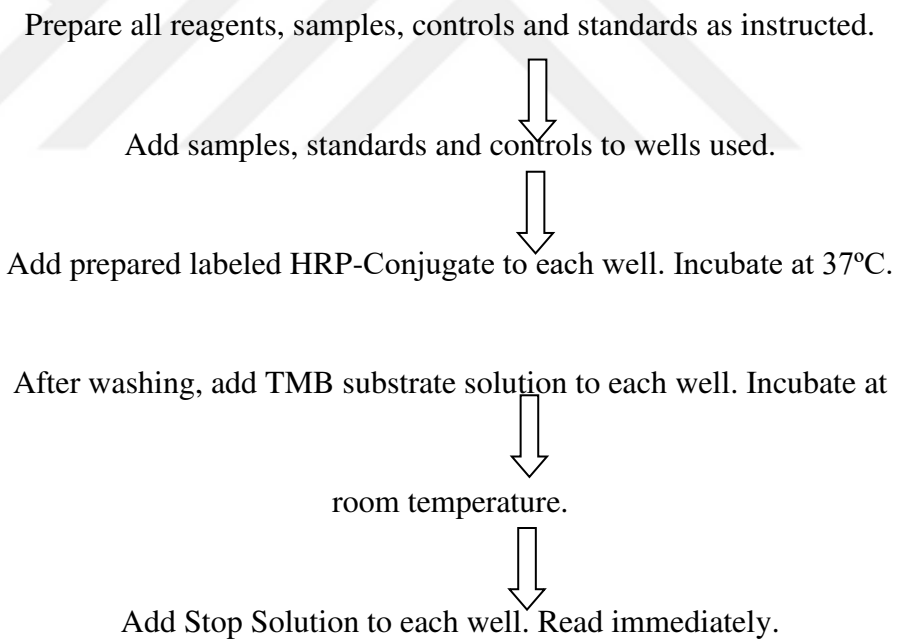
3.3 Test of study

- Age
- Weight
- BMI
- Testosterone hormone
- IL-6
- IL-8
- FSH
- LH
- Lipid peroxidation (MDA)
- Vitamin E
- Total cholesterol
- Triglyceride
- HDL, LDL and VLDL

3.3.1 Testosterone ELISA kit (ab108666)

A 96-well plate has been precoated with anti-Testosterone antibodies. Samples and the Testosterone-HRP conjugate are added to the wells, where Testosterone in the sample competes with the added Testosterone-HRP for antibody binding. After incubation, the wells are washed to remove unbound material and TMB substrate is then added which is catalyzed by HRP to produce blue coloration. The reaction is terminated by addition of Stop Solution which stops the color development and produces a color change from blue to yellow. The intensity of signal is inversely proportional to the amount of Testosterone in the sample and the intensity is measured at 450 nm. Testosterone (17β-Hydroxy-4-androstene-3-one) is a steroid hormone from the androgen group.

Protocol Summary



Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use for at least 30 minutes.

- At the end of the assay store the reagents immediately at 4°C; avoid long exposure to room temperature.
- Prepare only as much reagent as is needed on the day of the experiment.

Sample Preparation

The determination of Testosterone can be performed in urine, and plasma as well as in serum. If the assay is performed on the same day of sample collection, the specimen should be kept at 4°C; otherwise, it should be aliquoted and stored deep-frozen (-20°C). If samples are stored frozen, mix thawed samples gently for 5 minutes before testing. Urine may be used undiluted in this assay.

Assay Procedure

- Prepare all reagents, working standards, and samples as directed in the previous sections.
- Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
- Add 25 µL standards, control and samples into their respective wells. Add 100 µL Testosterone-HRP Conjugate to each well. Leave a blank well for substrate blank.
- Cover wells with the foil supplied in the kit and incubate for 1 hour at 37°C.
- Remove the foil, aspirate the contents of the wells and wash each well three times with 300 µL of 1X Wash Buffer. Avoid spill over into neighboring wells. The soak time between each wash cycle should be >5 sec. After the last wash, remove the remaining fluid by aspiration or decanting. Invert the plate and blot it against clean paper towels to remove excess liquid. Automatic washer: in case you use an automatic washer, it is advised to do 6 washing steps.
- Note: Complete removal of liquid at each step is essential for good assay performance.
- Add 100 µL TMB Substrate Solution into all wells.

- Incubate for 15 minutes at 37°C in the dark. Incubation at lower temperature may require longer incubation timing.
- Add 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. Shake the microplate gently. Any blue color developed during the incubation turns into yellow.
- Measure the absorbance of the sample at 450 nm against a reference wavelength of 620-630 nm within 30 min after addition of the Stop Solution or against blank within 5 minutes.

3.3.2 Human IL-6 ELISA kit (ab178013)

Storage and Stability: Store kit at +4°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Materials Supplied

- Item Quantity Storage Condition
- Human IL-6 Lyophilized Capture Antibody 1 vial +4ºC
- Human IL-6 Detector Antibody 10X 600 µL +4ºC
- Human IL-6 Lyophilized Recombinant Protein 2 Vials +4ºC
- Antibody Diluent 5BI 6 mL +4ºC
- Wash Buffer PT 10X 20 mL +4ºC
- TMB Substrate 12 mL +4ºC
- Stop Solution 12 mL +4ºC
- Sample Diluent NS 50 mL +4ºC
- Anti-tag coated microplate (12 x 8 well strips) 96 Wells +4ºC
- Plate Seal 1 +4º.

Reagent Preparation

Plasma: Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples into Sample Diluent NS and assay. Store un-diluted plasma samples at - 20ºC or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples into Sample Diluent NS and assay. Store un-diluted serum at -20ºC or below. Avoid repeated freeze-thaw cycles. Other tests we will used according accordance with the modus operandi specified by the manufacturing company.

3.4 Statistical analysis

For the statistical analysis, and because of the presence of more than one study group, the ANOVA one-way test was used, which is one of the tests of the statistical program SPSS 25. And through the use of this program, the averages between the variables in this study were reached. Also, in the same study, the Pearson test was used in order to find the correlations between the variables at P = 0.05.

4. RESULTS AND DISCUSSION

Aimed this study was aimed to assess the Evaluation of vitamin E, Lipid peroxidation, IL-6, IL-8 and some hormones in serum and seminal plasma (Oligozoospermia and azoospermia) of infertile men. And research into the extent of changes that occur in immunological tests, which may lead us to identify the causes of changes in some trace elements and important hormones in the formation of sperm in men. 155 samples were studied, where group A consisted of 50 men with azoospermia aged 18 and over, group B: consisted of 50 men with azoospermia and group C: consisted of 55 men who had no problems with azoospermia (control group). They were selected, which were divided according to the number of sperm into the patient's group (Oligozoospermia and azoospermia) and healthy groups (the control group) into three groups, and the results of the study were as follows:

4.1 The Results of Age, Weight and BMI with Three Groups Study

The results of the study concluded that there was a significant statistical for age when comparing group B (43.1858 ± 15.158) with the control group (26.8000 ± 6.0759), and the same for group C (42.1600 ± 8.7781) was statistically significant when comparing its results with the control group (26.8000 ± 6.0759) as in Table 4.1 and Figure 4.1. As for weight, there were statistically significant differences between group Bwhen compared to the control group (80.320±6.4467), while there are no statistically significant differences between group C when compared to group A (the control). As for body mass index, there are no statistically significant differences between group B (24.504 ±2.8527) and group C (25.924 ±3.4774) when compared to the control group (24.290 ±2.3195), as in (Table 4.1 and Figure 4.1).

Table 4.1 The results of statistic of age, weight and BMI

Age, weight and BMI descriptives								
Group study & Parameters		Mean	Std. Deviation	Std. Error	95%CIM		Minimum	Maximum
					Lower Bound	Upper Bound		
Age	Group AC	26.8000	6.0759	1.215	24.29	29.30	18.00	41.00
	Group BP	43.1858[a]	15.158	3.031	36.92	49.44	24.00	84.00
	Group CP	42.1600[a]	8.7781	1.755	38.53	45.78	29.00	61.00
	Total	37.3819	12.977	1.498	34.39	40.36	18.00	84.00
weight	Group AC	80.320	6.4467	1.289	77.65	82.98	68.00	92.00
	Group BP	84.560[b]	6.8073	1.361	81.75	87.36	69.00	99.00
	Group CP	91.800[a]	9.4604	1.892	87.89	95.70	79.00	112.00
	Total	85.560	8.9612	1.034	83.49	87.62	68.00	112.00
BMI	Group AC	24.290	2.3195	0.463	23.33	25.24	19.86	27.78
	Group BP	24.504[a]	2.8527	0.570	23.32	25.68	19.57	29.58
	Group CP	25.924[a]	3.4774	0.695	24.48	27.35	19.68	32.77
	Total	24.906	2.9730	0.343	24.22	25.59	19.57	32.77

95%CIM = 95% Confidence Interval for Mean

a= Significant P >0.05, b= N.S (P < 0.05)

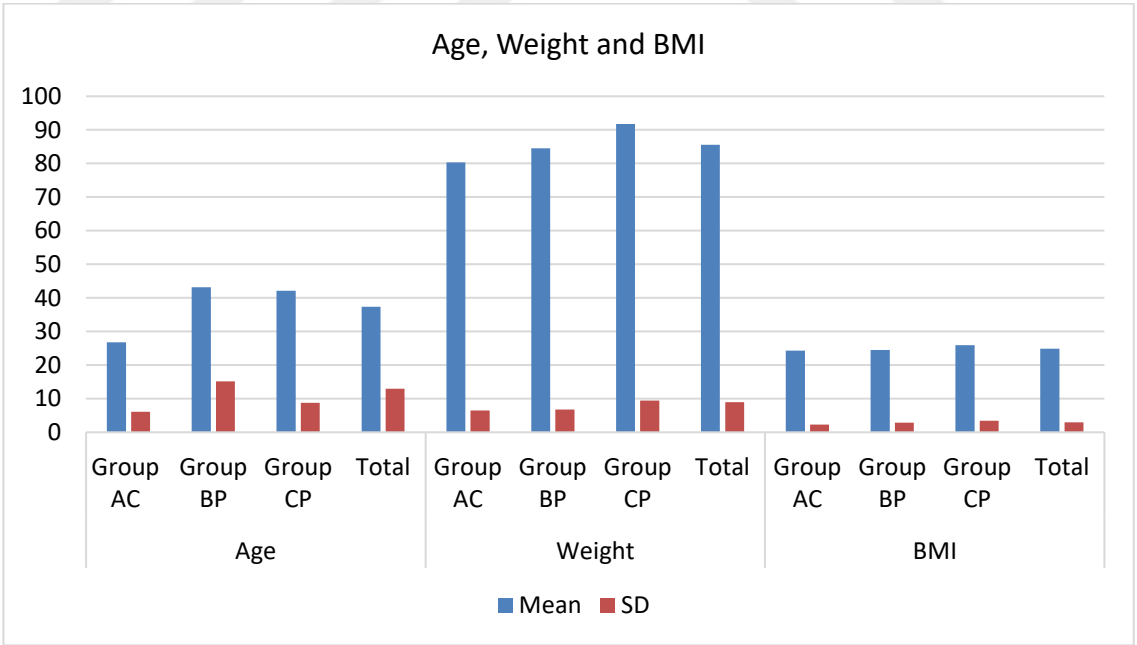


Figure 4.1 The graph of age, weight and BMI

This study provides additional proof that men who are overweight are more likely to experience infertility. Values might be overestimated because in the worst circumstances, infertile couples (Nguyen *et al.* 2007).

The median SHBG levels rose throughout age quartiles and fell as BMI increased (all P 0.001). More SHBG levels decreased with increasing BMI than SHBG gradually increased with age. The findings revealed that primary infertile males had a wide range of SHBG concentrations across age and BMI levels. The relationship between BMI and lower SHBG levels appears to be stronger than the relationship between aging and higher SHBG (Boeri *et al.* 2020).

According to studies done at the National Institute of Environmental Health Sciences (NIEHS), one of the National Institutes of Health, men with higher body mass index (BMI) were substantially more likely to be infertile than men of normal weight. The principal author of the study, Markku Sallmen, who is currently employed at the Finnish Institute of Occupational Health, states that the statistics "indicate that a 20-pound rise in men's weight may increase the likelihood of infertility by roughly 10%." A person's weight and height are used to calculate their BMI. For the majority of people, BMI gives a trustworthy estimate of body fatness and is used to check for weight categories that may cause health issues (Katib 2015).

4.2 The Results of Total Testeosterone with Three Groups Study

The results of total testosterone indicate that there are high significant differences between group B(430.36 ±102.73) and group C (541.08 ±122.10) when comparing their results with group A (the control group), as in (Table 4.2 and Figure 4.2).Infertility is one of the negative effects of testosterone therapy. By lowering levels of another hormone, follicle-stimulating hormone (FSH), which is crucial for promoting sperm formation, testosterone therapy reduces sperm production. The infertility brought on by testosterone therapy is typically reversible.

Table 4.2 The results of statistic of total testosterone

Total testeosterone descriptives								
Group study & Parameters		Mean	Std. Deviation	Std. Error	95%CIM		Minimum	Maximum
					Lower Bound	Upper Bound		
TT	Group AC	384.00	84.79	27.158	427.94	540.0	299.0	745.0
	Group BP	430.36[a]	102.73	28.547	371.44	489.2	209.0	857.0
	Group CP	541.08[a]	122.10	44.421	449.39	632.7	206.0	957.0
	Total	485.14	175.09	20.218	444.86	525.4	206.0	957.0
95%CIM = 95% Confidence Interval for Mean a= Significant P >0.05, b= N.S (P < 0.05)								

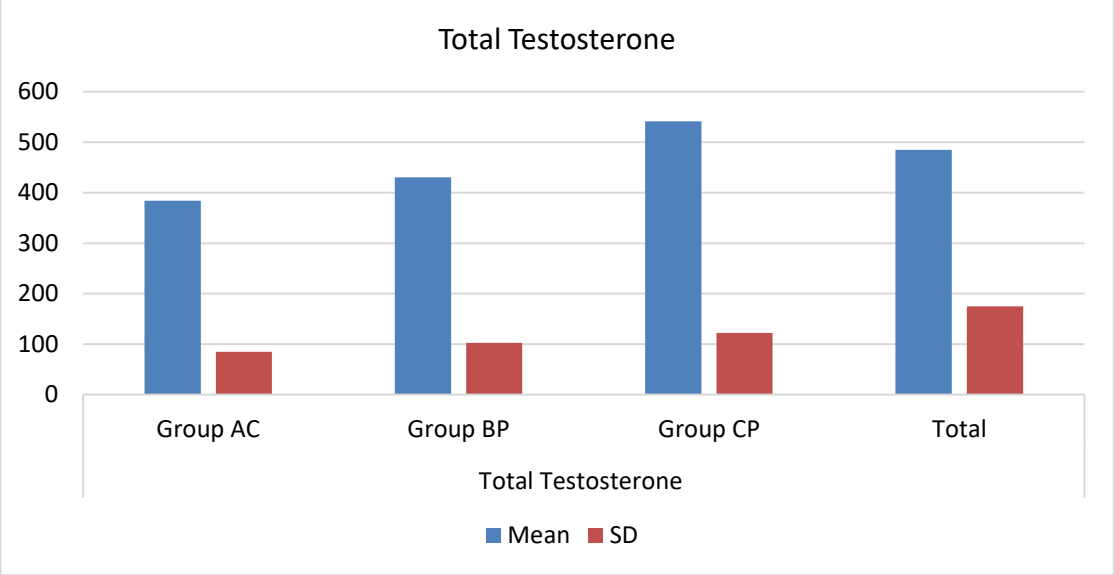


Figure 4.2 The graph of total testosterone

One participant in the study took a T.T. that was recommended by numerous medical professionals, including endocrinologists. The study discovered that men's semen parameters improved along with their testosterone levels. Within 6 months of quitting T, almost 2/3 of infertile men use T spermatogenesis (Samplaski *et al.* 2014).

Infertility is a result of low testosterone. Other hormones than testosterone are in fact responsible for stimulating sperm production. Although testosterone is necessary for the creation of sperm, the level in the testes where sperm are created is significantly higher

than the level in the circulation. It's possible for men with low or borderline T levels to still produce sperm. Men who have low T levels and low T symptoms may want to consider getting help. Men with low T levels can produce sperm by receiving medication in the form of injections, gels, patches, or implanted pellets (Ohlander *et al.* 2016).

4.3 The Results of Il-6 and Il-8 with Three Groups Study

As for interleukin-6, there were significant differences in group B when comparing its results to group A (the control group), while for group C (1.0801 ±0.75842), it indicated that there were non-significant differences when comparing its results to the control group, as in (Table 4.3 and Figure 4.3). The results of interleukin-8 indicated that there was a significant increase in its levels in group B when compared to group A (18.7793 ±9.79819). As for group C, the results indicated that there were significant differences, and they tended towards an increase, as in (Table 4.3 and Figure 4.4). These changes may be due to the large number of treatments given to the patient, which causes a direct effect on the immune variables.

Table 4.3 The results of statistic of IL-6 and IL-8

IL-6 and IL-8 descriptives								
Group study & Parameters		Mean	Std. Deviation	Std. Error	95%CIM		Minimum	Maximum
					Lower Bound	Upper Bound		
IL6	Group AC	1.2378	1.96206	0.392	0.427	2.047	0.34	10.48
	Group BP	2.3568[a]	1.44166	0.288	1.761	2.951	0.23	5.58
	Group CP	1.0801[a]	0.75842	0.151	0.767	1.393	0.08	2.58
	Total	1.5582	1.56095	0.180	1.199	1.917	0.08	10.48
IL8	Group AC	18.7793	9.79819	1.959	14.73	22.82	7.58	45.76
	Group BP	53.4800[a]	17.3159	3.463	46.33	60.62	27.00	84.00
	Group CP	45.4000[a]	23.2808	4.656	35.79	55.00	11.00	92.00
	Total	39.2198	22.9548	2.650	33.93	44.50	7.58	92.00
95%CIM = 95% Confidence Interval for Mean a= Significant P >0.05, b= N.S (P < 0.05)								

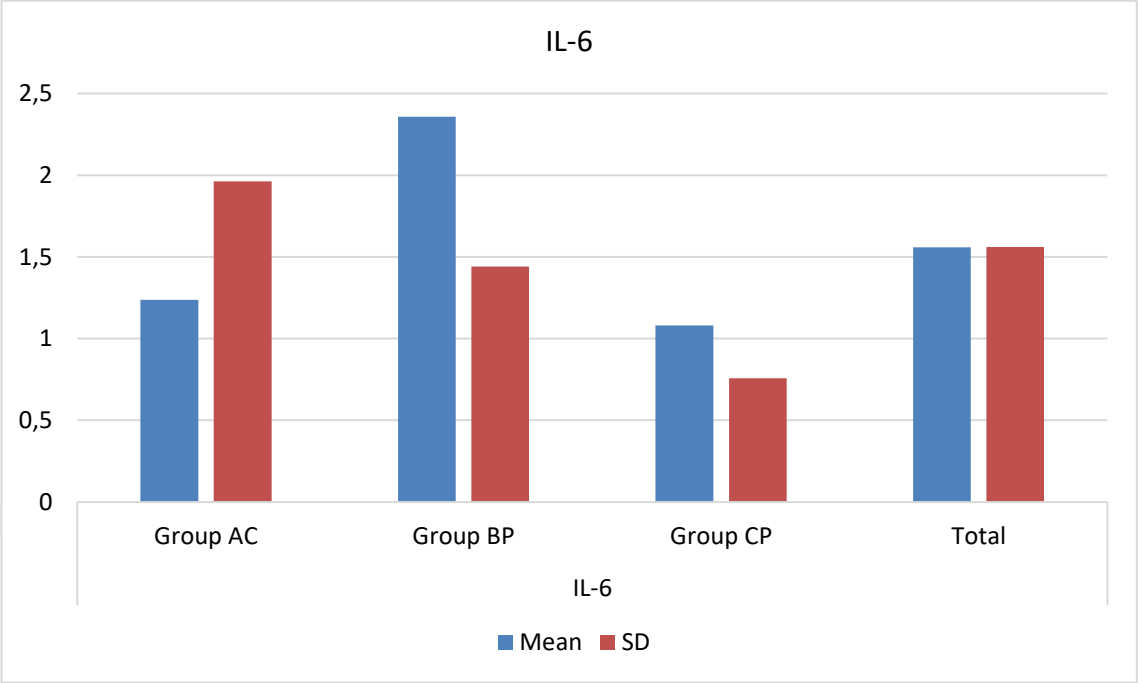


Figure 4.3 The graph of IL-6

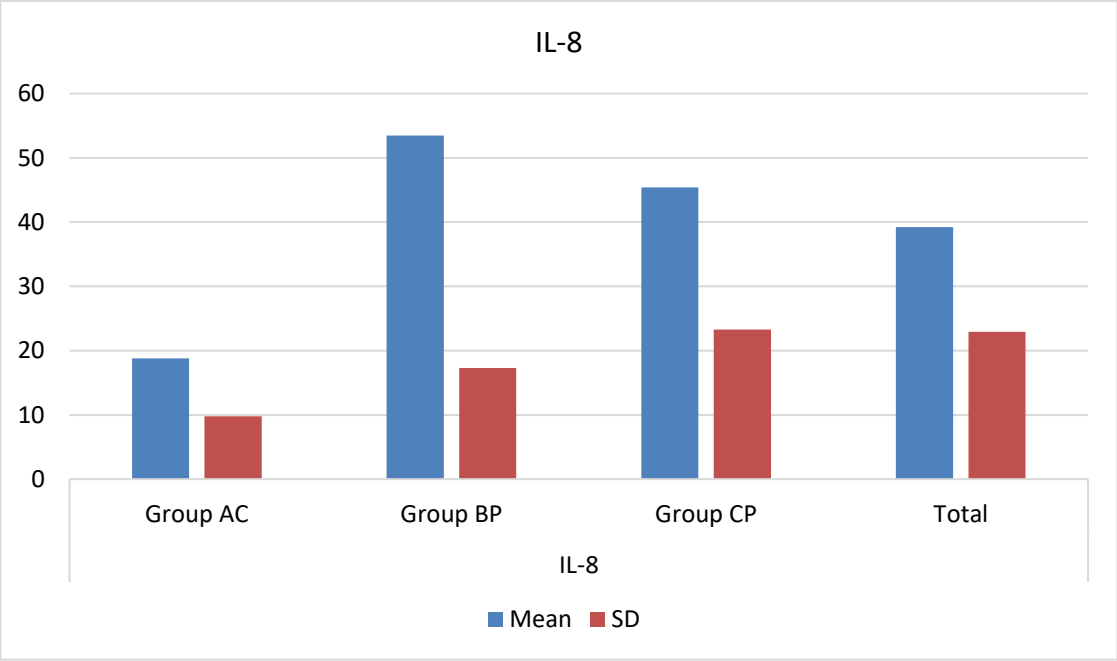


Figure 4.4 The graph of IL-8

The content of interleukin (IL)-8 and IL-6 in seminal plasma (SP) of subfertile males was evaluated in order to assess the association with other potential markers of

subclinical infection/inflammation, such as seminal leukocytes, and with semen quality in a prospective research. None of the individuals had any symptoms of genital tract infections. Two indicators of the quality of the semen that were negatively connected with the SP concentration of IL-8 were the quantity of mobile spermatozoa and the outcomes of the sperm migration test (motile sperm harvested after a swim-up procedure). Leukocyte ratio, leukocyte counts per ml, and leukocyte counts per ejaculate were all significantly correlated with IL-8 concentrations (P 0.0001) (P 0.001). All leukocytospermic samples had high IL-8 levels (2 ng/ml). Despite being much lower, the SP IL-6 concentration had a strong correlation with the IL-8 concentration (P 0.0001). There were significant connections between IL-8, IL-6, and the C3c. The bacterial colonization of semen samples did not correlate with interleukin levels. The results demonstrate a direct relationship between a number of pro-inflammatory cytokines and semen quality. Due to its significant association with seminal leukocytes and other potential inflammation markers, IL-8 may be used as a sensitive diagnostic for silent male genital tract infection (Eggert-Kruse *et al.* 2001).

In terms of sperm concentration (P> 0.05) or semen liquefaction time (P> 0.05), there were no discernible differences between the immunological infertility groups and the fertile control, but the former did have significantly lower permanent viability (P=0.05) and progressive motility (P=0.01) than the former. No seminal parameters between the immunological infertility group and other infertility groups demonstrated statistically significant differences (P> 0.05) (Shi *et al.* 2014).

4.4 The Results of FSH and LH with Three Groups Study

FSH results indicated that there were high significant differences in group B (11.7263 ±4.81034) when compared to group A (the control group), on the contrary, the results of group C (11.0379 ±4.17732) were close to group B, indicate to statistical significance when compared to control group, as in (Table 4.4 and Figure 4.5). As for LH, the results also indicated that there were high statistical and significant differences in group B when comparing its results with group A (the control group), there were lower statistical

differences between group Cwhen compared tothe control group, as in (Table 4.4 and Figure 4.6).

Table 4.4 The results of statistic of FSH and LH

FSH and LH descriptives								
Group study & Parameters		**Mean**	**Std. Deviation**	**Std. Error**	**95%CIM**		**Mini mum**	**Maxi mum**
					Lower Bound	**Upper Bound**		
FSH	**Group AC**	4.6620	1.81712	0.363	3.911	5.412	1.76	7.76
	Group BP	11.7263[a]	4.81034	0.962	9.740	13.71	4.58	21.68
	Group CP	11.0379[a]	4.17732	0.852	9.273	12.80	3.66	20.58
	Total	9.1164	4.95305	0.575	7.968	10.26	1.76	21.68
LH	**Group AC**	3.8471	1.74728	0.349	3.125	4.568	1.58	7.07
	Group BP	9.9755[a]	3.56844	0.760	8.393	11.55	1.98	16.58
	Group CP	7.0488[a]	3.17043	0.661	5.677	8.419	1.97	13.59
	Total	6.8252	3.81271	0.455	5.916	7.734	1.58	16.58
95%CIM = 95% Confidence Interval for Mean a= Significant P >0.05, b= N.S (P < 0.05)								

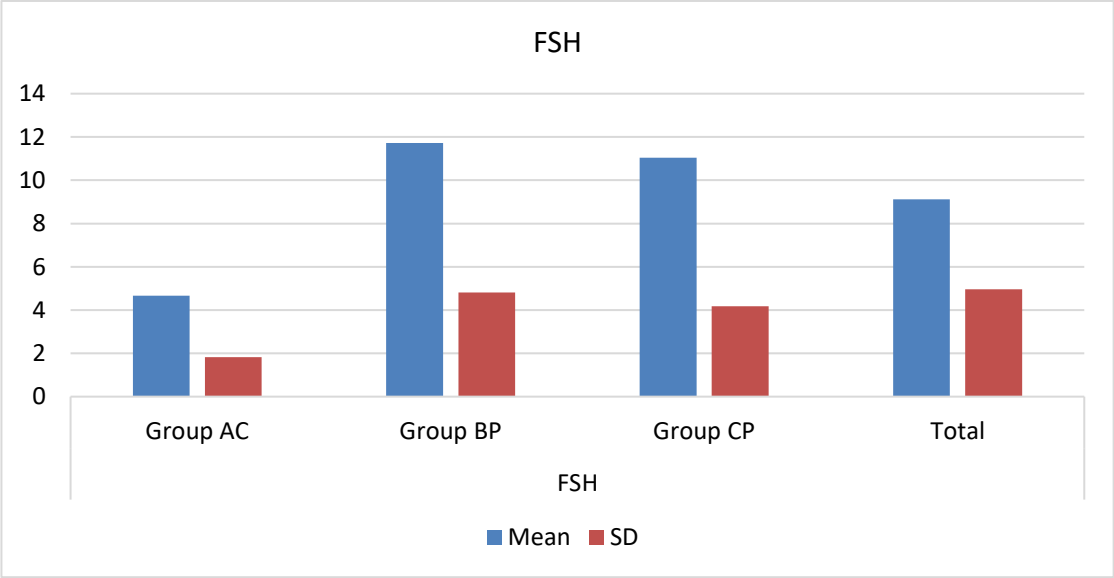


Figure 4.5 The graph of FSH

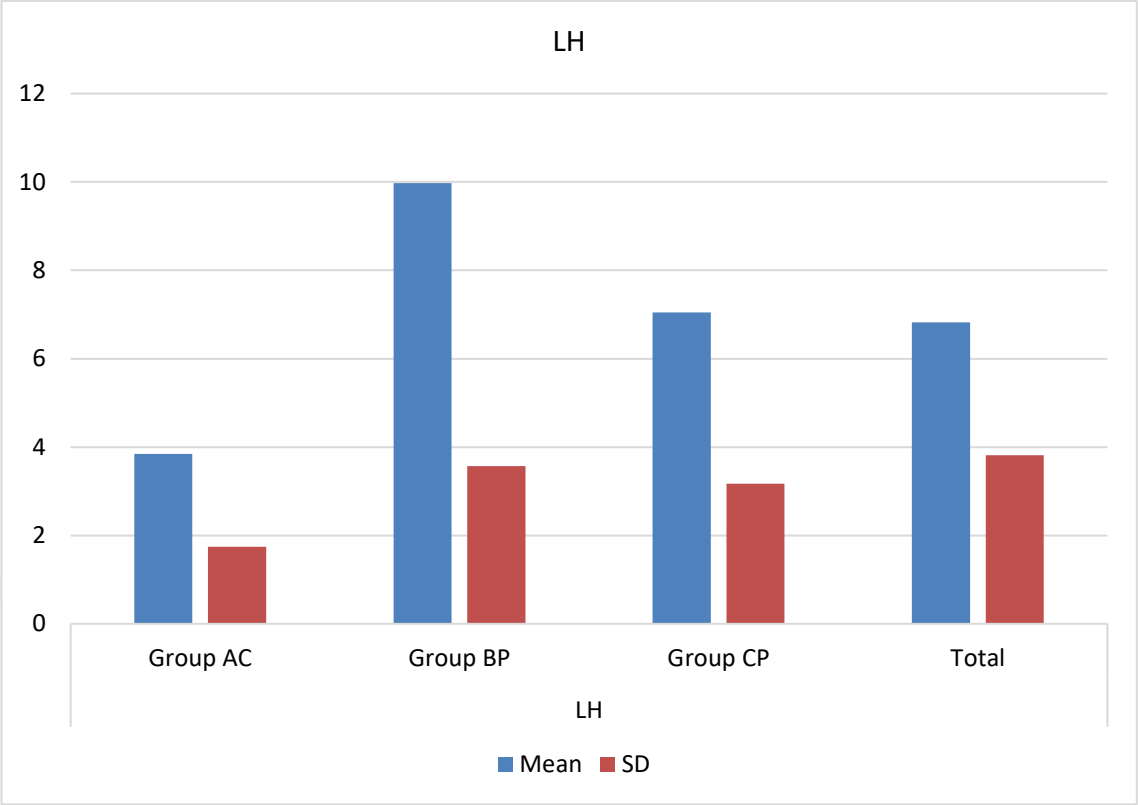


Figure 4.6 The graph of LH

Infertility is one of the negative effects of testosterone therapy. By lowering levels of another hormone, follicle-stimulating hormone (FSH), which is crucial for promoting sperm formation, testosterone therapy reduces sperm production. The infertility brought on by testosterone therapy is typically reversible (Ramasamy and Arora 2021).

Beginning with the physiological interactions between the testis and the hypothalamo-hypophysical unit, endocrinological elements of male infertility are examined. Testicular dysfunction and infertility are caused by the pituitary's inability to secrete follicle stimulating hormone (FSH) and luteinizing hormone (LH). Gonadotropin insufficiency, however, only makes for less than.5% of the causes of infertility in men. A hypothalmic or pituitary lesion should be sought after if deficits in FSH or LH are found. FSH, LH, and prolactin measurements are nevertheless helpful in the treatment of male infertility. The most crucial test is the measurement of serum FSH, which, when the concentration is correlated with sperm density, offers a useful index of the condition of the seminiferous epithelium. A high concentration associated with severe

oligospermia or azoospermia typically indicates untreatable infertility. About 30% of males with significant testicular injury had elevated LH and low testosterone levels, which are signs of interstitial cell failure. Prolactin levels are more frequently linked to impotence than infertility. It is frequently recommended to treat male infertility using hormones (Sokol 2009, Krausz 2011).

The treatment of individuals with hypogonadotropic hypogonadism to stimulate spermatogenesis, which enables the induction of a clinical pregnancy in the female partner, and ultimately the birth of a healthy child, is the accepted clinical indication for the use of FSH in male infertility. However, with novel hormonal treatment options now available for female infertility treatment, advances in therapy may be anticipated. In most nations, the use of FSH as a treatment for people with normogonadotropic idiopathic infertility and oligozoospermia is still regarded as experimental. FSH can greatly improve the likelihood of conception in these patients' female partners, but the impact size is quite small, according to recent meta-analyses. In order to choose the appropriate normogonadotropic patients with idiopathic infertility for FSH medication, predictive criteria for treatment effectiveness, including FSH pharmacogenetics, must be found (Behre 2019).

4.5 The Results of MDA and Vitamin E with Three Groups Study

Also, with regard to the AMD test, the results indicated that there was lower a statistical significance for group B (29.2821 ±15.9023) when comparing its results with the control group (21.7338 ±6.77437), while there were high statistical differences between group C and group A (the control group), as in Table (4.5 and Figure 4.7). The results of vitamin E also indicated that there were statistically significant clinical differences towards height in group B when comparing its results with the control group, while the results in group Cmore statistically signofocant with the control group, as in (Table 4.5 and Figure 4.8).

Table 4.5 The results of statistic of MDA and vitamin E

AMD and vitamin E descriptives								
Group study & Parameters		Mean	Std. Deviation	Std. Error	95%CIM		Minimum	Maximum
					Lower Bound	Upper Bound		
MDA	Group AC	21.7338	6.77437	1.354	18.93	24.53	11.68	33.67
	Group BP	29.2821[a]	15.9023	3.180	22.71	35.84	11.76	64.77
	Group CP	36.5542[a]	19.1633	3.832	28.64	44.46	8.55	80.57
	Total	29.1900	15.9094	1.837	25.52	32.85	8.55	80.57
VitE	Group AC	10.7818	3.59559	0.719	9.297	12.26	3.77	16.77
	Group BP	6.7916[a]	4.06304	0.812	5.114	8.468	1.58	15.85
	Group CP	7.9718[a]	3.94580	0.789	6.343	9.600	2.67	16.86
	Total	8.5151	4.17559	0.482	7.554	9.475	1.58	16.86

95%CIM = 95% Confidence Interval for Mean

a= Significant P >0.05, b= N.S (P < 0.05)

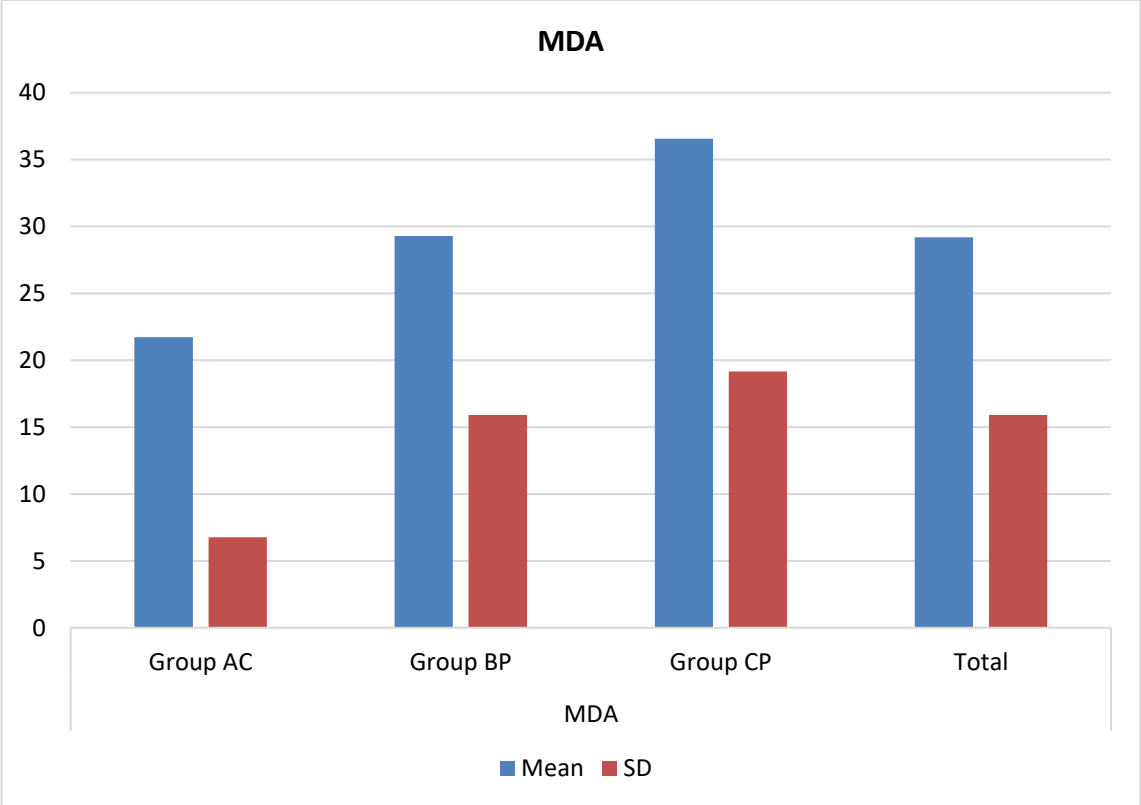


Figure 4.7 The graph of MDA

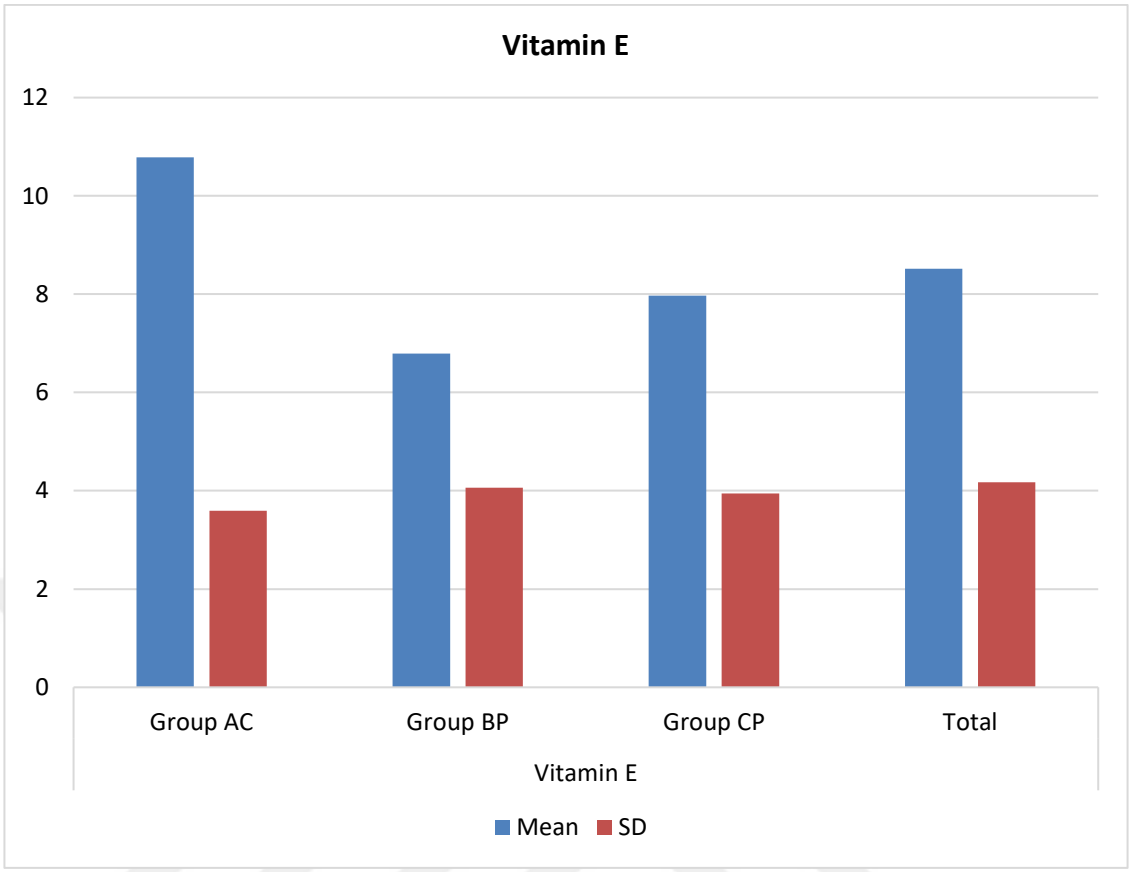


Figure 4.8 The graph of vıtamın E

Malondialdehyde (MDA) levels and the relationship with semen (MDA) were assessed in all patients of the infertile subgroup, and the present results showed that there is a negative relationship between the level of MDA and sperm motility percent active and a positive relationship between MDA and abnormal sperm morphology. This study also suggests that simvastation may be possible (Al-Hilli 2009).

Spectrofluorometry was used to calculate the levels of seminal MDA. In comparison to infertile groups, the fertile group displayed considerably higher values for sperm concentration, motility, and fertility index. Sperm motility, concentration, apoptosis, and fertility index did not differ significantly between the infertile groups. The percentage of necrosis in the infection group was significantly higher than that seen in the groups of fertile men, varicocele, and unexplained infertility (p 0.001). MDA levels considerably rose in the infection group when compared to the varicocele group, the group experiencing idiopathic infertility, and the group of fertile men (p 0.01), and in

the varicocele group when compared to the group experiencing idiopathic infertility (p 0.001). MDA levels were positively connected with sperm concentration (p 0.01), fertility index (p 0.05), and necrosis (p 0.001) in the infection group, but negatively correlated with motility (p 0.01). MDA levels in the varicocele group showed a positive correlation with necrosis and a negative correlation with immaturity (p 0.05). The idiopathic infertility group and the fertile men did not exhibit any association. As a conclusion, we propose that assessment of seminal MDA may serve as a useful marker for identifying the illnesses that cause a reduction in sperm motility, such as urogenital infections or inflammatory state (Collodel *et al.* 2015).

Comparing the VitE group to the control group, the men's sperm rate was clearly higher in the VitE group. Progressive motility, sperm concentration, morphology, and total sperm number all saw substantial improvements thanks to vitamin E. According to this study, VitE may help infertile men become pregnant more frequently and have healthier semen (Zhou *et al.* 2022).

4.6 The Results of Lipid Profile with Three Groups Study

The results of the study of the lipid profile profile found that there was a significant statistical significance for total cholesterol in group B (184.0400 ±48.5510) when compared with the control group, and the same for group C (210.0833 ±55.7961) was statistically slightly significant when comparing its results with the control group. As for triglycerides, there were statistically significant differences between group B (111.9600 ±28.2230) when compared to the control group, while there were slightly statistically significant differences between group C compared to group A (the control), and as for HDL and VLDL, there is no There are statistically significant differences between group B and group C when compared to the control group. The LDL, there were statistical differences between group B and group C when comparing their results to group A (the control group), as in T(able 4.6 and Figure 4.9).

Table 4.6 The results of statistic of lipid profile

Lipid profile descriptives								
Group study & Parameters		Mean	Std. Deviation	Std. Error	95%CIM		Mini mum	Maxi mum
					Lower Bound	Upper Bound		
Tc	Group AC	168.9200	23.3450	4.669	159.2	178.5	135.0	211.00
	Group BP	184.0400[b]	48.5510	9.710	163.9	204.0	132.0	308.0
	Group CP	210.0833[a]	55.7961	11.38	186.5	233.6	135.0	301.0
	Total	187.3784	47.1699	5.483	176.4	198.3	132.0	308.0
Tg	Group AC	88.2400	15.0590	3.011	82.02	94.45	46.00	113.00
	Group BP	111.9600[a]	28.2230	5.644	100.3	123.6	79.00	175.0
	Group CP	100.6000[b]	25.7665	5.153	89.96	111.23	57.00	157.0
	Total	100.2667	25.3437	2.926	94.43	106.0	46.00	175.0
HDL	Group AC	46.9504	6.78054	1.356	44.15	49.74	33.67	58.68
	Group BP	47.6400[b]	7.73455	1.546	44.44	50.83	32.00	61.00
	Group CP	47.3600[b]	10.4756	2.095	43.03	51.68	29.00	66.00
	Total	47.3168	8.36575	0.965	45.39	49.24	29.00	66.00
LDL	Group AC	104.3216	24.8705	4.974	94.05	114.58	69.02	140.5
	Group BP	114.0080[b]	46.2962	9.259	94.89	133.11	59.00	230.2
	Group CP	140.9272[a]	54.3433	10.86	118.4	163.3	69.20	229.0
	Total	119.7523	45.7887	5.287	109.2	130.2	59.00	230.2
VLDL	Group AC	17.6480	3.01180	0.602	16.40	18.89	9.20	22.60
	Group BP	22.3920[a]	5.64461	1.128	20.06	24.72	15.80	35.00
	Group CP	20.1200[b]	5.15332	1.030	17.99	22.24	11.40	31.40
	Total	20.0533	5.06875	0.585	18.88	21.21	9.20	35.00

95%CIM = 95% Confidence Interval for Mean
a= Significant P >0.05, b= N.S (P < 0.05)

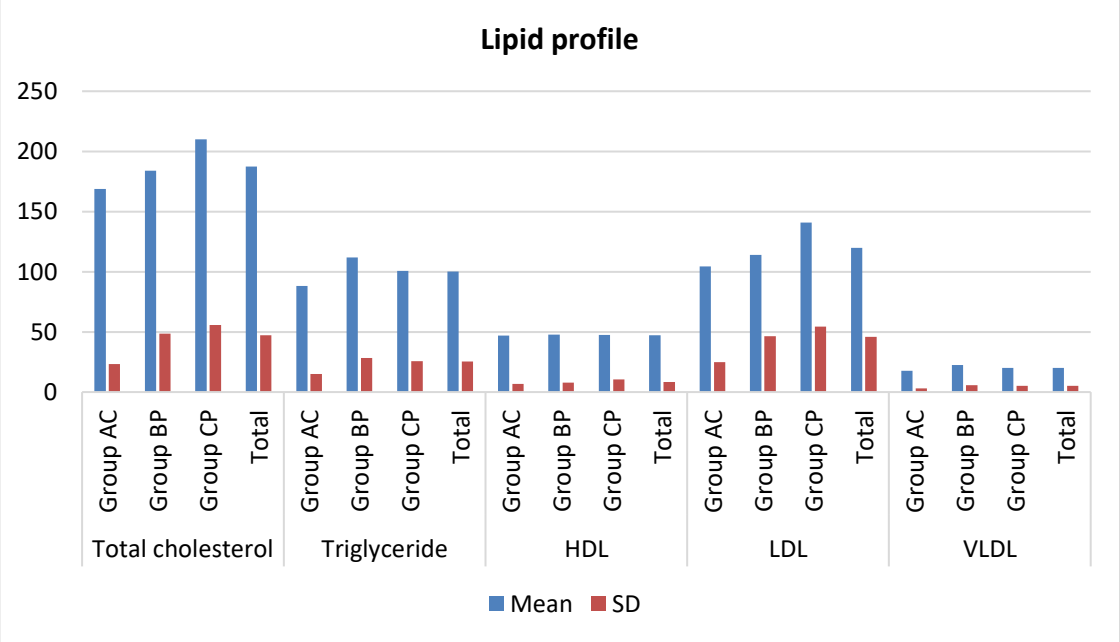


Figure 4.9 The graph of lipid profiie

Despite the fact that male infertility is common, the majority of cases are classified as idiopathic, which restricts the range of available treatments and increases the likelihood that assisted reproductive technologies will be used. As our study found that lipid stress has a significant impact on the death of male germ cells, we would want to emphasize the significance of lipid homeostasis in this context. Improving lipid metabolism and narrowing our knowledge of the many functions that lipids play in sperm activity are both necessary to increase sperm production (Walters *et al.* 2020).

An alarming correlation between male infertility and poor physical health has emerged over the past ten years, with strong evidence pointing to an increased risk of oncological disease, cardiovascular disease, metabolic disorders, and autoimmune diseases in men who have previously been diagnosed with subfertility. The infertile phenotype may be used as a sign of probable pathological disorders in this paradigm, which is worrying but may also open up a new avenue for important health change. The lack of knowledge of the molecular characteristics that connect infertility with comorbidities throughout the life course is one of the main limiting factors in this association. Enzymes involved in the oxidation of lipids may offer fresh insights into the molecular underpinnings of infertility and subsequent clinical diseases. To improve the early detection of disease states and give vital information about the disease risk of offspring created through assisted reproduction, it is imperative to expand research capability in this area (Burke *et al.* 2022).

Both industrialized and developing nations are experiencing an increase in lipid metabolic problems brought on by bad eating practices, with the "Western diet" having a detrimental effect on sperm quantity and quality. Under various pathophysiological situations referred to as dyslipidemia, dietary lipid imbalance might involve fatty acids, cholesterol, or both. The development of systemic oxidative stress, a well-known detrimental factor for the quality of male gametes and related with infertility, is the main characteristic of dyslipidemia. Polyunsaturated fatty acids (PUFA), which are particularly abundant in sperm and are crucial for optimal sperm physiology and reproductive outcomes, are also popular targets for oxidative drive. The effects of dietary cholesterol or other fatty acid excess on sperm composition and function in both

animals and humans are the main subject of this review. Following that, the connections between oxidative stress brought on by dyslipidemia and sperm failure are examined, along with potential preventive or therapeutic measures to maintain gamete quality, longevity when kept in cryobanks, and male fertility (Saez and Drevet 2019).

4.7 Least Significant Difference (LSD) For Parameters Study

LSD is a method used in ANOVA to create confidence intervals for all pairwise differences between the mean factor levels while controlling for the individual error rate to the level of significance you specify. In this study we collected the simultaneous confidence level and the probability that all confidence intervals contain the true difference of the chemical variables in our study as shown in Table 5.

4.8 The Correlation Between Parameters Study

In order to better understand the correlations between the variables in the study, we performed the Pearson test to understand these correlations, as in Table 5T between the variables and extending their use in early diagnosis of disease or follow-up and differentiating between the quality and level of patients

Table 4.7 Multiple Comparisons-LSD for age, weight and BMI

Dependent Variable	(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Significance	95% Confidence Interval	
						Lower Bound	Upper Bound
Age	Group AC	Group BP	-16.38584*	3.02760	.000	-22.4213	-10.3504
		Group CP	-15.36000*	3.02760	.000	-21.3954	-9.3246
	Group BP	Group AC	16.38584*	3.02760	.000	10.3504	22.4213
		Group CP	1.02584	3.02760	.736	-5.0096	7.0613
	Group CP	Group AC	15.36000*	3.02760	.000	9.3246	21.3954
		Group BP	-1.02584	3.02760	.736	-7.0613	5.0096
weight	Group AC	Group BP	-4.24000	2.17501	.055	-8.5758	.0958
		Group CP	-11.48000*	2.17501	.000	-15.8158	-7.1442
	Group BP	Group AC	4.24000	2.17501	.055	-.0958	8.5758
		Group CP	-7.24000*	2.17501	.001	-11.5758	-2.9042
	Group CP	Group AC	11.48000*	2.17501	.000	7.1442	15.8158
		Group BP	7.24000*	2.17501	.001	2.9042	11.5758
BMI	Group AC	Group BP	-.21473	.82641	.796	-1.8622	1.4327
		Group CP	-1.63415	.82641	.052	-3.2816	.0133
	Group BP	Group AC	.21473	.82641	.796	-1.4327	1.8622
		Group CP	-1.41943	.82641	.090	-3.0669	.2280
	Group CP	Group AC	1.63415	.82641	.052	-.0133	3.2816
		Group BP	1.41943	.82641	.090	-.2280	3.0669

Table 4.8 Multiple Comparisons-LSD for TT, IL-6 and IL-8

Dependent Variable	(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Significance	95% Confidence Interval	
						Lower Bound	Upper Bound
TT	Group AC	Group BP	53.64000	48.48232	.272	-43.0077	150.2877
		Group CP	-57.08000	48.48232	.243	-153.7277	39.5677
	Group BP	Group AC	-53.64000	48.48232	.272	-150.2877	43.0077
		Group CP	-110.7200*	48.48232	.025	-207.3677	-14.0723
	Group CP	Group AC	57.08000	48.48232	.243	-39.5677	153.7277
		Group BP	110.72000*	48.48232	.025	14.0723	207.3677
IL6	Group AC	Group BP	-1.11907*	.41644	.009	-1.9492	-.2889
		Group CP	.15768	.41644	.706	-.6725	.9878
	Group BP	Group AC	1.11907*	.41644	.009	.2889	1.9492
		Group CP	1.27676*	.41644	.003	.4466	2.1069
	Group CP	Group AC	-.15768	.41644	.706	-.9878	.6725
		Group BP	-1.27676*	.41644	.003	-2.1069	-.4466
IL8	Group AC	Group BP	-34.70074*	5.00093	.000	-44.6699	-24.7316
		Group CP	-26.62074*	5.00093	.000	-36.5899	-16.6516
	Group BP	Group AC	34.70074*	5.00093	.000	24.7316	44.6699
		Group CP	8.08000	5.00093	.111	-1.8892	18.0492
	Group CP	Group AC	26.62074*	5.00093	.000	16.6516	36.5899
		Group BP	-8.08000	5.00093	.111	-18.0492	1.8892

Table 4.9 Multiple Comparisons-LSD for FSH and LH

Dependent Variable	(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Significance	95% Confidence Interval	
						Lower Bound	Upper Bound
FSH	Group AC	Group BP	-7.06431*	1.08040	.000	-9.2186	-4.9101
		Group CP	-6.37589*	1.09159	.000	-8.5525	-4.1993
	Group BP	Group AC	7.06431*	1.08040	.000	4.9101	9.2186
		Group CP	.68842	1.09159	.530	-1.4882	2.8650
	Group CP	Group AC	6.37589*	1.09159	.000	4.1993	8.5525
		Group BP	-.68842	1.09159	.530	-2.8650	1.4882
LH	Group AC	Group BP	-6.12844*	.84650	.000	-7.8181	-4.4388
		Group CP	-3.20166*	.83666	.000	-4.8716	-1.5317
	Group BP	Group AC	6.12844*	.84650	.000	4.4388	7.8181
		Group CP	2.92678*	.86356	.001	1.2031	4.6504
	Group CP	Group AC	3.20166*	.83666	.000	1.5317	4.8716
		Group BP	-2.92678*	.86356	.001	-4.6504	-1.2031

Table 4.10 Multiple Comparisons-LSD for MDA and vıtamin E

Dependent Variable	(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Significance	95% Confidence Interval	
						Lower Bound	Upper Bound
MDA	Group AC	Group BP	-7.54831	4.21429	.077	-15.9493	.8527
		Group CP	-14.82039*	4.21429	.001	-23.2214	-6.4194
	Group BP	Group AC	7.54831	4.21429	.077	-.8527	15.9493
		Group CP	-7.27208	4.21429	.089	-15.6731	1.1290
	Group CP	Group AC	14.82039*	4.21429	.001	6.4194	23.2214
		Group BP	7.27208	4.21429	.089	-1.1290	15.6731
Vitamin E	Group AC	Group BP	3.99024*	1.09552	.001	1.8064	6.1741
		Group CP	2.80999*	1.09552	.012	.6261	4.9939
	Group BP	Group AC	-3.99024*	1.09552	.001	-6.1741	-1.8064
		Group CP	-1.18025	1.09552	.285	-3.3641	1.0036
	Group CP	Group AC	-2.80999*	1.09552	.012	-4.9939	-.6261
		Group BP	1.18025	1.09552	.285	-1.0036	3.3641

Table 4.11 Multiple Comparisons-LSD for lipid profile

Dependent Variable	(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Significance	95% Confidence Interval	
						Lower Bound	Upper Bound
Tc	Group AC	Group BP	-15.12000	12.61596	.235	-40.2755	10.0355
		Group CP	-41.16333*	12.74670	.002	-66.5795	-15.7471
	Group BP	Group AC	15.12000	12.61596	.235	-10.0355	40.2755
		Group CP	-26.04333*	12.74670	.045	-51.4595	-.6271
	Group CP	Group AC	41.16333*	12.74670	.002	15.7471	66.5795
		Group BP	26.04333*	12.74670	.045	.6271	51.4595
Tg	Group AC	Group BP	-23.72000*	6.70767	.001	-37.0915	-10.3485
		Group CP	-12.36000	6.70767	.069	-25.7315	1.0115
	Group BP	Group AC	23.72000*	6.70767	.001	10.3485	37.0915
		Group CP	11.36000	6.70767	.095	-2.0115	24.7315
	Group CP	Group AC	12.36000	6.70767	.069	-1.0115	25.7315
		Group BP	-11.36000	6.70767	.095	-24.7315	2.0115
HDL	Group AC	Group BP	-.68959	2.39744	.774	-5.4688	4.0896
		Group CP	-.40959	2.39744	.865	-5.1888	4.3696
	Group BP	Group AC	.68959	2.39744	.774	-4.0896	5.4688
		Group CP	.28000	2.39744	.907	-4.4992	5.0592
	Group CP	Group AC	.40959	2.39744	.865	-4.3696	5.1888
		Group BP	-.28000	2.39744	.907	-5.0592	4.4992
LDL	Group AC	Group BP	-9.68641	12.34513	.435	-34.2960	14.9232
		Group CP	-36.60561*	12.34513	.004	-61.2152	-11.9960
	Group BP	Group AC	9.68641	12.34513	.435	-14.9232	34.2960
		Group CP	-26.91920*	12.34513	.032	-51.5288	-2.3096
	Group CP	Group AC	36.60561*	12.34513	.004	11.9960	61.2152
		Group BP	26.91920*	12.34513	.032	2.3096	51.5288
VLDL	Group AC	Group BP	-4.74400*	1.34153	.001	-7.4183	-2.0697
		Group CP	-2.47200	1.34153	.069	-5.1463	.2023
	Group BP	Group AC	4.74400*	1.34153	.001	2.0697	7.4183
		Group CP	2.27200	1.34153	.095	-.4023	4.9463
	Group CP	Group AC	2.47200	1.34153	.069	-.2023	5.1463
		Group BP	-2.27200	1.34153	.095	-4.9463	.4023

Table 4.12 The correlation between parameters study (Age, weight, BMI, TT and IL-6)

Correlations																
		Age	**weıght**	**BMI**	**TT**	**IL6**	**IL8**	**FSH**	**LH**	**MDA**	**VıtE**	**Tc**	**Tg**	**HDL**	**LDL**	**VLDL**
Age	PC	1	.383**	.278*	.161	.021	.442**	.422**	.392**	.311**	-.344**	.049	.217	-.061	.032	.217
	Sig.2		.001	.016	.168	.856	.000	.000	.001	.007	.003	.681	.061	.606	.783	.061
	C	168.406	44.510	10.740	365.22	.432	131.71	27.257	19.099	64.2	-18.67	29.8	71.5	-6.572	19.201	14.305
	N	155	155	155	155	155	155	155	155	155	155	155	155	155	155	155
weıght	PC	.383**	1	.783**	.041	.053	.299**	.369**	.135	.201	-.116	.184	.060	.088	.154	.060
	Sig.2	.001		.000	.725	.653	.009	.001	.264	.083	.322	.117	.610	.453	.187	.610
	C	44.510	80.304	20.868	64.768	.739	61.561	16.432	4.410	28.7	-4.338	77.0	13.6	6.600	63.157	2.721
	N	155	155	155	155	155	155	155	155	155	155	155	155	155	155	155
BMI	PC	.278*	.783**	1	-.019	.119	.200	.221	-.067	.097	-.031	.143	.060	.049	.121	.060
	Sig.2	.016	.000		.872	.310	.086	.058	.581	.406	.789	.225	.608	.676	.300	.608
	C	10.740	20.868	8.839	-9.821	.551	13.616	3.210	-.734	4.60	-.390	19.9	4.53	1.221	16.502	.906
	N	155	155	155	155	155	155	155	155	155	155	155	155	155	155	155
TT	PC	.161	.041	-.019	1	-.044	.058	-.064	-.108	-.040	.016	-.103	-.154	-.177	-.051	-.154
	Sig.2	.168	.725	.872		.708	.622	.586	.373	.734	.891	.381	.187	.128	.664	.187
	C	365.226	64.768	-9.821	30659.	-12.0	232.30	-56.23	-70.06	-111	11.789	-855	-683	-259.5	-408.4	-136.7
	N	155	155	155	155	155	155	155	155	155	155	155	155	155	155	155
IL6	PC	.021	.053	.119	-.044	1	.233*	.246*	.212	-.149	-.090	-.065	.143	.054	-.088	.143
	Sig.2	.856	.653	.310	.708		.045	.034	.078	.202	.444	.581	.222	.646	.453	.222
	C	.432	.739	.551	-12.00	2.43	8.337	1.906	1.296	-3.74	-.585	-4.84	5.64	.703	-6.291	1.128
	N	155	155	155	155	155	155	155	155	155	155	155	155	155	155	155

Table 4.13 The correlation between parameters study (IL-8, FSH, LH, MDA and vitamin E)

		Age	weıght	BMI	TT	IL6	IL8	FSH	LH	MDA	VıtE	Tc	Tg	HDL	LDL	VLDL
IL8	PC	.442**	.299**	.200	.058	.233*	1	.472**	.302*	.416**	-.354**	.132	.115	.101	.093	.115
	Sig.2	.000	.009	.086	.622	.045		.000	.011	.000	.002	.264	.324	.390	.427	.324
	C	131.715	61.561	13.616	232.30	8.33	526.92	54.017	25.837	152	-33.93	141	67.1	19.322	97.880	13.434
	N	155	155	155	155	155	155	155	155	155	155	155	155	155	155	155
FSH	PC	.422**	.369**	.221	-.064	.246*	.472**	1	.363**	.303**	-.146	.074	.041	.065	.058	.041
	Sig.2	.000	.001	.058	.586	.034	.000		.002	.009	.214	.534	.727	.583	.625	.727
	C	27.257	16.432	3.210	-56.23	1.90	54.017	24.533	7.034	23.5	-3.045	16.9	5.09	2.675	12.909	1.019
	N	155	155	155	155	155	155	155	155	155	155	155	155	155	155	155
LH	PC	.392**	.135	-.067	-.108	.212	.302*	.363**	1	.125	-.294*	.071	.208	-.062	.061	.208
	Sig.2	.001	.264	.581	.373	.078	.011	.002		.301	.014	.560	.084	.612	.614	.084
	C	19.099	4.410	-.734	-70.00	1.29	25.837	7.034	14.537	7.57	-4.696	12.0	20.5	-1.990	9.949	4.101
	N	155	155	155	155	155	155	155	155	155	155	155	155	155	155	155
MDA	PC	.311**	.201	.097	-.040	-.149	.416**	.303**	.125	1	-.087	.403**	-.026	.119	.383**	-.026
	Sig.2	.007	.083	.406	.734	.202	.000	.009	.301		.458	.000	.827	.307	.001	.827
	C	64.241	28.700	4.609	-111.0	-3.70	152.02	23.532	7.576	253	-5.773	301	-10.3	15.900	279.08	-2.074
	N	155	155	155	155	155	155	155	155	155	155	155	155	155	155	155
VıtE	PC	-.344**	-.116	-.031	.016	-.090	-.354**	-.146	-.294*	-.087	1	.015	-.196	-.020	.049	-.196
	Sig.2	.003	.322	.789	.891	.444	.002	.214	.014	.458		.896	.093	.862	.678	.093
	C	-18.627	-4.338	-.390	11.789	-.585	-33.93	-3.045	-4.696	-5.77	17.436	3.02	-20.6	-.714	9.321	-4.138
	N	155	155	155	155	155	155	155	155	155	155	155	155	155	155	155

Table 4.14 The correlation between parameters study (Lipid profile)

		Age	weıght	BMI	TT	IL6	IL8	FSH	LH	MDA	VıtE	Tc	Tg	HDL	LDL	VLDL
Tc	PC	.049	.184	.143	-.103	-.065	.132	.074	.071	.403**	.015	1	.079	.213	.978**	.079
	Sig.2	.681	.117	.225	.381	.581	.264	.534	.560	.000	.896		.504	.068	.000	.504
	C	29.833	77.006	19.905	-855.1	-4.81	141.30	16.989	12.011	301.77	3.02	2225	91.6	84.618	2122.0	18.321
	N	155	155	155	155	155	155	155	155	155	155	155	155	155	155	155
Tg	PC	.217	.060	.060	-.154	.143	.115	.041	.208	-.026	-.196	.079	1	.000	-.046	1.000**
	Sig.2	.061	.610	.608	.187	.222	.324	.727	.084	.827	.093	.504		.999	.698	.000
	C	71.524	13.605	4.532	-683.6	5.64	67.169	5.095	20.504	-10.36	-20.6	91.6	642.	.017	-52.83	128.46
	N	155	155	155	155	155	155	155	155	155	155	155	155	155	155	155
HDL	PC	-.061	.088	.049	-.177	.054	.101	.065	-.062	.119	-.020	.213	.000	1	.037	.000
	Sig.2	.606	.453	.676	.128	.646	.390	.583	.612	.307	.862	.068	.999		.750	.999
	C	-6.572	6.600	1.221	-259.5	.703	19.322	2.675	-1.990	15.900	-.714	84.6	.017	69.986	14.346	.003
	N	155	155	155	155	155	155	155	155	155	155	155	155	155	155	155
LDL	PC	.032	.154	.121	-.051	-.088	.093	.058	.061	.383**	.049	.978**	-.046	.037	1	-.046
	Sig.2	.783	.187	.300	.664	.453	.427	.625	.614	.001	.678	.000	.698	.750		.698
	C	19.201	63.157	16.502	-408.4	-6.29	97.880	12.909	9.949	279.08	9.32	2122	-52.8	14.346	2096.6	-10.56
	N	155	155	155	155	155	155	155	155	155	155	155	155	155	155	155
VLDL	PC	.217	.060	.060	-.154	.143	.115	.041	.208	-.026	-.196	.079	1.0**	.000	-.046	1
	Sig.2	.061	.610	.608	.187	.222	.324	.727	.084	.827	.093	.504	.000	.999	.698	
	C	14.305	2.721	.906	-136.7	1.12	13.434	1.019	4.101	-2.074	-4.13	18.3	128.	.003	-10.56	25.692
	N	155	155	155	155	155	155	155	155	155	155	155	155	155	155	155

Pc = Pearson Correlation, Sig.2 = Significance(2-tailed), C = Covariance

5. CONCLUSIONS AND RECOMMENDATION

With increasing age, problems with sperm production increase in men, and dietary diversity and lifestyle may directly affect sperm formation. Also, weight had a direct effect on men in the two groups (infertile men) when compared with the control group. The mean body mass index changed little, indicating a slight effect on sperm count. Our study confirmed that low levels of testosterone may have a direct effect on the number of sperm, but after receiving treatments, it led to high levels of testosterone, but the problem remains the same, which indicates the association of infertility (sperm disorders) with another problem that may be hormonal. The quality and number of sperm increased in men who had high or normal vitamin E levels. The immunological problems had a direct effect on spermatogenesis, as the levels of interleukin-6 and interleukin-8 changed clearly in our study. The results demonstrate a direct relationship between a number of pro-inflammatory cytokines and semen quality. Due to its substantial association with seminal parameters and other potential inflammation markers, IL-8 may be used as a sensitive diagnostic for silent male genital tract infection. The study proved that disturbances in the metabolism of fats constitute direct problems in the process of sperm formation in men.

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