A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF ÇANKIRI KARATEKİN UNIVERSITY

USING CREATININE KINASE ENZYMES AS INDICATORS OF MATURE SPERM AND CORRELATION WITH SOME MONOSACCHARIDE IN SEMINAL FLUID

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
CHEMISTRY

BY

OMAR AHMED ABBAS ABBAS

ÇANKIRI

2022

USING CREATININE KINASE ENZYMES AS INDICATORS OF MATURE SPERM AND CORRELATION WITH SOME MONOSACCHARIDE IN SEMINAL FLUID

By Omar Ahmed Abbas ABBAS

December 2022

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

Advisor **:** Prof. Dr. Volkan EYÜPOĞLU
Co-Advisor **:** Asst. Prof. Dr. Wisam Mahmood MOHAMMED

Examining Committee Members:

Chairman **:** Assoc. Prof. Dr. Şevki ADEM
Chemistry
Çankırı Karatekin University

Member **:** Asst. Prof. Dr. Ümit YIRTICI
Medical Laboratory
Kırıkkale University

Member **:** Prof. Dr. Volkan EYÜPOĞLU
Chemistry
Çankırı Karatekin University

Approved for the Graduate School of Natural and Applied Sciences

Prof. Dr. İbrahim ÇİFTÇİ
Director of Graduate School

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Omar Ahmed Abbas ABBAS

ABSTRACT

USING CREATININE KINASE ENZYMES AS INDICATORS OF MATURE SPERM AND CORRELATION WITH SOME MONOSACCHARIDE IN SEMINAL FLUID

Omar Ahmed Abbas ABBAS

Master of Science in Chemistry

Advisor: Prof. Dr. Volkan EYÜPOĞLU

Co-Advisor: Asst. Prof. Dr. Wisam Mahmood MOHAMMED

December 2022

Physiological changes and different lifestyles are related to the general health and safety of the human being, the most important of which is the fertility factor. This study aimed to study the different biochemical components of seminal plasma in Anbar Governorate, which suffer from infertile males with oligospermia, and to know their association with the sperm CK enzyme. The number of patients subjected to this study was 80 patients with an average age of (20-50) years for both the group of patients with oligospermia and the control group. The study showed a significant increase in the level of CK enzyme in the group of patients with oligospermia compared to the control group (P=0.000). The study also showed that the level of monosaccharides for both fructose and mannose was significantly higher in the group of patients compared to the controls. While the study showed that the level of glucose sugar was unremarkably low in the group of patients compared to the controls. The body mass indices and age had a non-significant value, while the sperm count was very low in the patient's group compared to the control group. The results confirm that the causes of infertility and lack of sperm have many causes, whether they are acquired habits or changes in the reproductive system, and the necessity of conducting more studies to detect the causes of infertility.

2022, 57 pages

Keywords: Infertility, CK, Monosaccharide, Seminal fluid, Sperm

ÖZET

YETİŞKİN SPERMİN GÖSTERGESİ OLARAK KREATİNİN KİNAZ ENZİMLERİNİN KULLANILMASI VE SEMİNAL SIVIDAKİ BAZI MONOSAKKARİT İLE KORELASYON

Omar Ahmed Abbas ABBAS
Kimya, Yüksek Lisans
Tez Danışmanı: Prof. Dr. Volkan EYÜPOĞLU
Eş Danışman: Dr. Öğr. Üyesi Wisam Mahmood MOHAMMED
Aralık 2022

Fizyolojik değişiklikler ve farklı yaşam tarzları, en önemlisi doğurganlık faktörü olan insanın genel sağlık ve güvenliği ile ilgilidir. Bu çalışma, oligospermili infertil erkeklerden muzdarip Anbar Valiliği'ndeki seminal plazmanın farklı biyokimyasal bileşenlerini araştırmayı ve bunların sperm CK enzimi ile ilişkilerini bilmeyi amaçladı. Bu çalışmaya alınan hasta sayısı, hem oligospermili hasta grubu hem de kontrol grubu için yaş ortalaması (20-50) olan 80 hasta idi. Çalışma, kontrol grubuna kıyasla oligospermili hasta grubunda CK enzim düzeyinde önemli bir artış gösterdi (P=0,000). Çalışma ayrıca, hem fruktoz hem de mannoz için monosakkarit seviyesinin, kontrollere kıyasla hasta grubunda önemli ölçüde daha yüksek olduğunu gösterdi. Çalışma, kontrollere kıyasla hasta grubunda glikoz şekeri seviyesinin dikkat çekici derecede düşük olduğunu gösterdi. Hasta grubunda kontrol grubuna göre vücut kitle indeksleri ve yaş önemsiz bulunurken, sperm sayısı çok düşüktü. Sonuçlar, kısırlığın ve sperm eksikliğinin sebeplerinin ister kazanılmış alışkanlıklar olsun isterse üreme sistemindeki değişiklikler olsun birçok nedeni olduğunu ve kısırlığın nedenlerinin saptanması için daha fazla çalışma yapılması gerektiğini doğrulamaktadır.

2022, 57 sayfa

Anahtar Kelimeler: Kısırlık, CK, Monosakkarit, Seminal sıvı, Sperm

PREFACE AND ACKNOWLEDGEMENTS

I would like to thank my thesis advisor, Prof. Dr. Volkan EYÜPOĞLU and Asst. Prof. Dr. Wısam Mahmood MOHAMMD, for their patience, guidance and understanding.

Omar Ahmed Abbas ABBAS

Çankırı-2022

CONTENTS

LIST OF SYMBOLS

%	Percent
±	Plus minus
°C	Degrees Celcium
µL	Microliter
dL	Deciliter
IU	International unit
Kg	Kilogram
L	Liter
mg	Miligram
mL	Mililitter
nm	Nanometer

LIST OF ABBREVIATIONS

AR	Acrosome interaction
ATP	Adenosine triphosphate
AUA	American urological association
BMI	Body mass index
CK	Creatinine kinase
DMBS	D-mannose binding sites
DNA	Deoxyribonucleic acid
GLUTs	Glucose transporters
ICSI	Microscopic injection
IVF	Artificial insemination
QA	Quality assurance
SAS	Semen analyzes
UTIs	Urinary tract infection

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

In all living creatures, reproduction is a crucial biological occurrence. Any flaw that jeopardizes an organism's ability to reproduce will get a lot of attention from the scientific community since an organism's ability to reproduce is essential to the continued survival of all species. Reports from many regions of the world during the past five to six decades show that reproductive health indices have declined, especially in industrialized nations where lifestyles have changed.

Lifestyle factors refer to the changeable behavior and other lifestyles that affect the general health and well-being of individuals and psychological state, and its including fertility. Lifestyle factors that may be one of the causes of infertility have aroused great interest among researchers. Where many authors are have developed evidence of a relationship between lifestyle methods and infertility in women and men, including, Delayed childbearing due to pursuit of education or work, advancing age, smoking, eating a high-fat diet, drinking caffeine and alcohol, strenuous exercise, suspicious sexual practices, anxiety/depression, drug abuse, cell phones and exposure to radiation, among many others (Emokpae and Brown 2021)

Infertility, which affects around one in six couples, can be brought on by a variety of male and female causes (Sigman and Jarow 2007).

The idea of infertility definitively refers back to the incapability to have children. For this, couples who've had issues conceiving in greater than a year have to be taken into consideration much less fertile.

As stated by this definition, about 14% are sub-fertile of couples. To be greater precise, the time period sub-fertility method a male who has did not conceive after twelve months of normal unprotected sex with the equal associate and had a sperm rely of much less than 20 million/mL (Khan *et al.* 2008).

In 30% of cases, the female is blamed for the problem, in 30%, the male, in 30%, both, and in 10% of cases, the cause is not known. Semen analysis, which assesses sperm count, motility, and morphology, has historically been the only approach for evaluating male infertility. A man's semen and the sperm it contains are evaluated for particular traits through a semen analysis. It is a crucial part of the inquiry into male infertility, and how the results are interpreted has a big impact on how infertile couples are treated all around. Evaluation of ejaculate descriptive characteristics is the primary goal of semen analysis (spermatozoa floating in a combination of testicular, epididymal, and other accessory gland secretions). For an epidemiologist, the results of the semen analysis serve as the foundation for determining environmental risks, occupational exposure risks, and the impacts of medications and chemicals. The results are also predictive of prospective fertility and the potential reasons of infertility (WHO 1999).

Medical laboratories must validate their methodology to ensure accuracy and give an indication of the uncertainty of their results to help clinicians screen for infertile patients, despite the possibility that minor variations in semen volume may not have an impact on the clinical management of infertile men. Volume scaling can be difficult to the control as evidenced by not being included in external QA charts, and it appears inappropriate to use unknown methods as they contain significant errors (Matson *et al.* 2010).

1.1 Aims of the study

Infertile males in Anbar with oligospermia were the subject of the study, which sought to determine the various biochemical elements of seminal plasma and their relationships to the CK enzyme in semen.

The objectives covered are:

1. The use of creatinine kinase enzyme as an indicator of sperm maturation in infertile patients.

2. Assessing glucose levels in infertile individuals as a sign of sperm maturity
3. Fructose sugar's usage in infertile people as a gauge of sperm maturity.
4. assessing sperm maturity in infertile patients using mannose sugar.

2. LITERATURE REVIEW

2.1 Natural Fertility for Men

Infertility is one of mankind's oldest challenges, and it poses a serious danger to the species' continued existence. It is generally accepted that infertility sets in after a couple has been married for a year.

Male infertility accounts for around 15% of all infertility cases worldwide, whereas female infertility accounts for the remaining 85%. To put it simply, male infertility occurs when a man's sperm are unable to successfully fertilize a woman's egg. About half of all infertility cases may be traced back to the male (30% from the guy and 20% from the couple) (Vander *et al.* 1990).

Until recently, most of the reasons of infertility were the female partner's responsibility, but our knowledge of the efficacy and ineffectiveness of sperm has led to a dramatic growth in our notions of male infertility during the last three decades.

Because of the problems in sperm production and function that result from physical, hormonal, and genetic factors, all known information on male infertility may be found in the semen.

Fewer than 20 million sperm per milliliter of semen (a condition known as oligospermia) or poor sperm motility (where fewer than 60% of the sperms have a strong and forward movement) or a lack of normal spermatozoa (teratospermia) (where normal spermatozoa form fewer than 30%) are responsible for about 40% of infertility cases. in such instances, a microscopic study of the semen will reveal the problem.

Unfortunately, there are certain forms of infertility that cannot be identified via microscopic examination of sperm. Occasionally, a metabolic imbalance prevents

fertilization of the egg by healthy, normally shaped sperm that is of an adequate amount (Razvi *et al.* 1999).

Since the process by which sperm and egg interact is so intricate, the sperm's surroundings must be conducive to its survival and viability. Furthermore, the sperm's head cover contains specific enzymes that are necessary for the sperm to get access to and penetrate the egg. The release of these enzymes facilitates fertilization by allowing sperm to break through the egg's protective outer covering (the zona pellucida) and inject their genetic material directly into the egg's nucleus (Chia *et al.* 1998).

Any biochemical problem may prevent one of these processes from happening, leading to infertility. Although it may be challenging to identify these situations, it is important to remember that a normal result from a conventional microscopic semen study does not guarantee a man's fertility. The biochemical components imply that the normal results of the classic microscopic study of semen do not offer a guarantee of fertilization (Chia *et al.* 1998).

The reasons of infertility can't be determined by microscopic investigation. So, modern research has focused on dissecting the essential components of semen to identify the root reasons of male infertility. For lack of other studies, our nation has turned to this one to identify the biochemical signs that contribute to infertility in patients with low sperm count.

The inability of a couple to have children after one year of regular, infertility, which affects 15% of people of reproductive age, is clinically defined as unprotected sexual activity (Zegers-Hochschild *et al.* 2009, Mosher and Pratt 1991). A fault in semen analysis, such as a low or absent sperm count and low motility, is the most frequent cause of male infertility. Although andrology inquiry techniques have a wide range of diagnostic capabilities, they are prone to overestimate the genuine incidence of male variables in infertile couples. due to the increased prevalence of male infertility issues, a comprehensive assessment of males should be conducted early in marriage. A detailed medical history, physical exam, and at least two semen analyses should all be performed

on patients as part of a male fertility assessment (SAs), in accordance with established recommendations of the American Urological Association (AUA) (Jarow *et al.* 2010).

When a couple has been trying to get pregnant naturally for a while without any luck, a fertility assessment often kicks off. Prior to starting any therapies, they should get evaluated to find out if there are any issues that might prevent them from getting pregnant naturally. At least two semen analyses are part of the first laboratory evaluation for male infertility to gauge reproductive potential.

Since there is a lot of variation across laboratories, it is better to utilize the same ones for several tests. The findings, however, serve as a proxy for the likelihood of conception rather than measuring fertility directly (Jequier 2010). In recent years, International research have demonstrated that hereditary factors might contribute to an increase in infertility, bodily functions or anatomical structures many environmental influences, as well as daily accumulated ones, such as advancing age, chronic diseases, smoking, drinking alcohol, and change in an individual's sex and diet behaviors, affect semen quality and fertility, and this leads to a diversity of causes of male infertility in different regions (Kazemijaliseh *et al.* 2015, Macaluso *et al.* 2010, Ombelet 2011). Understanding the causes of male infertility in each specific area can help infertility professionals to develop studies and make appropriate plans (Taghipour and Latifnejad 2019).

2.2 The Most Important Factors Affecting Male Fertility

2.2.1 Aging

The majority of men, including healthy individuals, see a reduction in blood testosterone levels with age, this decline likely begins around the age of thirty. The andropause is the term used to describe the 1% annual reduction in testosterone levels that occurs after the age of 30. Technically speaking, though, symptomatic hypogonadism in aging men would be a better way to describe the reduction in

testosterone. Hypogonadism isn't described through any particular stage of serum androgens due to the fact the testosterone stage that reasons disorder varies broadly amongst individuals. The signs related to symptomatic hypogonadism in growing old men consist of reduced libido, reduced bone mineral density, reduced muscle mass, extended fats mass, critical obesity, emotional irritability, dysphoria and erectile disorder, insulin resistance (Mahat *et al.* 2016).

Researchers have revealed that sperm physiology is significantly affected by both age and sexual abstinence.

It's better for men than women since they can help start a family far beyond the age of 40 (Amann 2008).

However, as people become older, the germinal epithelium deteriorates (Wang *et al.* 1993), the number of Leydig cells declines (Johnson 1986), and the activities of these cells negatively impact spermatogenesis due to a fall in testosterone level (Harman *et al.* 2001).

Furthermore, aged males have been shown to have an effect on sperm quality and spermatogenesis in animal experiments (Eskenazi *et al.* 2003). The elderly are at increased risk for erectile dysfunction due to the drop in nitric oxide level that occurs with aging. In addition, poor sperm quality in older men may lead to miscarriages and the transmission of autosomal dominant diseases. (Dain *et al.* 2011, Kidd *et al.* 2001).

Alterations in sex hormone production and sperm physiology were shown to be positively correlated with testicular size and histological alterations as men aged. However, the cutoff age for male spermatogenesis has not been determined (Mahmoud *et al.* 2003, Sampson *et al.* 2007).

Additionally, in the evolving lifestyle situation, delayed motherhood is an impending issue, and specific couples will need an ART in order to conceive (Wiener *et al.* 2012).

According to World Health Organization standards, age-related impacts on semen parameters have been seen in several retrospective investigations in the United States, China, and India (Kidd *et al.* 2001, Zhu *et al.* 2011, Mukhopadhyay *et al.* 2010).

2.2.2 Smoking

There is a relationship between the number of cigarettes smoked each day and the percentage of sperm cells that are mobile and have a typical morphology. Additionally, smoking has been linked to an increase in reactive oxygen species, which contributes to oxidative stress in the environment. This oxidative strain may potentially harm sperm by poisoning it and surpassing the seminal plasma's antioxidant capacity. One study found that smoking decreases the viability, motility, sperm concentration, and morphology of the semen, among other factors (Mahat *et al.* 2016).

According to a different study, smoking causes sperm to become less plentiful and more likely to have morphological flaws. but at the same time it showed that the average acidity, sperm motility, and the production of hormones that contribute to reproduction had no effect in the same group of unfertilized men (Bundhun *et al.* 2019).

Cigarette smoke contains a wide variety of toxic chemicals, including nicotine, carbon monoxide, cadmium, and other odd compounds, all of which may be damaging to sperm (Zenzes 2000).

Cigarette smoke toxins may decrease mitochondrial activity in sperm and alter the chromatin structure of human sperm, both of which negatively affect the sperm's capacity for fertilization in vivo (Sharma *et al.* 2013, Calogero *et al.* 2009).

The most prevalent criteria used in clinical settings to determine fertility are motility, concentration, and morphology; all three have been linked to cigarette smoking (Künzle *et al.* 2003, Al-Matubsi *et al.* 2011).

There is inconclusive data; some research has shown no impact on semen quality (Trummer *et al.* 2002, Li *et al.* 2009).

2.2.3 Obesity for a couple

Previous research has shown that fertility is reduced in women who are overweight. Obesity also plays a role in male fertility. According to a research conducted on farmers and their spouses in the United States, fertility drops by about 10% every time body weight goes up by 10 kilos, and the significant effect on men whose body mass index is greater than 32. Men with a body mass index of more than 25 also showed a significant decline in the quantity of normally motile sperm, and it was shown that men with a significant increase in thigh and belly fat have worse quality semen. Infertility risk is influenced by both a low and high body mass index (BMI), according to a prior study conducted in Norway (Mahat *et al.* 2016).

Lack of understanding of the underlying process through which male obesity might decrease fertility. Obesity has been linked to impaired secretion of sex hormones, sperm generation, and sperm maturation due to its effect on luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which in turn impairs the functioning of the Sertoli and Leydig cells. Having a higher BMI (Maghsoumi *et al.* 2020).

Moreover, it has been connected with obesity in men. Male reproductive abnormalities. Studies have demonstrated that endocrine problems are linked to obesity, and that losing weight may correct these issues, so it's probable that these hormones play a significant role.

Inhibin-B and anti-Mullerian hormone (AMH) have both been studied extensively as indicators of spermatogenesis since they are virtually exclusively generated by Sertoli cells.

Obesity has been linked to reduced levels of inhibin-B, which has been proven to be favorably connected with fertility. However, there are no studies linking anti-Mullerian hormone (AMH) to obesity, and the data that do exist are inconsistent (Håkonsen *et al.* 2011).

2.2.4 Diet (alcohol and caffeine)

Alcohol use has a significant impact on all aspects of the reproductive system. Caffeinated beverage consumption can also affect male fertility. Men who drink more than three cups of tea each day are linked to reduced fertility, according to a study in this field (Mahat *et al.* 2016).

Higher levels of testosterone and sex hormone-binding globulin are found in males who consume large amounts of coffee, as caffeine likely alters glucose-degrading Sertoli cells and oxidation, which interferes with men's ability to fertilize. Caffeine may appear indirectly through affecting the pituitary gland, hypothalamus and gonads, or through direct toxic effects on the vegetative epithelium. Diagnosed also through injury to the testicle or through oxidative stress (Ricci *et al.* 2017).

2.2.5 Chemicals and stress

Recent investigations have demonstrated an increased risk of male infertility connected with exposure to damaging physical and chemical elements. Male fertility may be negatively impacted by a number of industrial solvents, including glycol ethers and carbon disulfide. Semen quality shows changes in males exposed to hydrocarbons such as xylene, benzene, and toluene, including liquefaction capacity, viscosity, sperm motility, sperm count, and percentage of normal-shaped sperm compared to unexposed males. Industrial and construction workers and farmers also suffer from an increase in infertility rates due to increased exposure to stress, psychological and nervous pressure that affects the adrenal glands, as well as sunlight and pesticides (Mahat *et al.* 2016).

2.2.6 Genital tract infections

A well-known illness known as male reproductive system infections causes deterioration of the function of the male glands as well as poor quality sperm in terms of mobility and form. As a result, this infection is regarded as one of the major potential causes of male infertility. Semen volume, -glucosidase, fructose, and zinc levels are all much lower in seminal plasma when the male reproductive system is infected. This results in epididymis, seminal vesicles, and prostate secretary function being compromised (Mahat *et al.* 2016).

Male reproductive health may be negatively impacted by genital inflammation in a number of ways. DNA fragmentation, reactive oxygen species generation, and anti-sperm antibodies are all direct or indirect effects of inflammation that negatively impact spermatogenesis and sperm function (Haidl *et al.* 2015).

Inflammation of the male urogenital tract is often caused by infection. Infertility therapy with ART may be hampered by untreated infection (Zeyad *et al.* 2018).

UTIs are linked to up to 35% of male infertility issues. Asymptomatic azoospermia is one possible reason for male infertility. Male urinary tract infections and sexually transmitted infections are also possible (Rybar *et al.* 2012).

Spermatogenesis and fertility in men may be negatively impacted by testicular, epididymal, and prostate infections (Rusz *et al.* 2012).

The amount of leukocytes in the seminal plasma also rises when there is a urogenital tract infection (leukocytes in the sperm) (Akgul *et al.* 2018).

Environmental contaminants, vaginal items used during the course of treatment, alcoholic and tobacco product use, certain medicines, and surgical procedures are only few of the many causes of leukopenia (Domes *et al.* 2012).

However, bacterazoospermia and polyspermy seem to be strongly linked in infertile men.

Multiple pathways are adversely impacted by germs and leukocytosis on male fertility. These pathways include spermatogenesis, sperm function determination, and reproductive system failure (Esmailkhani *et al.* 2018).

Germs may boost sperm motility via cellular interactions, sperm adhesion, and agglutination (Keck *et al.* 1998).

Escherichia coli is a pathogenic bacterium that inhibits sperm motility (Huwe *et al.* 1998).

In addition, leukocytes in spermatozoa may affect sperm activity by inducing cytokines and producing reactive oxygen species. DNA damage in sperm may be caused by lipid peroxidation in the plasma membrane, which is correlated with an elevated quantity of reactive oxygen species (ROS) in seminal plasma (Agarwal *et al.* 2018).

Seminal plasma has been found to be altered by spores and leukocytes, which in turn prevents fertilization (Altmäe *et al.*, 2019). Anti-sperm antibodies, which are formed when an infection or inflammatory pathway breaks through the blood-testis barrier, may have a negative impact on sperm's ability to do their job and a couple's ability to have children (Fraczek and Kurpisz 2015).

2.2.7 Treatments

Primary infertility may occur when using medications. There are many treatments that have a significant effect a direct toxic effect on the gonads or on the hypothalamic-pituitary-gonadal axis, which leads to only impaired fertility in men (Mahat *et al.* 2016).

Evidence suggests that pharmaceutical medications and long-term pharmaceuticals may alter key aspects of the sperm development process. Some medications can lessen sexual desire and the capacity to ejaculate and maintain an erection. Paroxetine, a selective serotonin reuptake inhibitor used to treat depression, was shown to have a substantial long-term impact on sperm DNA fragmentation (Brezina *et al.* 2012).

It has been established that the use of alpha-adrenergic blockers, one of the most widely recommended classes of drugs for the treatment of lower urinary tract syndrome caused by benign prostatic hyperplasia, may have an effect on sperm ejaculation (Hellstrom and Sikka 2006).

Carbamazepine, phenytoin, and valproate were shown to have sperm-related adverse effects in studies. These included aberrant sperm morphology, reduced motility, decreased sperm count, and decreased testicular volume. Interaction between antiepileptic medicines and sex hormones may be the cause of their adverse effects (Herzog *et al.* 1986).

The quality of sperm may be impacted by exogenous medicines that interfere with the hypothalamic-pituitary-gonadal axis. Low thyroid hormone levels or shifts in prolactin concentration, both of which may be side effects of certain drugs, are known to have a negative impact on sperm quality (Rajender *et al.* 2011). To improve muscular bulk, many people turn to anabolic steroids, which have been linked to a higher risk of infertility (Tan and Scally 2009, Lombardo *et al.* 2005).

2.2.8 Chromosomal and genetic defects

10-15% of severe male infertility is due to genetic reasons, which include single gene abnormalities and chromosomal anomalies. Any flaw in the genetic condition may result in varied degrees of male reproductive impairment since it has the power to alter sperm creation, disrupt the gonads' normal growth, and limit sperm motility and fertilization (Mahat *et al.* 2016).

2.2.9 Varicocele

Varicose veins are an expansion of the testicular veins within the glial plexus, the shape of the seminal lamina that carries the male testicles. Males who seek examination from infertility counselors frequently have varicocele, which is a significant correctable cause of infertility. According to the updated Human Report, 15% of the general population was found to suffer from varicocele including adolescents and adults but it is significantly more among men who go to infertility clinics and the percentage ranges between 30-40% (Figure 2.1). A varicocele usually occurs on the left side. There are multiple causes of varicocele, the left and right spermatic veins have different anatomical structures, and the seminal vessels lack valves. These features are the most well-known, which causes reduced blood flow, and the pressure of the left renal vein, which causes partial obstruction (Mahat et al. 2016).

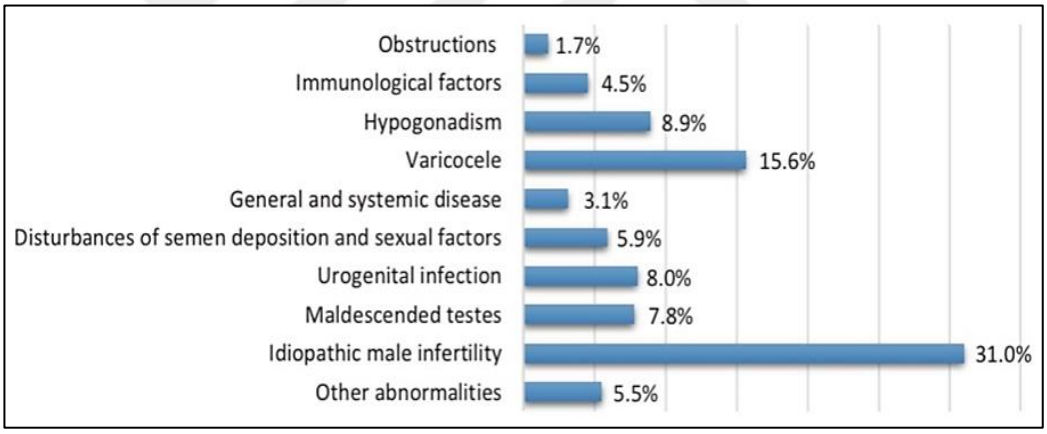

Figure 2.1 Diagram of factors affecting male infertility (Andersen 2017)

2.2.10 Scrotum temperature

Underwear and its types affect the temperature of the scrotum and the quality of semen, as wearing tight underwear is associated with a high temperature of the scrotum. Also, activity and physical work have an effect on the high temperature of the scrotum. Walking and driving for hours on end also affect the temperature of the scrotum. Sperm production, the maturation of sperm cells and spermatids require a minimum

temperature of 1°C and a maximum of 28°C below normal body temperature (Mahat *et al.* 2016).

This was diagnosed through research that worked on people, which led to artificially bringing the testicles near or inside the inguinal canal and causing a rise in the temperature of the scrotum, and consequently, the testicle temperature rose close to the normal body temperature (Mieusset *et al.*1987, Mieusset and B'ujan 1994). As a result of this process, spermatogenesis is disrupted and thus leads to a decrease in semen quality.

2.3 Semen

A concentrated suspension of spermatozoa is retained in the epididymis where it is combined and diluted with liquid secretions from the accessory genitals before being released in many batches to form semen. The seminal fluid which is released during male ejaculation, usually consists of approximately (2-5) mL with an average range of spermatozoa of round (20) million per milliliter of ejaculation. This fluid appears as creamy, viscous, yellowish, slightly or grayish fluid, which at first goes through the clotting process and then becomes more liquid. Combining the semen volumes demonstrates that the bulbar glands (Coopers), the epididymis, and the prostate contribute just slightly to the semen volume, which is composed of secretions from accessory organs at roughly 90% of the volume. Semen has two main quantifiable features:

Total Sperm Count: This measures the amount of sperm produced by the testicles and the post-testicular airway system's arm,

The total fluid volume that the different auxiliary glands contribute: This represents the glands' secretory activity. Sperm function is also influenced by the characteristics of sperm, such as their vitality, motility, and morphology, as well as the make-up of semen.

A sperm plasma membrane protects the head, midsection, and tail of the mature spermatozoon. The acrosome, which is made up of granules with enzymes, is found in the skull along with the nucleus, which has densely packed DNA. When the enzymes come into touch with the oocyte, they are released, allowing the oocyte membrane to be penetrated (Figure 2.2). The mitochondria in the mid-piece, which are propelled by the sperm tail's motile force, create the energy required for cell movement (Andersen 2017).

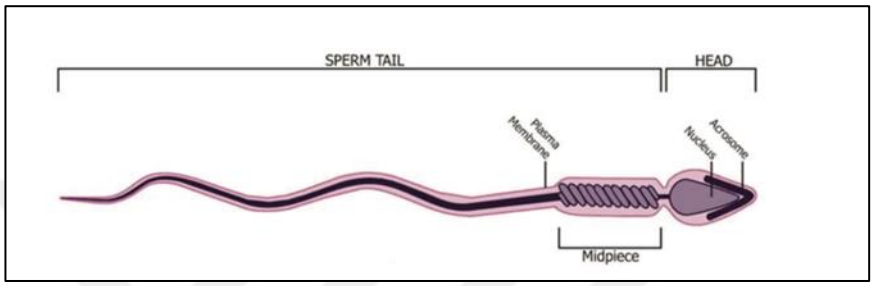

Figure 2.2 Mature sperm. Illustration of a sperm with the head, midsection and tail covered by a plasma membrane. The nucleus and acrosome are located in the head. The middle part contains mitochondria that provide the energy needed for movement (Andersen 2017)

Seminal vesicles, testis, prostate, epididymis, and bulbourethral glands all contribute to the combination of secretions used to ejaculate spermatozoa (Tocci and Lucchini 2010).

Boluses of semen are discharged. The spermatozoa-rich initial part of the ejaculate also comprises prostate and epididymis secretions (Mortimer 1994).

The second fraction, which mostly consists of seminal vesicle secretion and contains fewer spermatozoa (Andersen 2017).

2.4 Environmental Factors

2.4.1 Air pollution

Recently studies have shown that air pollution has a negative impact on reproductive outcomes in men and women, as it significantly affects the quality of men's semen. Air pollution has been observed to cause increased sperm (DNA) fragments, changes in sperm morphology, and decreased sperm motility (Jurewicz *et al.* 2018). Some tests reported that the level of air pollution had a significant association with a decrease in volume, concentration, forward motility, and the rate of normal sperm formation in the body. It was also shown that polluted air increases the DNA breakdown index in sperm, which affects fertility and decreases it in men (Zhang *et al.* 2020). Various gaseous air pollutants and semen quality have a high link, and exposure to sulfur dioxide (SO2) and nitrogen dioxide (NO2) has a negative impact on sperm concentration and motility, according to a new study, which appeared in the first stage of great aggression, so the danger of these toxic gases in The early stages of sperm growth and formation (Wang *et al.* 2020).

The results of another study indicated that the progressive movement of sperms, and their concentration, in men working in gates and highways was very low compared to the residents of the same area. Unaffected genetics and hence have a major impact on these men's semen quality in comparison to controls (Rosa *et al.* 2003, Calogero *et al.* 2011).

2.4.2 Exposure to environmental chemicals

People are exposed to a variety of harmful chemicals daily. Recent research have demonstrated that exposure to environmental toxins that cause infertility affects the male reproductive system, which is one of the critical human body systems most dangerously affected by chemicals (Dissanayake *et al.* 2019). Recent research has shown that mothers' exposure to chemicals that cause endocrine disruption during

pregnancy, especially pesticides, heavy metals and phthalates, which affect semen quality, volume and sperm count in their sons at puberty, as high exposure to heavy metals causes low sperm concentration (Istvan *et al.* 2021).

2.4.3 Exposure to high heat

It is one of the environmental factors that men are exposed to in the workplace or due to climate change. In order to keep sperm production in the testicles regular, temperature is crucial. The temperature of the scrotum decreases by (2-4) degrees Celsius from the normal body temperature. Any influence, whether internal or external, that leads to an increase in the temperature of the testicles will affect the process of sperm formation and thus lead to infertility when males. Moreover, it has been observed that an increase in temperature of the testes by 1 to 1.5 degrees Celsius can lead to impaired spermatogenesis (oligospermia, azoospermia, tratozoospermia), and morphological abnormalities of the sperm (Kumar and Singh 2022).

2.5 Semen Sample Test

That an accurate clinical evaluation of an infertile couple requires knowledge of sperm abnormalities that impair conception, which is provided by the use of sophisticated sperm testing. Regular semen analysis offers important insights on sperm production from the testis, sperm viability and motility, the male reproductive system's armor, the implantation of accessory organs, and ejaculation and emission. Although the results of this test are undoubtedly helpful for the first evaluation of an infertile man, they are insufficient as a fertility test, and they offer no information on the sperm's functional capacity to fertilize an egg or undergo later fertilization.

the development procedures necessary for fertilization In actuality, unless the male has azoospermia, the findings of semen analysis cannot separate yeast from the infertile population. Semen analysis is crucial for assessing the results of medication or environmental toxic research (Lamb 2010).

As a result, tests have been created to find sperm function anomalies in male infertility. The capacity of a single sperm to deliver the right chromosomal complement to an egg can be considered of as the function of sperm in its widest meaning. Sperm production must be sufficient for this, and they must also have proper motility and morphology (and these parameters are generally evaluated in a routine semen analysis). The sperm also has to be able to get past the cervical mucus, the uterus, and into the oviducts' ampulla. It is necessary for fertilization and early embryonic development, although its function is unclear. Over the past 25 years, flaws in any of these intricate processes discussed might result in male infertility. For the purpose of finding anomalies in these processes, tests have been created. These tests may be used in clinical trials for drug testing as well as evaluations of possible toxicity. Although pre-treatment examination might help avoid overtreatment with the most expensive technology and unexpected IVF failures for men with normal symptoms, the clinical usage of these tests substantially declined with the introduction of ICSI (Andersen 2017).

2.6 Monosaccharides

The carbonyl group (a functional group in which a carbon atom is doubly linked to an oxygen atom, C = O) and the number of carbon atoms in a monosaccharide are used to categorize them (C). Monosaccharides are classified as tri(3C), tetra(4C), pentagonal(5C), and hexoses depending on the length of the C chain (6C). The most prevalent and plentiful monosaccharide among all sugars, including glycogen, starch, and cellulose, is glucose. Galactose is a monosaccharide found in fruit and honey, while fructose is a monosaccharide found in sucrose (table sugar) and lactose in milk. High fructose corn syrup has recently become one of the primary sources of fructose in foods (Stevens 2014).

Carbohydrates are the most important sources of energy for the occurrence of most processes in the body, including the process of fertilization. The zona pellucida must first be recognized and bound to by the sperms in order for the sperm to be able to penetrate it and fertilize the egg by secreting acrosomal contents (Yanagimachi 1994).

There is strong evidence that the key mediator in the interaction of the sperm and the egg is the carbohydrate. The sperm's carbohydrate surface-binding proteins specifically connect to glycoconjugates in the zona pellucida, and this causes signal transduction pathways called acrosome interaction (AR) to release. This is the theory behind how this event occurs (Töpfer-Petersen 1999, Benoff 1997, Oehninger 2003).

2.6.1 Glucose

The primary metabolic substrate for the synthesis of tissue energy is glucose. The mechanisms that regulate glucose synthesis and glucose utilisation keep normal blood glucose levels within this constrained range. It is the level of glucose in the blood or plasma at which a person exhibits a particular reaction to the aberrant environment brought on by insufficient glucose transport to a target organ (eg, the brain).

The blood glucose level should be evaluated in the context of the clinical circumstance, taking into account the counter-regulatory hormone responses and intermediate metabolites. Hypoglycemia should thus be thought of as a continuum (Szablewski 2011).

A crucial process in the development of sperm is the passive glucose transfer over the blood-testicular barrier, which is mediated by glucose transporters (GLUTs). The presence of GLUTs is also detected in mature sperm cells, which need transporters to take up active sources necessary for maintaining both basic cell activity and specialised tasks like sperm motility and fertilization (Bucci *et al.* 2011).

Using the glucose oxidase technique, scientists found that while human seminal plasma initially included high levels of both glucose and fructose, the latter was gradually replaced by the former (Hornstein 1961).

It was formerly thought that this enzymatic approach might be used to determine glucose levels in the blood, but it was subsequently discovered that a number of other chemicals interfere with this process.

Sperm, which may use glucose for a variety of functions, are greatly aided by its presence in human semen. Normal trace quantities of glucose, however, are invisible. An essential function for glucose in sperm metabolism seems plausible.

In contrast, human semen includes trace quantities of other monosaccharides including (galactose, ribose, ribulose) (Eliasson 1965).

Due to the fact that glucose may be produced from glycogen or oligosaccharides by enzymatic breakdown of these molecules, its origin in semen is unclear. However, after 6 hours of incubation, no enzyme was detected in the glucose content of the seminal plasma. Thus, it is less probable that this route is important for gluconeogenesis in human semen (Eliasson 1965).

2.6.2 Fructose

Humans naturally consume fructose, which is mostly found in fruit, honey, and to a lesser extent vegetables. Both the free form of sugar and the disaccharide sucrose are present in these sources, in varying amounts.

Thus, the use of enzymatically processed sucrose, also known as invert sugar, and corn syrup, which are both based on hydrolyzed cornstarch and contain substantial levels of free fructose, to sweeten diverse meals quickly expanded, leading to an excessive consumption of fructose. Studying the effects of nutrition on consumer health is incredibly challenging due to the complexity of the diet, and it is not always easy to transfer findings from animal tests to people due to the need for meticulous research.

Reviewing earlier research is crucial despite these challenges. Without a doubt, fructose plays a distinctive role in the metabolic process and behaves differently from glucose, which is crucial for human health. Therefore, a variety of physiological effects must be considered. Excessive fructose consumption has been linked to malignant tumors, cardiovascular diseases, obesity, and signs of the metabolic syndrome (Biró 2018).

Fructose is found in high concentration in seminal plasma which provides nourishment and energy to the mitochondria of sperm (Histewart *et al.* 2004).

Sperm are found in the fluid seminal plasma that makes up the substance known as semen. Both the epididymis and the accessory glands release the seminal plasma, which is the liquid component of the semen, before to and during ejaculation. A complicated fluid called seminal plasma is responsible for moving ejaculated sperm from the testicles to the egg, which is their intended destination. Along with protecting and nourishing the sperm as they go through the female reproductive system, seminal plasma also transports them. The seminal plasma is made up of a variety of biological substances, including intracellular enzymes, proteins, antioxidants, metabolites, and mineral elements as well as fructose, glucose, and cholesterol (Raines 1998).

- Fructose activity in seminal plasma:

High fructose sugar seems to have a more noticeable impact on ill populations than healthy ones. A shortage of sperm means reduced sugar intake, hence diseased groups have higher sugar concentrations while having less sperm than healthy ones (Moon *et al.* 1970).

Due to the presence of a significant number of dead or slow-moving sperm that do not consume the sugar molecules, the process of glycolysis slows down, leading to a high concentration of fructose with a reduction in the number of active sperm (Mayes 1993).

2.6.3 Mannose

Due to its capacity to conceal the bacterial adhesin FimH and so inhibit its adherence to urothelial cells, the natural sugar d-mannose is regarded as an antibiotic alternative. The potential that d-mannose displays "antibiotic-like" action via affecting bacterial growth and metabolism or by choosing FimH variations has not yet been researched, despite its widespread use. In compared to d-glucose, d-fructose, and l-arabinose, d-mannose rated as the least favored carbon source to sustain bacterial metabolism and development. Since urine may physically detect little levels of glucose, Overall, our findings show that d-mannose is an effective substitute for UPEC-related UTI prevention and therapy (Scribano *et al.* 2020).

Because (Tesarik *et al.* 1991) used a fluorinated neoglycoprotein containing a residue of the sugar D-mannose to describe the first identification of D-mannose binding sites (MBS) on the surface of sperm, there is a strong association between sperm and d-mannose. In order to identify between patients who are having IVF who are "fertile" and "sterile," it has been discovered that there is a rise in the number of sperm that express MBS during amplitude (Hershlag *et al.* 1998).

The role of D-mannose in the interaction of sperm with the egg has also been researched, noting that MBS can be both a manifestation of the capacity to fertilize sperm in vitro and a target for its modulation (Teixeira and Borges 2012).

2.6.4 Metabolism of monosaccharides in sperm

The highest developed mammalian cells are those seen in sperm. The primary function of sperm is to transpose male DNA into female DNA through a sequence of events that indicate its movement down the female genital canal and its capacity to fertilize (Rodriguez-Martinez 2007).

The primary purpose of the sperm cells' energy is to maintain motility until full amplitude and then to engage with particles (Flesch and Gadella 2000, Yanagimachi 1994).

Because they cannot move inside the testicle due to epididymal maturation, sperm cells require energy to increase and sustain their efficiency in motility (Yanagimachi 1994).

Adenosine triphosphate (ATP) is largely consumed in sperm to maintain motility, save for a few metabolites, such lactate and citrate. Therefore, carbohydrates like glucose, mannose, and fructose are the major sources of energy for sperm. The two primary metabolic processes that contribute to energy production are anaerobic glycolysis and oxidative phosphorylation. (Bucci *et al.* 2011).

Numerous species have oxidative stress or glycolysis inhibitors, and each route by itself can sustain motility (Storey 2004).

The pentose phosphate route, mitochondrial oxidative phosphorylation, or glycolysis can all be used to process sperm. The species, oxygen concentration, and availability of the hexamer all influence the prevailing route. The sperm really possesses a mitochondrial sheath in the center, where oxidative reactions may take place (Bucci *et al.* 2011).

Therefore, the majority of the most crucial glycolytic enzymes are found in the area of the tail that is linked to the fibrous sheath (Bucci *et al.* 2011, Eddy *et al.* 2003, Miki 2007).

2.7 Creatine Kinase

bidirectional phosphotransfer between the ATP/ADP and Creatine/Phosphocreatine systems is catalyzed by this member of the phosphagen kinase family. The energy homeostasis of the tissue cells of excitable tissues, which need high energy fluxes, is

significantly influenced by CK, which is abundantly expressed in these tissues. Since K Lohman's discovery of the creatine kinase response in muscle tissue in 1934, it has been the subject of much research. The enzyme has clinical significance, and its concentrations are frequently utilized to detect acute myocardial infarction. There are two cytosolic and two mitochondrial CK isomers among the four major isomers. Under physiological circumstances, both tissue types (muscle or brain cells) exist as homodimers (MM-CK and BB-CK) as well as heterogeneous dimers (MB-CK), which are made up of either muscle (M) or brain (B) monomers.

CK is also found in non-muscular cells such as photoreceptor cells in the retina, brain cells, kidneys, placenta, pancreas, and thyroid gland. Significant amounts of the CK (B-CK and Mi-CK) and the total creatine (Cr) are also found in sperm (Wallimann and Hemmer 1994).

CK enzyme level as an indicator of infertility:

Investigations of the CK enzyme in human ejaculate demonstrated the identification of CK isotopes in sperm as well as in the seminal plasma.

The seminal plasma of CK was identified as B-CK, which may be secreted from additional glands, prostate or seminal vesicles.

The appearance of the CK in seminal plasma is accompanied by the presence of substrates, mainly Cr, which led to the assumption of an external energy source for human sperm through the CK interaction.

This was achieved by the detected substrates (PCr, Cr, ATP, ADP) in seminal vesicle fluids or high Cr levels in the testis, while PCr was not detected in human semen by 3'P-NMR spectroscopy (Kaldis *et al.* 1997, Wallimann and Hemmer 1994).

While PCr supplied to isolated human sperm seems to enhance sperm motility and the velocity (Fakih *et al.* 1986), it is still not known whether exogenous PCr can be taken up by healthy sperm and subsequently used intracellularly as a rapid source of energy for ATP regeneration, or its metabolism by extracellular CK.

In the latter case, its positive effect on sperm motility is mediated by PCr through an indirect process. Therefore, extracellular protein kinases have been found in some mammalian semen (Lee *et al.* 1988) that could be involved in phosphorylation of plasma membrane surface proteins during sperm maturation.

This process requires exogenous ATP which can theoretically be delivered by CK from epididymal epithelial cells to the sperm surface (Kaldis *et al.* 1997, Wallimann and Hemmer 1994).

2.7.1 The level of creatinine kinase (CK) enzyme activity in the sperm

An increase in cytoplasmic waste and a fault in the activity of the sperm are related with a reduction in the likelihood of fertilization, both of which are indicated by a rise in CK enzyme activity. Meiosis, a process of cell division, is triggered in Sertoli cells, causing them to divide and eventually create sperm (Kingsley 1942).

Cells undergo cytoplasmic ejection during the synthesis Sertoli cell stage, which is necessary for the cell to change into a sperm (spermiation) (Dandekar and Parkar 1999, Vigue *et al.* 1992).

Prior to entering the seminiferous tubules, a typical, mature sperm will discharge extra cytoplasmic components into the lumenal area as waste. The term "cytoplasmic waste" is used to describe these byproducts. One distinguishing feature of these sperm is a marked reduction in the activity of the enzyme responsible for breaking down cytoplasmic trash (CK). Some abnormalities in the fertilization process result in the cytoplasmic components staying with the sperm.

After fertilization, the sperm's cytoplasmic waste collects in the sperm's central region, where it forms an uneven cytoplasmic mass that surrounds the mitochondria (Huszar *et al.* 1998).

Reduced sperm count is caused by the sperm being released from the germinal epithelium along with the extra cytoplasm (Dandekar and Parkar 1999).

A sperm is considered to have an irregular form when the volume of its cytoplasmic waste is three times larger than its head. This waste is referred to as a cytoplasmic droplet (Sidhu *et al.* 1998).

Electrons leaking out of damaged mitochondria produce active oxygen species, leading to central edema (Huszar *et al.* 1998).

Because the efficiency of CK in the creation of (ATP) is based on three processes, increasing its activity results in an increase in electron production, which in turn leads to an increase in the generation of active oxygen species (creatine phosphate and ADP).

Second, it uses ATP to produce glucose-6-phosphate in the presence of phosphoric acid (Hexokinase).

Third, glucose-6-phosphate is converted to 6-phosphogluconate by the oxidation catalyzed by phosphate dehydrogenase (G6PDH). This is a crucial part of the (Hexosemonophosphate) transformation because it results in the production of NADPH, which is a major source of electrons responsible for the production of free radicals (ROS) and the weakening of the sperm production process, which increases cytoplasmic waste during the differentiation stage (Huszar *et al.* 2000).

Increasing CK coenzyme levels results in a corresponding rise in activity. The high activity of the CK enzyme in persons with few sperms of the severe kind is explained by cytoplasmic waste and a deficiency in the sperm's activity, which leads to a

considerable reduction in the process of creating sperm. Extreme, as its enzyme activity rate was 7.491, or around 10–12 times more than the other two brands (Agarwal 2001).

A morphological abnormality, located in the sperm's middle, where the cytoplasmic waste is stored, is linked to the oxidative disintegration of the sperm.

Keeping a lot of cytoplasmic trash around will boost the efficiency of (NADPH), which in turn will increase the creation of free radicals (G6PDH). The membrane of the sperm organ is an oxidase, which destroys fats (Sidhu *et al.* 1998).

An sign of healthy sperm function and the efficient transport of biochemical molecules across the membrane is the membrane's ability to remain intact. A sperm's ability to interchange important chemical compounds like fructose, which the sperm requires to make the energy it needs for movement, will be weakened if the sperm membrane is damaged as a result of the beginning of the fat oxidation process.

In addition, fat oxidation reduces ion exchange across the sperm membrane, and as ions are essential to the sperm's continued normal motility, this results in low sperm activity (Sidhu *et al.* 1998).

2.7.2 Seminal plasma creatine kinase activity

Many cells, including sperm, testicular cells, and the cells of the reproductive organs, exude creatinine into the semen as a byproduct of producing Cr and PCr (Dandekar and Parkar 1999).

Since it requires the help of other cells in order to be secreted, it is not considered vital to the diagnosis, and its concentration has no effect on the sperm count.

Since the internal ratio of (Cr) to (PCr) remains constant, changes in the concentration of (ATP) have no effect on the equilibrium between (Cr) and (PCr) in the cell (PCr). That it is independent of the efficacy (Dandekar and Parkar 1999).

3. MATERIALS AND METHODS

3.1 Study Design

The study was conducted on patients who had infertility problems, and semen samples were taken from the general population, aged between 20-50 years, in Anbar Governorate. Then the quality of the semen was assessed using two parameters: macroscopically and microscopic examination of semen analysis using world health organization criteria.

3.1.1 Study groups

This study included the collection of 80 samples, aged between 20-50 years, and the samples were divided into two groups as follows:

- Group A: consists of 55 oligospermic individuals aged 20 to 50.
- Group B: includes 25 natural controls (fertilized male).

Males with zero animals were excluded.

3.1.2 Control group

This study included 25 normal controls (fertilized males) aged between (20-50) who did not have any problems. Table 3.1 and Table 3.2 show all the chemicals and tools used in this study.

Table 3.1 Chemical used in the study

NO.	Kit	Company, Origin
1	Glucose	Gesan-Italy
2	Fructose	Diamond-Jordan
3	Mannose	Ireland
4	Creatine Kinase (CK)	Biolabo_ France

Table 3.2 Tools employed in the study

NO.	Materials	Company and origin
1	Semi-Auto	Gesan - Italy
2	Water bath	Joanlab - Chine
3	Centrifuge	Dragon-Lab - Chine
4	Micro Pipette (100-1000)uL	Dragon-Lab - Chine
5	Refrigerator	Turkey (beko)

3.2 Sampling

Semen was produced by masturbation after three to five days of abstinence from people living in Anbar Governorate/Iraq.

The samples were frozen in plastic test tubes in the refrigerator until use. Upon examination, samples are left for 20 to 60 minutes until liquefaction occurs at room temperature.

Semen quality was then assessed using two parameters: macroscopically and microscopically examination of semen analysis using world health organization criteria.

3.3 Methods

3.3.1 Determination of glucose in semen

Use the GOD-POD kit to test the amount of glucose in serum, plasma, and urine.

Principle: In the presence of glucose oxidase (GOD), glucose is converted to gluconic acid and hydrogen peroxide. This one-use peroxidase (POD) to react with phenol and 4-aminophenazone to produce a colored molecule whose intensity is directly correlated with the sample's glucose level.

3.3.2 Determination of fructose in semen

The main energy source for sperm metabolism (a glycolytic, anaerobic process), fructose, is crucial for sperm motility maintenance. A biomarker for seminal vesicle function is fructose. Low testosterone or insufficient seminal vesicles cause a low fructose content.

Principle: Fructose converted to Oxymethyl furfural when heated with concentrated hydrochloric acid in the presence of resorcinol, which forms a red-orange color. The color formed can be directly measured photometrically.

3.3.3 Determination of mannose in semen

The "mannan-type" polysaccharides 1,4-β-D-mannans, D-galacto-D-mannans, D-gluco-D-mannans, and D-galacto-D-gluco-D-mannans all include D-mannose as their primary sugar component. Though seldom found free in nature or as a component of straightforward oligosaccharides, it is a significant component of the carbohydrate core of glycoproteins.

Principle: Hexokinase (HK), adenosine-5'-triphosphate (ATP), and D-mannose phosphorylate each other to produce mannose-6-phosphate (M-6-P) and adenosine-5-diphosphate simultaneously (ADP) (Equation 3.1).

$$\text{D-Manose} + \text{ATP} \xrightarrow{\text{HK}} \text{M-6-P} + \text{ADP} \qquad (3.1)$$

3.3.4 Determination of CK level in semen

This test works at Wavelength: 340nm Temperature: 37°C

Let the reagent and sample stand at room temperature. After mixing, set a 2-minute timer to begin recording the first absorbance at 340 nm. Every minute for three minutes, record the absorbance again.

Calculate AAbs/min, the rate at which absorbance changes.

Principle: Oliver first described an enzymatic technique, which Rosalki and subsequently Szasz adapted (Equation 3.2, 3.3, and 3.4).

$$\text{Creatinephosphate} + \text{ADP} \xrightarrow{\text{CK}} \text{Creatine} + \text{ATP} \tag{3.2}$$

$$\text{D\_Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{ADP} + \text{G\_6\_Phosphate} \tag{3.3}$$

$$\text{G\_6\_Phosphate} + \text{NADP}^{+} \xrightarrow{\text{G6-PDH}} \text{6\_Phosphate} + \text{NADPH} + \text{H}^{+} \tag{3.4}$$

At 340 nm, a rise in absorbance proportional to the amount of CK activity in the samples is detected.

4. RESULTS AND DISCUSSION

4.1 Levels of Glucose in Patients and Controls

Figure 4.1 the results showed that there was a non-significant decrease in glucose in patients compared to controls (30.5G1 ± 10.2 vs 29.5G2 ± 5.3 mg/dL, P-value = 0.5).

As shown in Table 4.1, levels of glucose values are slightly lower in patients comparing levels of controls. In another study, the results showed a significant decrease in glucose in patients compared to controls in semen concentration, in addition to total sperm count and motility (Baccetti *et al.* 2002).

High or low glucose has been significantly associated with the reproductive health of men, which leads to male infertility. It has been reported that low semen quality was the main reason for low male fertility in diabetic patients (Agbaje *et al.* 2007).

Studies have shown a significant decrease in the sperm concentration, and total sperm count in diabetic group compared to the normal group, which indicates poor semen quality by affecting spermatogenesis or inhibiting sperm vitality.

This makes them more sensitive to changes in blood glucose. Low glucose that affects male fertility may occur due to increased levels of oxidative stress, inflammatory factors, endocrine disorders, and insulin resistance in the body (Baccetti *et al.* 2002).

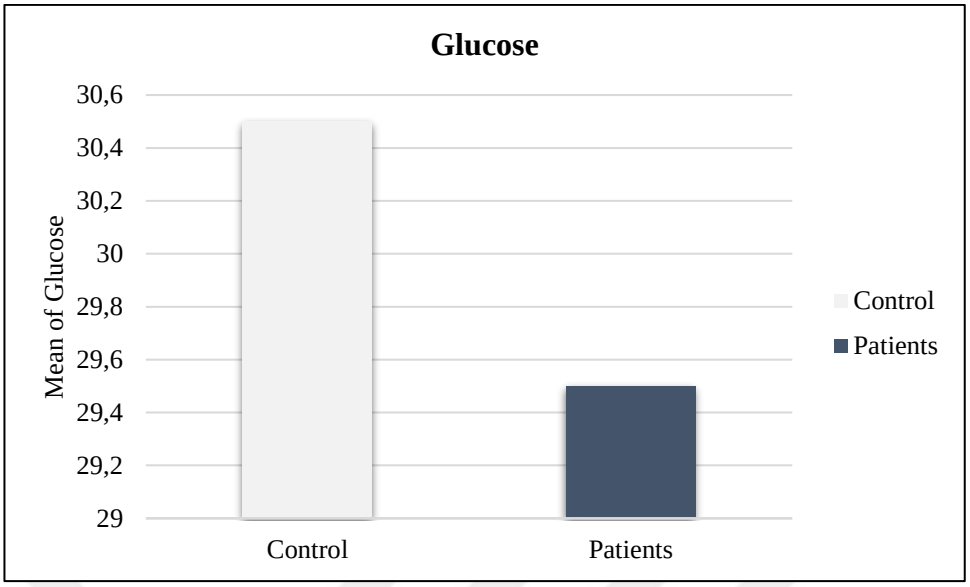

Figure 4.1 Semen glucose levels between patient group and control

4.2 Levels of Fructose in Patients and Controls

Figure 4.2 current results show there was significant level fructose Patients increased in comparison to controls (413.6G1 ± 76.1 VS 508.5G2 ± 118.7 mg/dL, P-value = 0.001).

As shown in Table 4.1, the results show a significant increase in the fructose in patients compared to controls. In a previous study, the same results were obtained indicating a high level of fructose in patients (Said *et al.* 2009).

The results are showed that the concentration of fructose increases with the decrease in the concentration of sperms and vice versa, as fructose is a storehouse of energy that sperms use for metabolism and movement.

High fructose concentration may be caused by reduced sperm count, abnormal sperm morphology, or low activity of sperm, resulting in low fructose use. The study also showed that the level of fructose is negatively related to the number of sperm and can be reduced by improving sperm motility and treating abnormalities and inflammation in the seminal vesicle.

Previous research has shown that abnormally shaped sperms may be weak or unable to move due to a urinary tract infection, so they use low fructose.

Fructose plays an important vital role in balancing the osmotic balance of semen which will affect membrane activity and sperm morphogenesis (Toragall *et al.* 2019).

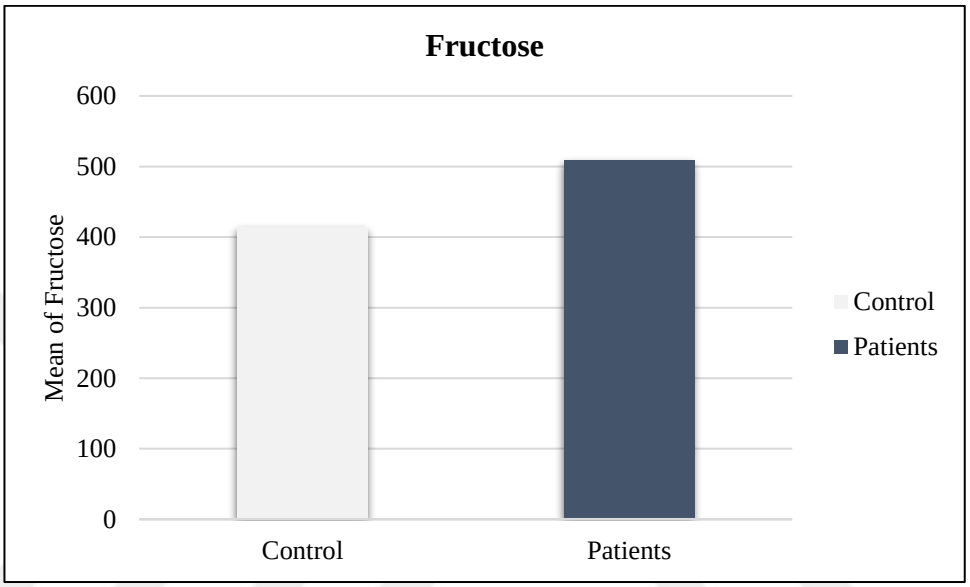

Figure 4.2 Levels of fructose in semen between patient group and control

4.3 Levels of Manose in Patients and Controls

The present results show that there is a significant increase in mannose receptors in patients compared to controls (17.5G1 ± 1.9 VS 22.3G2 ± 2.4 mg/dL, P-value = 0.000), as shown in Figure 4.3.

In Table 4.1, the results show a significant increase in mannose receptors in patients compared to the controls.

In another study, the effect of toxic metals in seminal plasma showed an increase in the expression level of the mannose receptors with zinc as well as cholesterol in the sperm

membrane and it decreased inversely with the lead levels, while cobalt had no effect on mannose receptors

A biological sign of the impact of heavy metals and organic toxicants on sperm fecundity is the expression of the mannose receptor on the outside of human sperm's plasma membrane (Benoff *et al.* 2000).

Another study reports that a mannose receptor-induced the acrosome interaction test may be useful in assessing the sperm function before fertilization (Benoff *et al.* 1997).

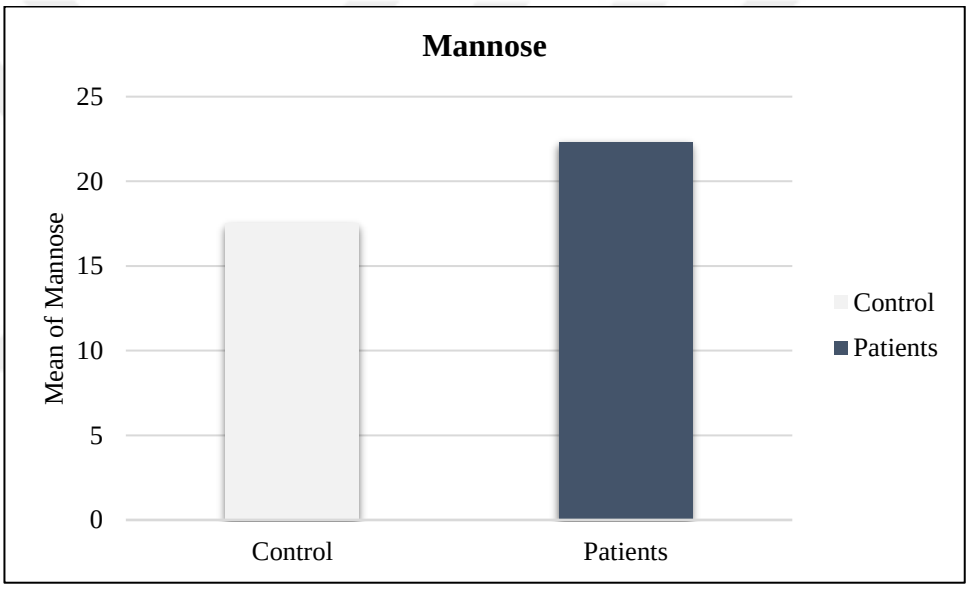

Figure 4.3 Levels of mannose in semen between group of patients and control

Table 4.1 Level of monosaccharides in semen of patients compared to the G1

parameter	**G1(Control) Mean ±SD**	**G2(patients) Mean ±SD**	**P-Value**
Glucose	30.5 ± 10.2	29.5 ± 5.3	0.587
Fructose	413.6 ± 76.1	508.5 ± 118.7	0.001
Manose	17.5 ± 1.9	22.3 ± 2.4	0.0001

4.4 Levels of Creatinine kinase in Patients and Controls

The results showed that there was a significantly higher creatinine kinase levels in patients compared to controls (480.8G1 ± 122.7 VS 612.4G2 ± 141.5 IU/L, P-value = 0.000). As shown in Table 4.2 and Figure4.4, the results show that there is a significant increase in the level of creatinine kinase enzyme in patients compared to controls.

The same results were obtained in a previous study, which indicated a significant increase in the level of creatinine enzyme in patients compared to controls, where the study showed that the activity of CK enzyme in semen depends on the number, morphology and movement of sperm and that the relationship between sperm number and the enzyme is an inverse relationship (Zeqiraj and Gashi 2014).

This biochemical marker can be used in the clinical assessment and the possibility of sperm cell fertilization where this marker represents an important diagnostic feature. The high activity of CK produced by gonads may be due to the presence of cytoplasmic remnants in immature sperm cells. Therefore, CK enzyme activity represents In the semen, there is a strong chemical indication of a decrease in the number of sperm cells and a decrease in their motility, and consequently, a decrease in the chances of fertilization in males (Huszar and Vigue 1993, Ergur *et al.* 2001).

There are some studies that showed that the level of Ck in the semen was very low in men who suffer from oligospermia. This is inconsistent with the current results of our study which showed that Ck enzyme activity was at a higher level in infertile patients compared to controls (AI-LNaqeeb and Fakhrildin 2015).

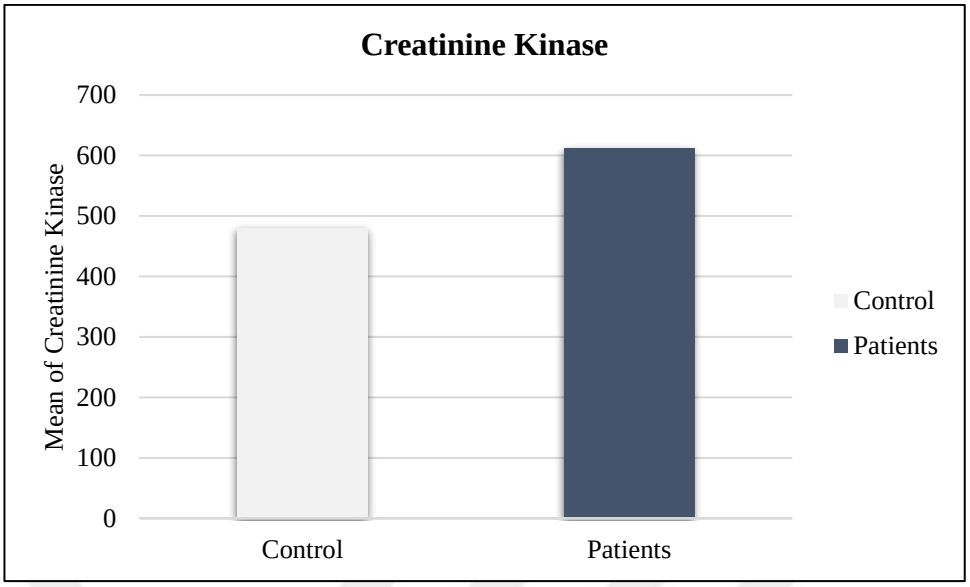

Figure 4.4 Levels of creatine kinase enzyme in semen between group of patients and control

Table 4.2 Level of creatinine kinase in the semen of patients compared to the control group

parameter	**G1(Control) Mean ±STD**	**G2(patients) Mean ±STD**	**P_ Value**
Creatinine (CK)	480.8 ± 122.7	612.4 ± 141.5	0.0001

4.5 Age

Figure 4.5 the results showed non-significant values for the average age of the patients compared to the control group (31.1G1 ± 7.2 vs. 31.8G2 ± 6.1 year, P-value = 0.54).

In Table 4.3, the results of average age had insignificant values in the group of patients compared to the controls, considering that the average age between the two groups was normal and very close.

In another study, the results showed that semen quality and fertility are strongly correlated with age and aging, where the greatest effect of age was on sperm motility, with a medium effect on semen volume and the least effect of age on sperm numbers.

Age-related changes affecting semen quality could be explained by physiological changes in the reproductive system, such as hardening of the testicular tube lumen, decreased semen volume due to lack of seminal vesicles, changes in the prostate glands resulting from smooth muscle atrophy, decreased water and protein levels, or changes in the epididymis. Which plays an important role in sperm maturation, or it may be acquired changes with age such as continuous smoking, external symptoms or diseases acquired due to occupational stress (Eskenazi *et al.* 2003).

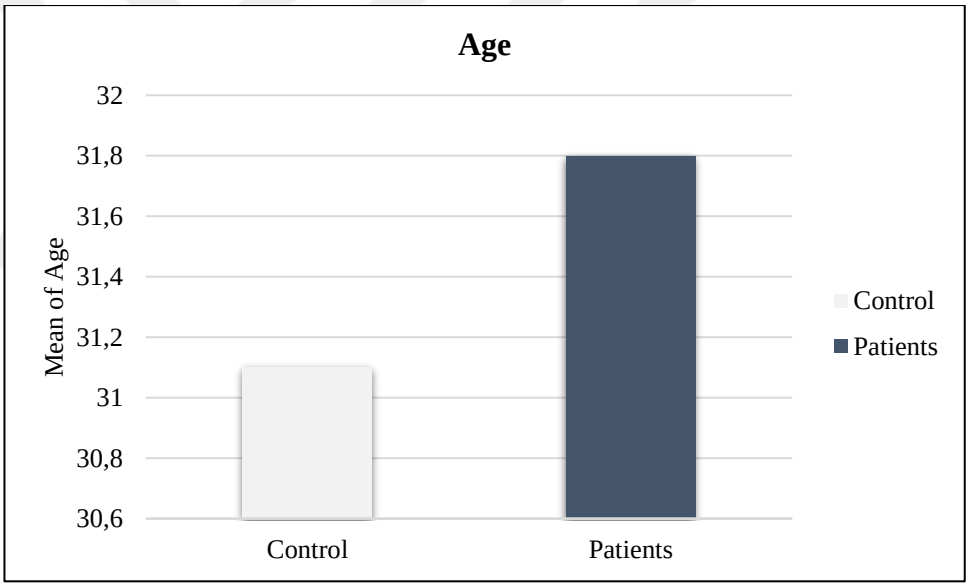

Figure 4.5 Age levels between patient group and control

4.6 Body Mass Index

Figure 4.6 the present results showed non-significant values of body mass index in patients compared to the control group (24.9G1 ± 2.6 vs 25.3G2 ± 3.5 Kg, P-value = 0.47).

In Table 4.3, the results showed that BMI was not significant in patients compared to control levels. In another study, semen quality in overweight and obese men may be lower compared to a normal BMI, which may be one of the causes of fertility and infertility.

This study also showed an inverse relationship between BMI, total sperm count, cell concentration and motility, which may be due to a marked change in the shape of sex hormones (Sekhavat and Moein 2010).

In our current study, the results of body mass index (BMI) were of non-significant value considering that the mean weight between the group of patients and controls was very close and normal.

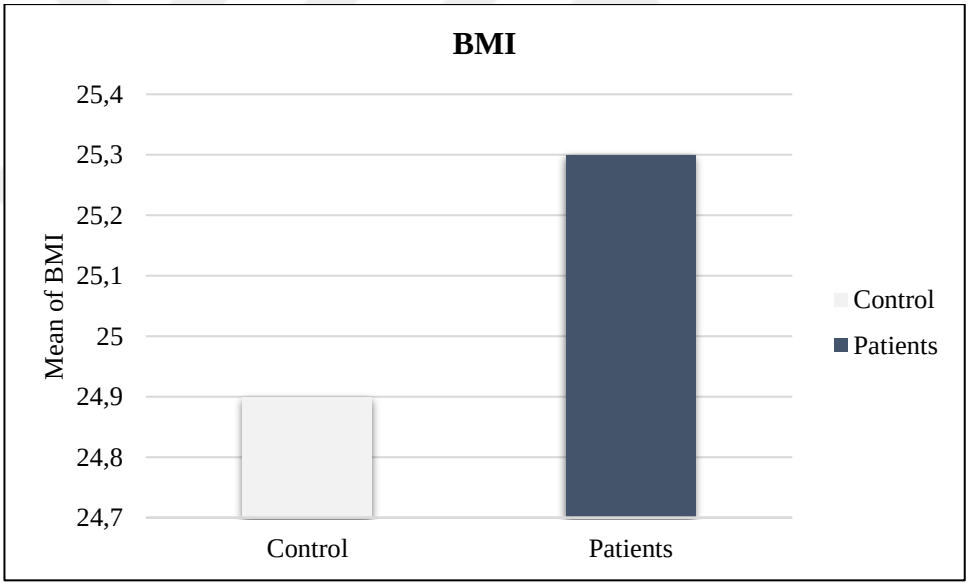

Figure 4.6 Comparison between patient group and the control body mass index

4.7 Sperm Count

Table 4.3 our current study shows that there is a significant decrease in total sperm count in patients compared to controls (115.1G1 ± 66.4 vs 14.2G2 ± 5.4, P-value = 0.005) as shown in Figure4.7.

Another research of European males revealed a general decline in sperm concentration of 32.5% during the previous 50 years. According to some study, environmental and lifestyle variables that have a detrimental impact on male fertility may also have an impact on semen quality.

Possible causes of low sperm concentration, which play an important role in determining reproductive health and affect fertility, are reproductive abnormalities in men, environmental and lifestyle factors, such as environmental exposure and stress, chronic medications or the sexually transmitted diseases, as well as our changing diet. over time.

Or it may be through acquired habits such as smoking, drinking alcohol and drugs that impair fertility by interfering with sperm formation and movement, change in the DNA of animals, and change in hormonal regulation, which reduces the ability of sperm to fertilize (Sengupta *et al.* 2018).

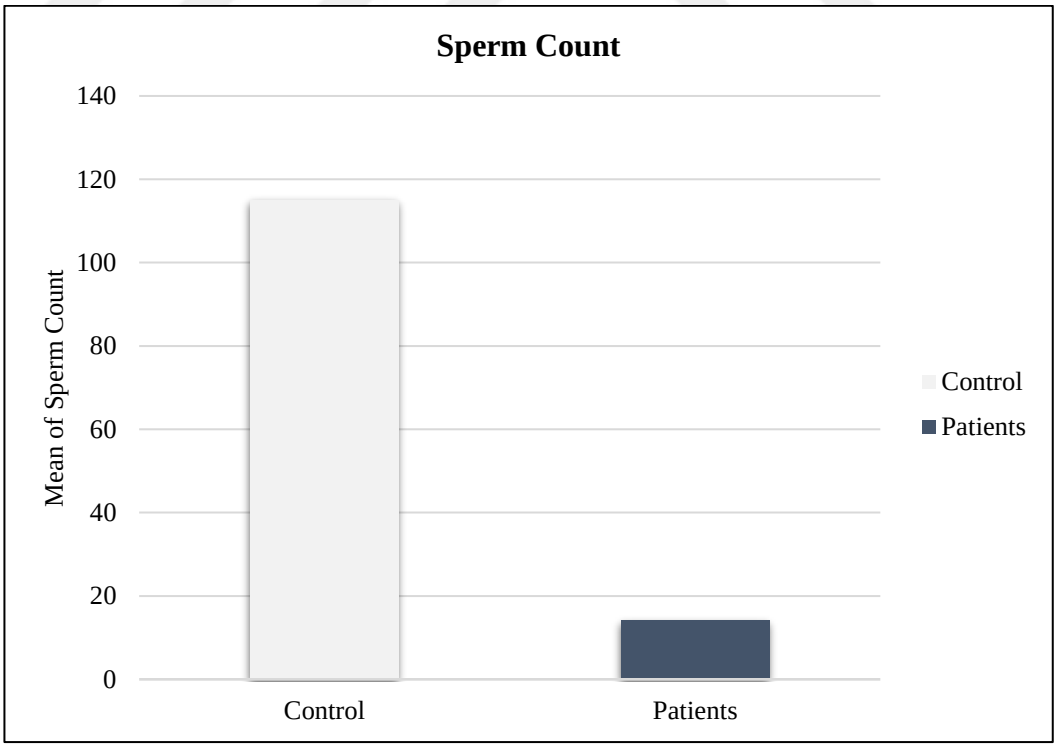

Figure 4.7 Sperm count in semen between patient group and control

Table 4.3 Mean and SD of body mass index and age and sperm count in this study

parameter	**G1(Control) Mean ±STD**	**G2(patients) Mean ±STD**	**P_ Value**
Age	31.1 ± 7.2	31.8 ± 6.1	0.54
BMI	24.9 ± 2.6	25.3 ± 3.5	0.47
Sperm count	115.1 ± 66.4	14.1 ± 5.4	0.005

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusion

1. The level of glucose in semen showed little difference in the group of patients compared to the controls.
2. The levels of fructose in the semen showed a significant difference in group of the patients compared to controls.
3. The mannose level had a significant difference in the group patients compared to the control group.
4. The results showed a significant difference in the level of creatinine kinase enzyme in semen among the patients group compared to the control group.
5. The average age showed an unremarkable effect due to the average ages, which were close between the group of patients and the controls.
6. BMI was not significant due to the close weights between patients group and the control group.
7. The percentage of sperm in the semen also showed a significant decrease in the group of patients compared to the controls.
8. The current study's findings revealed an antagonistic correlation between CK and sperm count.
9. The current study's findings revealed an antagonistic link between fructose, mannose, and sperm count.

5.2 Recommendation

1. More studies should be conducted to detect the effect of CK enzyme in the semen of infertile patients.
2. It is necessary to follow and adhere to international health standards, especially in laboratories, in determining the causes of infertility in men and in a wider geographical area.
3. Early periodic examinations are necessary in order to assess the risks of infertility in the future.

4. Acquired factors and technology have an effect that may exceed changes in the reproductive system and genetic factors in reducing fertility in men, so focus must be on them.

REFERENCES

Agarwal, A. 2001. Creatine Kinase: A New Marker of Sperm Quality. The cevel clinic, 2: 13.

Agarwal, A., Rana, M., Qiu, E., AlBunni, H., Bui, A. D. and Henkel, R. 2018. Role of oxidative stress, infection and inflammation in male infertility. Andrologia, 50(11): e13126.

Agbaje, I., Rogers, D. A., McVicar, C. M., McClure, N., Atkinson, A. B., Mallidis, C. and Lewis, S. E. M. 2007. Insulin dependant diabetes mellitus: implications for male reproductive function. Human Reproduction, 22(7): 1871-1877.

AI-LNaqeeb, A. A. and Fakhrildin, M. B. M. R. 2015. Effect of serum and seminal amh on sperm production for oligozoospermic and azoospermic men.

Akgul, A., Kadioglu, A., Koksal, M. O., Ozmez, A. and Agacfidan, A. 2018. Sexually transmitted agents and their association with leucocytospermia in infertility clinic patients. Andrologia, 50(10): e13127.

Al-Matubsi, H. Y., Kanaan, R. A., Hamdan, F., Salim, M., Oriquat, G. A. and Al-Hanbali, O. A. 2011. Smoking practices in Jordanian people and their impact on semen quality and hormonal levels among adult men. Central European journal of public health, 19(1): 54.

Altmäe, S., Franasiak, J. M. and Mändar, R. 2019. The seminal microbiome in health and disease. Nature Reviews Urology, 16(12): 703-721.

Amann, R. P. 2008. The cycle of the seminiferous epithelium in humans: a need to revisit?. Journal of andrology, 29(5): 469-487.

Andersen, J. M. 2017. Semen quality in relation to body mass index, sperm fatty acid composition, and anti-Müllerian hormone.

Baccetti, B., La Marca, A., Piomboni, P., Capitani, S., Bruni, E., Petraglia, F. and De Leo, V. 2002. Insulin-dependent diabetes in men is associated with hypothalamo-pituitary derangement and with impairment in semen quality. Human Reproduction, 17(10): 2673-2677.

Benoff, S. 1997. Carbohydrates and fertilization: an overview. Molecular human reproduction, 3(7): 599-637.

Benoff, S., Cooper, G. W., Centola, G. M., Jacob, A., Hershlag, A. and Hurley, I. R. 2000. Metal ions and human sperm mannose receptors. Andrologia, 32(4-5): 317-329.

Benoff, S., Hurley, I. R., Mandel, F. S., Paine, T., Jacob, A., Cooper, G. W. and Hershlag, A. 1997. Use of mannose ligands in IVF screens to mimic zona pellucida-induced acrosome reactions and predict fertilization success. Molecular human reproduction, 3(10): 839-846.

Biró, G. 2018. Human biological characteristics of fructose. Journal of Food Investigation–Vol, 64(1).

Brezina, P. R., Yunus, F. N. and Zhao, Y. 2012. Effects of pharmaceutical medications on male fertility. Sciences, 3: 12-13.

Bucci, D., Rodriguez-Gil, J. E., Vallorani, C., Spinaci, M., Galeati, G. and Tamanini, C. 2011. GLUTs and mammalian sperm metabolism. Journal of andrology, 32(4): 348-355.

Bundhun, P. K., Janoo, G., Bhurtu, A., Teeluck, A. R., Soogund, M. Z. S., Pursun, M. and Huang, F. 2019. Tobacco smoking and semen quality in infertile males: a systematic review and meta-analysis. BMC public health, 19(1): 1-11.

Calogero, A. E., La Vignera, S., Condorelli, R. A., Perdichizzi, A., Valenti, D., Asero, P. and De Rosa, M. 2011. Environmental car exhaust pollution damages human sperm chromatin and DNA. Journal of endocrinological investigation, 34(6): e139-e143.

Calogero, A., Polosa, R., Perdichizzi, A., Guarino, F., La Vignera, S., Scarfia, A. and Vicari, E. 2009. Cigarette smoke extract immobilizes human spermatozoa and induces sperm apoptosis. Reproductive biomedicine online, 19(4): 564-571.

Chia, S. E., Tay, S. K. and Lim, S. T. 1998. What constitutes a normal seminal analysis? Semen parameters of 243 fertile men. Human reproduction (Oxford, England): 13(12): 3394-3398.

Dain, L., Auslander, R. and Dirnfeld, M. 2011. The effect of paternal age on assisted reproduction outcome. Fertility and sterility, 95(1): 1-8.

Dandekar, S. P. and Parkar, G. M. 1999. Correlation between creatine kinase activity, lipid-peroxidation and water test in male infertility. Journal of postgraduate medicine, 45(2): 42.

Dissanayake, D. M. I. H., Keerthirathna, W. L. R. and Peiris, L. D. C. 2019. Male infertility problem: a contemporary review on present status and future perspective. Gender and the Genome, 3: 2470289719868240.

Domes, T., Lo, K. C., Grober, E. D., Mullen, J. B. M., Mazzulli, T. and Jarvi, K. 2012. The incidence and effect of bacteriospermia and elevated seminal leukocytes on semen parameters. Fertility and Sterility, 97(5): 1050-1055.

Eddy, E. M., Toshimori, K. and O'Brien, D. A. 2003. Fibrous sheath of mammalian spermatozoa. Microscopy research and technique, 61(1): 103-115.

Eliasson, R. 1965. Accurate determination of glucose in human semen. Reproduction, 9(3): 325-330.

Emokpae, M. A. and Brown, S. I. 2021. Effects of lifestyle factors on fertility: practical recommendations for modification. Reproduction and Fertility, 2(1): R13-R26.

Ergur, A. R., Dokras, A., Giraldo, J., Kovanci, E., Jones, E. and Huszar, G. 2001. Sperm cellular maturity and the treatment choice of IVF or ICSI: the predictive value of the sperm HspA2 chaperone protein ratio. Fertility and Sterility, 76(3): S245.

Eskenazi, B., Wyrobek, A. J., Sloter, E., Kidd, S. A., Moore, L., Young, S. and Moore, D. 2003. The association of age and semen quality in healthy men. Human reproduction, 18(2): 447-454.

Eskenazi, B., Wyrobek, A. J., Sloter, E., Kidd, S. A., Moore, L., Young, S. and Moore, D. 2003. The association of age and semen quality in healthy men. Human reproduction, 18(2): 447-454.

Esmailkhani, A., Akhi, M. T., Sadeghi, J., Niknafs, B., Bialvaei, A. Z., Farzadi, L. and Safadel, N. 2018. Assessing the prevalence of Staphylococcus aureus in infertile male patients in Tabriz, northwest Iran. International Journal of Reproductive BioMedicine, 16(7): 469.

Fakih, H., MacLusky, N., DeCherney, A., Wallimann, T. and Huszar, G. 1986. Enhancement ofhuman sperm motility and velocity in v i m : Effects of calcium and creatine phosphate. Fertil. Steril. 46, 938-944

Flesch, F. M. and Gadella, B. M. 2000. Dynamics of the mammalian sperm plasma membrane in the process of fertilization. Biochimica et Biophysica Acta (BBA)-Reviews on Biomembranes, 1469(3): 197-235.

Fraczek, M. and Kurpisz, M. 2015. Mechanisms of the harmful effects of bacterial semen infection on ejaculated human spermatozoa: potential inflammatory markers in semen. Folia Histochemica et Cytobiologica, 53(3): 201-217.

Haidl, F., Haidl, G., Oltermann, I. and Allam, J. P. 2015. Seminal parameters of chronic male genital inflammation are associated with disturbed sperm DNA integrity. Andrologia, 47(4): 464-469.

Håkonsen, L. B., Thulstrup, A. M., Aggerholm, A. S., Olsen, J., Bonde, J. P. andersen, C. Y. and Ramlau-Hansen, C. H. 2011. Does weight loss improve semen quality and reproductive hormones? Results from a cohort of severely obese men. Reproductive health, 8(1): 1-8.

Harman, S. M., Metter, E. J., Tobin, J. D., Pearson, J. and Blackman, M. R. 2001. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. The Journal of Clinical Endocrinology and Metabolism, 86(2): 724-731.

Hellstrom, W. J. and Sikka, S. C. 2006. Effects of acute treatment with tamsulosin versus alfuzosin on ejaculatory function in normal volunteers. The Journal of urology, 176(4): 1529-1533.

Hershlag, A., Scholl, G. M., Jacob, A., Mandel, F. S., Guhring, P., Paine, T. and Benoff, S. 1998. Mannose ligand receptor assay as a test to predict fertilization in vitro: a prospective study. Fertility and sterility, 70(3): 482-491.

Herzog, A. G., Seibel, M. M., Schomer, D. L., Vaitukaitis, J. L. and Geschwind, N. 1986. Reproductive endocrine disorders in men with partial seizures of temporal lobe origin. Archives of neurology, 43(4): 347-350.

Histewart, A. B., Anderson, W., Delves, G., Lwaleed, B. A., Birch, B. and Cooper, A. 2004. Prostasomes: a role in prostatic disease?. BJU international, 94(7): 985-989

Hornstein, O. 1961. Vergleichende untersuchungen zum fructose-und glucosegehalt des menschlichen spermaliquors. Archiv für klinische und experimentelle Dermatologie, 212(3): 257-274.

Huszar, G. and Vigue, L. 1993. Incomplete development of human spermatozoa is associated with increased creatine phosphokinase concentration and abnormal head morphology. Molecular reproduction and development, 34(3): 292-298.

Huszar, G., Patrizio, P., Vigue, L., Willets, M., Wdjker, C., Adhoot, D. and Johnson, L. 1998. Cytoplasmic extrusion and the switch from creatine kinase B to M isoform

are completed by the commencement of epididymal transport in human and stallion spermatozoa. Journal of andrology, 19(1): 11-20.

Huszar, G., Stone, K., Dix, D. and Vigue, L. 2000. Putative creatine kinase M-isoform in human sperm is identifiedas the 70-kilodalton heat shock protein HspA2. Biology of Reproduction, 63(3): 925-932.

Huwe, P., Diemer, T., Ludwig, M., Liu, J., Schiefer, H. G. and Weidner, W. 1998. Influence of different uropathogenic microorganisms on human sperm motility parameters in an in vitro experiment. Andrologia, 30(S1): 55-59.

Istvan, M., Rahban, R., Dananche, B., Senn, A., Stettler, E., Multigner, L. and Garlantézec, R. 2021. Maternal occupational exposure to endocrine-disrupting chemicals during pregnancy and semen parameters in adulthood: results of a nationwide cross-sectional study among Swiss conscripts. Human Reproduction, 36(7): 1948-1958.

Jarow, J., Sigman, M., Kolettis, P. N., Lipshultz, L. R., Mcclure, R. D. and Nangia, A. K. 2010. The optimal evaluation of the infertile male: AUA best practice statement. American Urological Association Education and Research.

Jequier, A. M. 2010. Semen analysis: a new manual and its application to the understanding of semen and its pathology. Asian journal of andrology, 12(1): 11.

Johnson, L. 1986. Spermatogenesis and aging in the human. Journal of Andrology, 7(6): 331-354.

Jurewicz, J., Dziewirska, E., Radwan, M. and Hanke, W. 2018. Air pollution from natural and anthropic sources and male fertility. Reproductive Biology and Endocrinology, 16(1): 1-18.

Kaldis, P., Kamp, G., Piendl, T. and Wallimann, T. 1997. Functions of creatine kinase isoenzymes in spermatozoa. In Advances in Developmental Biology 1992. Academic Press., 5: 275-312.

Kazemijaliseh, H., Tehrani, F. R., Behboudi-Gandevani, S., Hosseinpanah, F., Khalili, D. and Azizi, F. 2015. The prevalence and causes of primary infertility in Iran: a population-based study. Global journal of health science, 7(6): 226.

Keck, C., Gerber-Schäfer, C., Clad, A., Wilhelm, C. and Breckwoldt, M. 1998. Seminal tract infections: impact on male fertility and treatment options. Human Reproduction Update, 4(6): 891-903.

Khan, M. S., Ali, I., Tahir, F. and Khan, G. M. 2008. Simultaneous analysis of the three hormones involved in spermatogenesis and their interrelation ratios. Pakistan Journal of Pharmaceutical Sciences, 21(4).

Kidd, S. A., Eskenazi, B. and Wyrobek, A. J. 2001. Effects of male age on semen quality and fertility: a review of the literature. Fertility and sterility, 75(2): 237-248.

Kingsley, G. R. 1942. The direct biuret method for the determination of serum proteins as applied to photoelectric and visual colorimetry. Journal of Laboratory and Clinical Medicine, 27: 840-845.

Kumar, N. and Singh, A. K. 2022. Impact of environmental factors on human semen quality and male fertility: a narrative review. Environmental Sciences Europe, 34(1): 1-13.

Künzle, R., Mueller, M. D., Hänggi, W., Birkhäuser, M. H., Drescher, H. and Bersinger, N. A. 2003. Semen quality of male smokers and nonsmokers in infertile couples. Fertility and sterility, 79(2): 287-291.

Lamb, D. J. 2010. Semen analysis in 21st century medicine: the need for sperm function testing. Asian journal of andrology, 12(1): 64.

Lee, H. J., Fillers, W. S. and Iyengar, M. R. 1988. Phosphocreatine, an intracellular high-energy compound, is found in the extracellular fluid of the seminal vesicles in mice and rats. Proceedings of the National Academy of Sciences, 85(19): 7265-7269.

Lee, H. J., Fillers, W. S. and lyengar, M. R. 1988. Phosphocreatine, an intracellular high-energy compound, is found in the extracellular fluid of the seminal vesicles in mice and rats. Proc. Natl. Acad. Sci. USA 84,7265-7269.

Li, Y., Lin, H., Ma, M., Li, L., Cai, M., Zhou, N. and Cao, J. 2009. Semen quality of 1346 healthy men, results from the Chongqing area of southwest China. Human Reproduction, 24(2): 459-469.

Lombardo, F., Sgro, P., Salacone, P., Gilio, B., Gandini, L., Dondero, F. and Lenzi, A. 2005. Androgens and fertility. Journal of endocrinological investigation, 28(3): 51-55.

Lu, J. C., Huang, Y. F. and Lü, N. Q. 2010. WHO Laboratory Manual for the Examination and Processing of Human Semen: its applicability to andrology

laboratories in China. Zhonghua nan ke xue= National journal of andrology, 16(10): 867-871.

Macaluso, M., Wright-Schnapp, T. J., Chandra, A., Johnson, R., Satterwhite, C. L., Pulver, A. and Pollack, L. A. 2010. A public health focus on infertility prevention, detection, and management. Fertility and sterility, 93(1): 16-e1.

Maghsoumi-Norouzabad, L., Javid, A. Z., Aiiashi, S., Hosseini, S. A., Dadfar, M., Bazyar, H. and Dastoorpur, M. 2020. The impact of obesity on various semen parameters and sex hormones in Iranian men with infertility: A cross-sectional study. Research and Reports in Urology, 12: 357.

Mahat, R. K., Arora, M., Bhale, D. V., Holkar, S., Kumar, S. and Yadav, T. 2016. Risk factors and causes of male infertility-a review. Biochem Anal Biochem, 5(2): 271

Mahmoud, A. M., Goemaere, S., El-Garem, Y., Van Pottelbergh, I. N. G. E., Comhaire, F. H. and Kaufman, J. M. 2003. Testicular volume in relation to hormonal indices of gonadal function in community-dwelling elderly men. The Journal of Clinical Endocrinology and Metabolism, 88(1): 179-184.

Matson, P. L., Myssonski, K., Yovich, S., Morrison, L., Irving, J. and Bakos, H. W. 2010. The density of human semen and the validation of weight as an indicator of volume: a multicentre study. Reproductive biology, 10(2): 141-153.

Mayes, P. A. 1993. Intermediary metabolism of fructose. The American journal of clinical nutrition, 58(5): 754S-765S.

Mieusset, R. and B'ujan, L. O. U. I. S. 1994. The potential of mild testicular heating as a safe, effective and reversible contraceptive method for men. International journal of andrology, 17(4): 186-191

Mieusset, R., Bujan, L., Mansat, A., Pontonnier, F. and Grandjean, H. 1987. Hyperthermia and human spermatogenesis: enhancement of the inhibitory effect obtained by ‘artificial cryptorchidism’. International journal of andrology, 10(4): 571-580.

Miki, K. 2007. Energy metabolism and sperm function. Society of Reproduction and Fertility supplement, 65, 309-325.

Moon, K. H., Osborn, R. H., Yannone, M. E. and Bunge, R. G. 1970. Relationship of testosterone to human seminal fructose. Investigative urology, 7(6): 478-486.

Mortimer, D. 1994. Practical Laboratory Andrology Oxford University Press, Oxford.

Mosher, W. D. and Pratt, W. F. 1991. Fecundity and infertility in the United States: incidence and trends. Fertility and sterility, 56(2): 192-193.

Mukhopadhyay, D., Varghese, A. C., Pal, M., Banerjee, S. K., Bhattacharyya, A. K., Sharma, R. K. and Agarwal, A. 2010. Semen quality and age-specific changes: a study between two decades on 3,729 male partners of couples with normal sperm count and attending an andrology laboratory for infertility-related problems in an Indian city. Fertility and sterility, 93(7): 2247-2254.

Oehninger, S. 2003. Biochemical and functional characterization of the human zona pellucida. Reproductive biomedicine online, 7(6): 641-648.

Ombelet, W. 2011. Global access to infertility care in developing countries: a case of human rights, equity and social justice. Facts, views & vision in ObGyn, 3(4): 257.

Raines, R. T. 1998. Ribonuclease a. Chemical reviews, 98(3): 1045-1066.

Rajender, S., Monica, M. G., Walter, L. and Agarwal, A. 2011. Thyroid, spermatogenesis, and male infertility. Front Biosci (Elite Ed): 3: 843-55.

Razvi, K., Chew, S., Yong, E. L., Kumar, J. and Ng, S. C. 1999. The clinical management of male infertility. Singapore medical journal, 40(4): 291-297.

Ricci, E., Viganò, P., Cipriani, S., Somigliana, E., Chiaffarino, F., Bulfoni, A. and Parazzini, F. 2017. Coffee and caffeine intake and male infertility: a systematic review. Nutrition journal, 16(1): 1-14.

Rodriguez-Martinez, H. 2007. Role of the oviduct in sperm capacitation. Theriogenology, 68, S138-S146.

Rosa, M. D., Zarrilli, S., Paesano, L., Carbone, U., Boggia, B., Petretta, M. and Lombardi, G. 2003. Traffic pollutants affect fertility in men. Human Reproduction, 18(5): 1055-1061.

Rusz, A., Pilatz, A., Wagenlehner, F., Linn, T., Diemer, T., Schuppe, H. C. and Weidner, W. 2012. Influence of urogenital infections and inflammation on semen quality and male fertility. World journal of urology, 30(1): 23-30.

Rybar, R., Prinosilova, P., Kopecka, V., Hlavicova, J., Veznik, Z., Zajicova, A. and Rubes, J. 2012. The effect of bacterial contamination of semen on sperm chromatin integrity and standard semen parameters in men from infertile couples. Andrologia, 44, 410-418.

Said, L., Galeraud-Denis, I., Carreau, S. and Saad, A. 2009. Relationship between semen quality and seminal plasma components: Alpha-glucosidase, fructose and citrate in infertile men compared with a normospermic population of Tunisian men. Andrologia, 41(3): 150-156.

Sampson, N., Untergasser, G., Plas, E. and Berger, P. 2007. The ageing male reproductive tract. The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland, 211(2): 206-218.

Scribano, D., Sarshar, M., Prezioso, C., Lucarelli, M., Angeloni, A., Zagaglia, C. and Ambrosi, C. 2020. D-Mannose treatment neither affects uropathogenic Escherichia coli properties nor induces stable fimh modifications. Molecules, 25(2): 316.

Sekhavat, L. and Moein, M. R. 2010. The effect of male body mass index on sperm parameters. The Aging Male, 13(3): 155-158.

Sengupta, P., Borges Jr, E., Dutta, S. and Krajewska-Kulak, E. 2018. Decline in sperm count in European men during the past 50 years. Human & experimental toxicology, 37(3): 247-255.

Sharma, R., Biedenharn, K. R., Fedor, J. M. and Agarwal, A. 2013. Lifestyle factors and reproductive health: taking control of your fertility. Reproductive biology and endocrinology, 11(1): 1-15.

Sidhu, R. S., Hallak, J., Sharma, R. K., Thomas, A. J. and Agarwal, A. 1998. Relationship between creatine kinase levels and clinical diagnosis of infertility. Journal of assisted reproduction and genetics, 15(4): 188-192.

Sidhu, R. S., Sharma, R. K. and Agarwal, A. 1998. Relationship between creatine kinase activity and semen characteristics in subfertile men. International Journal of Fertility and Women's Medicine, 43(4): 192.

Sigman, M. and Jarow, J. P. 2007. Male infertility. Campbell-Walsh Urology. 9th edition.,609-53.

Stevens, C. E. 2014. Digestive system-AccessScience from McGraw-Hill Education http://www. accessscience. com/content/digestive-system/194600.

Storey, B. T. 2004. Mammalian sperm metabolism: oxygen and sugar, friend and foe. International Journal of Developmental Biology, 52(5-6): 427-437.

Szablewski, L. 2011. Glucose homeostasis–mechanism and defects. Diabetes- Damages and Treatments, 2.

Taghipour, A. and Latifnejad, R. R. 2019. The association of socio-demographic characteristics of infertile men and women with their attitude towards donation procedures.

Tan, R. S. and Scally, M. C. 2009. Anabolic steroid-induced hypogonadism–towards a unified hypothesis of anabolic steroid action. Medical hypotheses, 72(6): 723-728.

Teixeira, A. M. and Borges, G. F. 2012. Creatine kinase: structure and function. Brazilian Journal of Biomotricity, 6(2): 53-65.

Tesarik, J., Mendoza, C. and Carreras, A. 1991. Expression of D-mannose binding sites on human spermatozoa: comparison of fertile donors and infertile patients. Fertility and sterility, 56(1): 113-118.

Tocci, A. and Lucchini, C. 2010. WHO reference values for human semen. Human reproduction update, 16(5): 559-559.

Toragall, M. M., Satapathy, S. K., Kadadevaru, G. G. and Hiremath, M. B. 2019. Evaluation of seminal fructose and citric acid levels in men with fertility problem. Journal of human reproductive sciences, 12(3): 199.

Töpfer-Petersen, E. 1999. Carbohydrate-based interactions on the route of a spermatozoon to fertilization. Human reproduction update, 5(4): 314-329.

Trummer, H., Habermann, H., Haas, J. and Pummer, K. 2002. The impact of cigarette smoking on human semen parameters and hormones. Human Reproduction, 17(6): 1554-1559.

Vander, A. J., Sherman, J. H., Luciano, D. S. and Van Wynsberghe, D. M. 1990. Human physiology: the mechanisms of body function, pp. 563-603, New York: McGraw-Hill.

Vigue, C., Vigue, L. and Huszar, G. 1992. Adenosine Triphosphate (ATP) Concentrations and ATP/Adenosine Diphosphate Ratios in Human Sperm of Normospermic, Oligospermic, and Asthenospermic Specimens and in their Swim-up Fractions: Lack of Correlation Between ATP Parameters and Sperm Creatine Kinase Concentrations. Journal of andrology, 13(4): 305-311.

Wallimann, T. and Hemmer, W. 1994. Creatine kinase in non-muscle tissues and cells. Molecular and cellular biochemistry, 133(1): 193-220.

Wang, C. H. R. I. S. T. I. N. A., Leung, A. N. D. R. E. W. and Sinha-Hikim, A. P. 1993. Reproductive aging in the male brown-Norway rat: a model for the human. Endocrinology, 133(6): 2773-2781.

Wang, X., Tian, X., Ye, B., Zhang, Y., Li, C., Liao, J. and Ma, L. 2020. Gaseous pollutant exposure affects semen quality in central China: a cross-sectional study. Andrology, 8(1): 117-124.

Wiener-Megnazi, Z., Auslender, R. and Dirnfeld, M. 2012. Advanced paternal age and reproductive outcome. Asian journal of andrology, 14(1): 69.

World Health Organisation. 1999. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. Cambridge university press.

Yanagimachi, R. 1994. Fertility of mammalian spermatozoa: its development and relativity. Zygote, 2(4): 371-372.

Zegers-Hochschild, F., Adamson, G. D., De Mouzon, J., Ishihara, O., Mansour, R., Nygren, K. and Van der Poel, S. 2009. The international committee for monitoring assisted reproductive technology (ICMART) and the world health organization (WHO) revised glossary on ART terminology, 2009. Human reproduction, 24(11): 2683-2687.

Zenzes, M. T. 2000. Smoking and reproduction: gene damage to human gametes and embryos. Human Reproduction Update, 6(2): 122-131.

Zeqiraj, A. and Gashi, Z. 2014. Creatine kinase activity in human seminal fluid. Int. J. Innov. Sci. Eng. Technol, 1: 3.

Zeyad, A., Hamad, M. F. and Hammadeh, M. E. 2018. The effects of bacterial infection on human sperm nuclear protamine P1/P2 ratio and DNA integrity. Andrologia, 50(2): e12841.

Zhang, J., Cai, Z., Ma, C., Xiong, J. and Li, H. 2020. Impacts of outdoor air pollution on human semen quality: a meta-analysis and systematic review. BioMed research international, 2020.

Zhu, Q. X., Meads, C., Lu, M. L., Wu, J. Q., Zhou, W. J. and Gao, E. S. 2011. Turning point of age for semen quality: a population-based study in Chinese men. Fertility and sterility, 96(3): 572-576.

CURRICULUM VITAE

Personal Information

Name and Surname : Omar Ahmed Abbas ABBAS

Education

MSc	Çankırı Karatekin University Graduate School of Natural and Applied Sciences Department of Chemistry	2020-2022
Undergraduate	Anbar University Faculty of Sciences Department of Chemistry	2013-2017