Republic of Iraq

Ministry of Higher Education and Scientific Research

University of Kerbala /College of Medicine

Department of Chemistry and Biochemistry

Assessment of Galectin-3 Binding Protein and Heat Shock Protein 70 and Their Relations with Antioxidant Biomarkers in a Sample of Iraqi Men

A thesis

Submitted to the Council of the College of Medicine, University of Kerbala, in Partial Fulfillment of the Requirements for the Degree of Master in clinical chemistry and Biochemistry

By

Ahmed Abd Aljaleel Saeed

Bachelor's degree, College of Science, Chemistry Branch, University of Kerbala 2010-2011

Supervised By

Asst. prof.

Dr. Shaymaa Z . Nada

Chemistry and Biochemistry
College of Medicine/ University of Kerbala

Professor

Dr. Wasan Ghazi AlSafi

College of Medicine
University of Kerbala

2022 A.D **1444 A.H.**

وَلَقَدۡ خَلَقۡنَا ٱلۡإِنسَٰنَ مِن سُلَٰلَةٖ مِّن طِينٖ (١٢) ثُمَّ جَعَلۡنَٰهُ نُطۡفَةٗ فِي قَرَارٖ مَّكِينٖ (١٣) ثُمَّ خَلَقۡنَا ٱلنُّطۡفَةَ عَلَقَةٗ فَخَلَقۡنَا ٱلۡعَلَقَةَ مُضۡغَةٗ فَخَلَقۡنَا ٱلۡمُضۡغَةَ عِظَٰمٗا فَكَسَوۡنَا ٱلۡعِظَٰمَ لَحۡمٗا ثُمَّ أَنشَأۡنَٰهُ خَلۡقًا ءَاخَرَۚ فَتَبَارَكَ ٱللَّهُ أَحۡسَنُ ٱلۡخَٰلِقِينَ (١٤)

سورة المؤمنون

Supervisors Certification

We certify that this M.Sc. thesis entitled: -

Assessment of Galectin-3 Binding Protein and Heat Shock Protein 70 and Their Relations with Antioxidant Biomarkers in a Sample of Iraqi Men

Was prepared under our supervision at the College of Medicine/University of Kerbala, as a partial fulfillment of the requirement for the Degree of Master in clinical chemistry and Biochemistry

Assist prof.
Dr. Shaymaa Z . Nada
College of Medicine
University of Kerbala

Professor.
Dr. Wasan Ghazi AlSafi
College of Medicine
University of Kerbala

In the view of the available recommendation, we forward this M.Sc. thesis for debate by the examining committee

Dr. Atheer Hamid Al-Ghanimi

Head of Chemistry and Biochemistry Department

College of Medicine – University of Kerbala

Examination Committee

We certify that we have read this thesis entitled

" Assessment of Galectin-3 Binding Protein and Heat Shock Protein 70 and their Relations with Sperm Antioxidant Biomarkers in a Sample of Iraqi Men"

and as examining committee examined the student **"Ahmed Abd Aljalil Saeed"** in its content and in our opinion it is adequate with "Excellent" as a thesis for the degree of master in science of Clinical Biochemistry.

Prof. Dr. Manal Kamal Rasheed

College of Medicine / Chemistry and Biochemistry

Baghdad University

(Chairman)

Asst. Prof. Dr. Mona Qasem Mahmood

College of Medicine/ University of Kerbala

(Member)

Lecturer. Maher Abbood Mukheef

College of Medicine/ University of Kerbala

(Member)

Asst. Prof. Dr. Shyamaa Z. Nada

Chemistry and Biochemistry
College of Medicine/ University of Kerbala
(Member and Supervisor)

Prof. Dr. Wasan Ghazi AlSafi

College of Medicine
University of Kerbala
(Member and Supervisor)

Approved for the College Council

Professor Dr. Riyadh Dhiyud Mahdi Al-Zbaidi

Dean of Medicine College/ University of Karbala

Acknowledgment

First and foremost, I thank God for his mercy and a blessing has given me during my studies and in completing this letter During every period of my life.

I owe a special appreciation to the College of Medicine, Department of Chemistry and Clinical Chemistry/ Karbala University that Give me the opportunity to go to their higher studies.

I would like to express my gratitude to my supervisor, the late Professor, Dr. Shaima Al-Zaid Nada, may God have mercy on her soul, for suggesting this topic and for her great support in completing this study.

Special thanks go to my supervisor, Professor Wasan Al-Safi for support throughout the study.

I would like to express my deepest gratitude and appreciation to all Members of the Department of Chemistry and Clinical Chemistry throughout the study period.

Special thanks go to Dr. Musa Al-Alaaq for his assistance and support in collecting samples from his center. Special thanks to the Alrsool specialist laboratory and the Alnaqaa specialist laboratory.

Special thanks are to all patients participating in this study without them, there will be no research.

Finally, I am so grateful for all the support I have received from My colleagues and friends throughout my school years

Ahmed, 2022

Dedication

THIS THESIS IS DEDICATED TO THE SPIRIT OF MY FATHER.
TO MY MOTHER, BROTHERS AND SISTERS FOR THEIR CONTINUOUS SUPPORT AND LOVE.
THIS STUDY IS DEDICATED TO MY SUPPORTING WIFE FOR MY LIFE
ENCOURAGEMENT AND LOVE.
FINALLY, I DEDICATE THIS STUDY TO MY CHILDREN SHUBER AND ALI

Ahmed, 2022

List of contents

List of Tables

List of Figures

List of abbreviations

Abbreviation	Description
ADP	Adenosine di-phosphate
ATP	Adenosine tri-phosphate
ART	Assisted Reproductive Technology
ASAs	Anti-sperm antibodies
AUC	Area Under the Curve
$HCO3^-$	Bicarbonate
BMI	Body Mass Index
Ca2+	Calcium
CRDs	Carbohydrate Recognition Domains
CAT	Catalase
cAMP	Cyclic Guanosine Monophosphate
DNA	Deoxyribonucleic acid
FSH	follicle-stimulating hormone
GAL-3BP	Galectin-3 binding protein
GnRH	Gonadotropin-Releasing Hormone
GHS	genital heat stress
GSH	Glutathione
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GPx	Glutathione Peroxidase
SBD	C-terminal substrate-binding domain
Hsp70	Heat shock protein 70
H2O2	Hydrogen Peroxide
HPT axis	hypothalamic–pituitary–testicular axis
HOO.	Hydro Peroxy Radical
IUI	Intrauterine Insemination

ICSI	Intra Cytoplasmic Sperm Injection
IVF	In Vitro Fertilization
LH	luteinizing hormone
LacNAc	Glycoconjugates Containing N-acetyllactosamine
ROO.	Lipid Peroxide Radical
LPO	Lipid peroxidation
O_2	Oxygen
OS	Oxidative stress
O_2^-	superoxide anion radical
O3	Ozone
OH^-	Hydroxyl Radical
P-value	Probability
PTK	Protein Tyrosine kinase
PUFAs	polyunsaturated fatty acids
PTPase	phosphor tyrosine phosphatase PTPase
PKA	Protein Kinase A
ROS	Reactive oxygen
ROC	Receiver Operator Characteristic
SD	Standards Deviation
SOD	Superoxide Dismutase
SDF	Sperm DNA Fragmentation
TPR	Tetra Trico Peptide-Repeat domain
Trx	Thioredoxin
TAOS	Total anti-Oxidant
WHO	World Health Organization
NADPH	Nicotinamide Adenine Dinucleotide
NAD	N-terminal Aggregating Domain
NBD	N-terminal nucleotide-binding domain
Zn	Zinc

Summary

It is clear that the integrity of sperm is critical for the correct transfer of genetic materials to future generations and the integrity of the sperm is responsible for fertilization. The reactive oxygen species are important signalling molecules in physiological processes, perhaps it also plays a role in pathological processes involving the reproductive system and reproduction if present in excessive quantities. Antioxidants are chemicals that lessen or prevent the effects of free radicals, thereby reducing their reactivity. Measurement of antioxidant indices of semen is used in the evaluation and the detection of the biochemical causes of male infertility.

The main purpose of this study was to identify Biomarkers that aid in the diagnosis and monitoring of male reproductive capacity. this study performed The antioxidant assessment in seminal plasma, heat shock protein 70 and Galectin-3 binding protein, clinical status, and the lifestyle of people with reproductive problems.

A case control study was conducted on 119 semen samples, 31men with asthenozoospermia, 28 men with oligoasthenozoospermia, while another 60 healthy controls. Semen samples were collected from Iraqi patients who attended infertility clinics for infertility diagnosis and assisted reproductive technologies in Karbala for the period between November 2021 and May 2022 .

All participants' questionnaire responses were put on a data sheet and given a serial identity number. Errors were prevented by using multiple entries. The Statistical Package for the Social Sciences, version 28.0, was used to create the data analysis for this project (IBM, SPSS, Chicago, Illinois, USA). On the participant data from each group, descriptive statistics were run. Mean SD was used to illustrate values for continuous variables. The Shapiro test was used to examine the data distribution. Odds ratios (ORs) and a 95% Confidence Interval

Range, which were computed using a no conditional logistic regression, were used to estimate the connection between the components that had been examined. Analytical statistical tests were used to confirm that there were significant variations in categorical variables between the parameters. Results of every hypothesis test with a two-sided p-value of 0.05 or lower were deemed statistically significant

The average semen total antioxidant level showed the highest level in the control group (3.53±0.64U/ml), and the lowest level was associated with oligoasthenozoospermia (1.88 ± 0.15 ng/l). The results of this study also showed that the average level of heat shock protein 70, the lowest, was observed in the case of the control group (40.17±1.94 ng/L) and the highest level was associated with a decrease in oligoasthenozoospermia. 50.02 ± 7.39 ng/L). As for the average rate of Galectin-3 binding protein level, it was not statistically significant between the control group and Asthenozoospermea and Oligoasthenozoospermia, Heat shock protein 70 was associated with the total redox state of semen significant inverse correlation

There is a correlation between heat shock protein 70 and total semen antioxidants, and this indicates the importance of measuring each of them, in cases of infertility and not being satisfied with one over the other.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1. Infertility

Infertility is a worldwide reproductive health problem, which affects approximately 15% of couples, with male factor infertility dominating nearly 50% of the affected population (1). Approximately 48.5 million couples, experience infertility worldwide. The half of cases of infertility 50% have been determined to be caused by males, who are alone to blame for 20–30% of the cases. (2).

Some factors that determine the occurrence of pregnancy are the genomic quality of the oocyte and sperm, implantation capability of the embryo and the endometrial receptivity; Most biological systems depend on preservation of equilibrium. Within the male reproductive system, oxidative harmony is crucial to fertility, reactive oxygen species (ROS) make functional contributions at appropriate concentrations, but quickly become destructive if left unchecked (3). Oxidative stress, which arises from an unbalance between reactive oxygen species and protective antioxidants, influences the entire reproductive lifespan of men and women (4). Reactive oxygen species may act as key signalling molecules in physiological processes, but in excess, uncontrolled levels may also mediate pathological processes involving the reproductive tract/reproduction (5).

Galectin-3-binding protein (GAL-3BP) (is a secretory lectin in human seminal plasma that is well known for its action on cell adhesion) (6). GAL-3BP is a lectin that is transported to the surface of sperm during post-testicular maturation by extracellular vesicles found in human seminal plasma, ejaculated spermatozoa, the testis, and the epididymis. After that, sperm-bound galectin-3 is crucial for spermatozoa—zona pellucid binding (7).

Heat shock protein 70 (HSP): (is a member of HSPs family, which is spread in all living organisms, whether eukaryotic or prokaryotic) (8). (Hsp70) plays a crucial part in boosting cells' tolerance to environmental changes and pathologic circumstances in plasma sperm, protecting cells against cellular stress including

heat stress (9). Members of the HSP70 family appear to be one of the key molecules on the sperm cell membrane surface that are connected to several sperm processes such capacitation, fertilization, and motility (10).

The aim of this study is to investigate the relationship between total antioxidants, Galectin 3 binding protein and heat shock protein 70 for male infertility, and effect of total antioxidants, Galectin 3 binding protein and heat shock protein 70 levels on male infertility

1.1. Male Infertility

1.1.1. Definition

Infertility is described as the failure to get pregnant following a year of routine, unprotected sexual activity (11). Regardless of the outcome, infertility can be primary when there has never been a pregnancy or secondary when the male was previously fertile, but is now unable to conceive. Primary and secondary infertility affect 67%-71% and 29%-33%, respectively, of infertile people (12). Male factor infertility contributes to about half of all cases of infertility and affects around one in 20 men in the reproductive age group (13). Male infertility is primarily caused by abnormalities in the semen and/or sperm parameters. These abnormalities include reduced spermatozoa concentration (oligozoospermia), decreased sperm motility (asthenozoospermia), decreased sperm vitality (necrozoospermia), abnormal sperm morphology (teratozoospermia), complete lack of semen with ejaculation (aspermia), and including the lack of spermatozoa (azoospermia) (14).

1.1.2. The Causes of Male Infertility:

1.1.2-A- Pre-testicular factors

Pre-testicular factors include those that prevent the testicles from receiving appropriate support, such as hypogonadism caused by a variety of conditions, such as kallman syndrome, and include these conditions. Undiagnosed and untreated celiac disease can also decrease semen quality and result in immature secondary sex characteristics, hypogonadism, and hyperprolactinemia, which may cause impotence and a decrease in libido. Obesity decreases Kiss1 expression, which changes the release of gonadotropin-releasing hormone (GnRH) (15). It is advised that both males and females should have their underlying celiac disease evaluated as part of an effective screening for infertility. A number of medications, including anabolic steroids, chemotherapy, cimetidine, phenytoin, spironolactone, and sulfasalazine, may have an impact on the spermatogenesis process, lower follicle stimulating hormone (FSH) levels, or significantly lower sperm motility, which can have an impact on both the testicles and the pre-testicles (16).

1.1.2-B- Testicular factors

Infections of the testes, such as acute and chronic epididymo-orchitis that are both specific and non-specific, cancers of the testis, surgical procedures on the testes, congenital testicular issues, undescended testes/cryptorchidism, and testicular injury from direct or indirect trauma or torsion are a few of the testicular factors. Other factors include age, Y chromosome genetic abnormalities, Klinefelter syndrome, measles, hydrocele, infection, and testicular exposure to radiation therapy, chemotherapy, and other gonadotoxic medications and chemicals (16); (17).

1.1.2-C- Post-testicular factors

The post-testicular causes of infertility include obstruction or stricture of the ductal system of the male reproductive tract (figure 1.1). In contrast with testicular disorders, post-testicular abnormalities are commonly treatable. Thus, restoration of fertility potential may be possible (18).

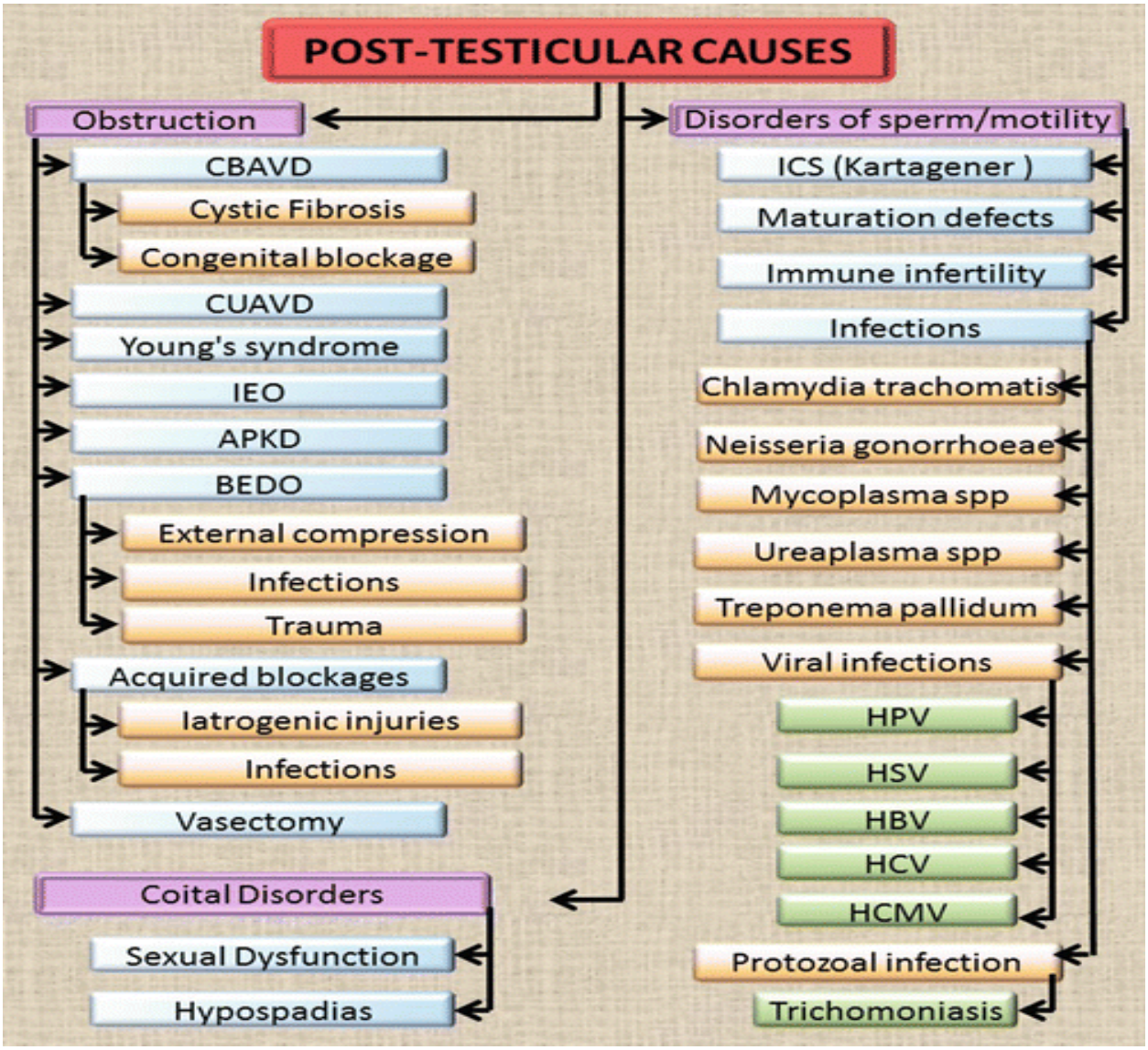


Figure (1.1) Post-testicular causes of male infertility (18).

APKD adult polycystic kidney disease, BEDO bilateral ejaculatory duct obstruction, CBAVD congenital bilateral absence of the vas deferens, CUAVD congenital unilateral absence of vas deferens, IEO idiopathic epididymis obstruction, ICS immotile cilia syndrome, HPV human papillomavirus, HSV herpes simplex virus, HBV hepatitis B virus, HCV hepatitis C virus, HCMV human cytomegalovirus

1.1.2. D- Immunological causes

The immune system plays an important role in infertility. Immunological causes are responsible for more than 10% (4% to 15%) of infertility cases. Anti-sperm antibodies (ASAs) are present in less than 2% of fertile and 10% of infertile men (19). Anti-sperm antibodies which are immunoglobulins directed against antigens of the sperm surface, Anti-sperm antibodies affect sperm function impairment of sperm penetration into cervical mucus, inhibition of sperm capacitation, incomplete acrosomal reaction, disruption of sperm-egg binding, and disorder in egg fertilization (20). There are cases of anti-sperm antibody-associated infertility, such as some varicocele cases, testicular torsion and inflammation (21).

1.2. Life Style Factors of Male Infertility

Alongside an increasing prevalence of couple and male infertility, evidence suggests that there is a global declining trend in male fertility parameters over the past few decades. This may, at least in part, be explained through detrimental lifestyle practices and exposures. These include alcohol and tobacco consumption, use of recreational drugs (e.g., cannabis, opioids and anabolic steroids), poor nutritional habits, obesity and metabolic syndrome, genital heat stress (e.g., radiation exposure through cell phones and laptops, prolonged periods of sitting, tight-fitting underwear and recurrent hot baths or saunas) (figur1.2), exposure to endocrine-disrupting chemicals (e.g., pesticide residue) and psychological stress (22).

Common mechanisms associated with a reduction in spermatogenesis and/ or steroidogenesis due to unfavourable lifestyle practices include inflammation and oxidative stress locally or systemically. It is recommended that relevant lifestyle practices are investigated in clinical history of male infertility cases, particularly in

unexplained or idiopathic male infertility. Appropriate modification of detrimental lifestyle practices is further suggested and recommended in the management of

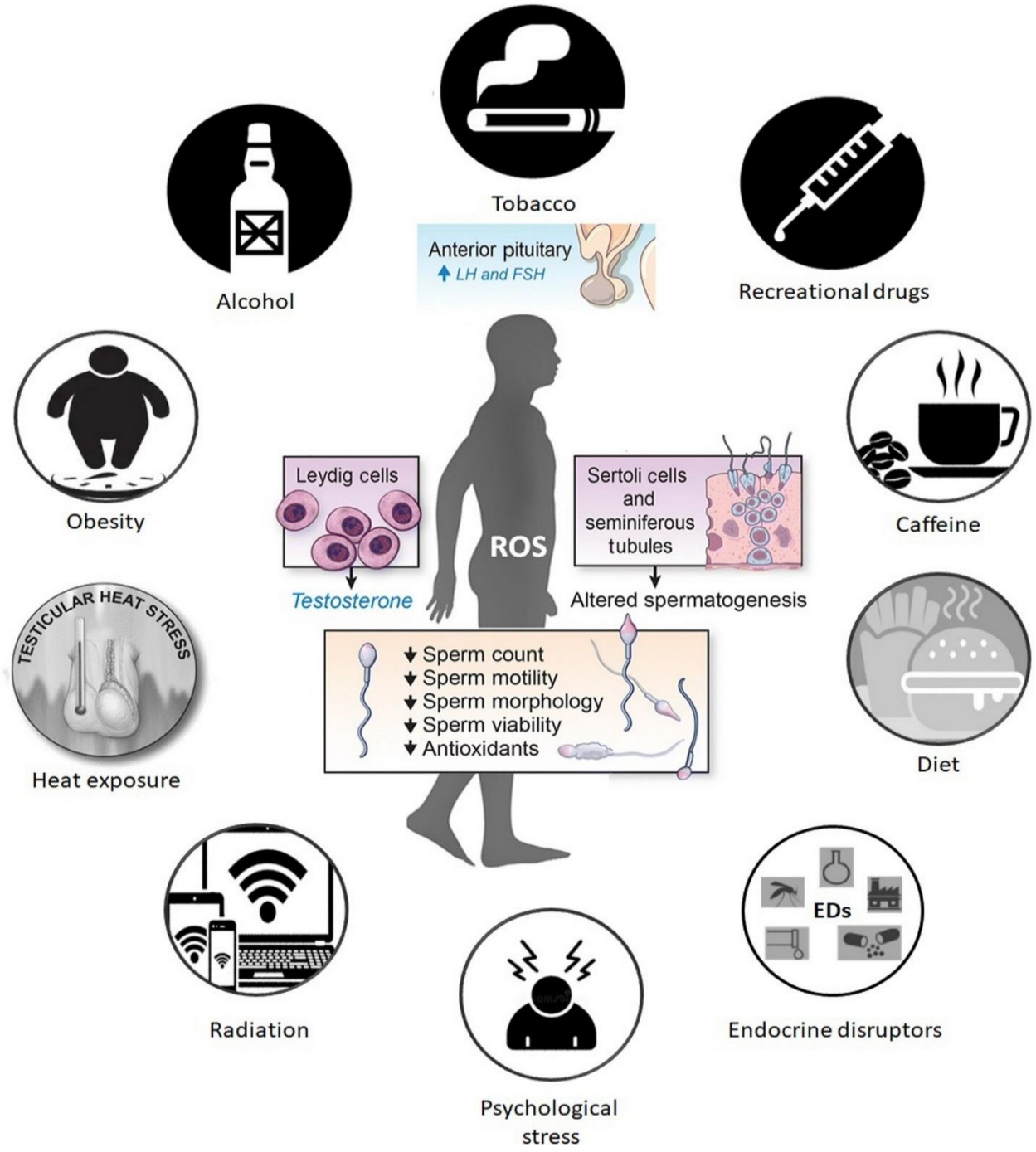


Figure (1.2) Lifestyle factors and their effects on male fertility (22).

FSH, follicle-stimulating hormone; LH, luteinizing hormone; ROS, reactive oxygen species

A- Smoking

In the US, roughly 21% of men of reproductive age consume tobacco frequently, Smoking exposes guys to around 4,700 potentially mutagenic substances, mainly polycyclic aromatic hydrocarbons among other hazardous chemicals, which have a negative impact on fertility and fecundity. However, the effect on male reproduction is typically overlooked (23). In moderate and heavy smokers, oligozoospermia, asthenozoospermia, and teratozoospermia are frequently observed (24).

Negative effects of smoking on sperm parameters that include reduced sperm concentration, motility and vitality, increased abnormal morphology, seminal leucocyte concentration and DNA fragmentation, alongside reduced capacitation and acrosome reactions (25). Smoking induces oxidative stress in the testes, affecting spermatogenesis and steroidogenesis, this leads to impaired chromatin DNA integrity, oocyte binding, as well as epigenetic modifications transmitted to the next generation (25). By altering the physiological epigenetic regulation, paternal smoking increases the risk of genetic disease, malformation and cancer in the progeny (27).

B- Alcohol

The parameters of spermatogenesis and semen, such as sperm concentration, motility, and morphology, are adversely affected by acute or moderate (20–25 units per week) alcohol intake (26)(table 1.1). Only 12% of chronic alcoholic males are found to have normozoospermia, compared to 37% of non-alcoholic men, which is related with oligozoospermia, asthenozoospermia, and teratozoospermia (27). Understanding of the effects of ethanol on the reproductive system has been enhanced by studies in mice and rats. A long-term imbalance in the oxidant/antioxidant ratio is caused by chronic exposure to ethanol, which

causes oxidative stress and a decrease in enzymatic antioxidants. Apoptosis, DNA fragmentation, and chromatin dissociation are associated with this (28). Chronic alcohol intake is further correlated with epigenetic modulations and the transfer of these modifications to the next generation, which is emerging as an important contributor to alcohol-related fetal growth defects via modified paternal DNA methylation (29).

C- Advanced Paternal Age (APA)

Male fertility generally starts to reduce around age 40 to 45 years when sperm quality decreases. Age-related morphological changes in the testis, such as a reduction in the number of germ cells, Leydig cells, and Sertoli cells, as well as structural changes, such as the constriction of the seminiferous tubules, cause men's testicular function and metabolism to decline. With advancing male age, free and total testosterone concentrations slowly decline, resulting in primary hypogonadism. As men age, ROS build up in their sex cells, causing oxidative stress and sperm DNA damage. Additionally, it accelerated apoptosis in aged testes (30).

D- Diet

Male fertility may be impacted by specific dietary factors, and there is growing evidence linking poor semen quality to dietary elevation. High-fat diets prevent conception by altering the sperm cells' physical and molecular makeup as well as that of the developing fetus and offspring (31). Studies have suggested that reproductive health can be enhanced by modification of dietary intakes such as fruits and vegetables, legumes, and fish That associated with buffer sperm quality (32); (33).

E- Environmental Factors:

Temperature affects spermatogenesis, with the ideal temperature being roughly 2 degrees Celsius below body temperature. Genital heat stress (GHS), which is exacerbated by elevated scrotal temperature, reduces spermatogenesis (34). Workers may be at risk for heat stress and GHS due to occupational hazards like high workplace temperatures (such as those found in the glass, foundry, and steel industries, as well as in kitchens, bakeries, and mines) (35). GHS negatively correlates with semen quality and is considered a major contributor in male infertility (34); (36).

The mechanism of GHS-induced spermatogenic impairments includes induction of oxidative stress with high reactive oxygen species (ROS) concentration and reduced antioxidants in the genital tract and ejaculate, sperm chromatin disintegration, DNA fragmentation, sperm mitochondrial dysfunction and increased rate of germ cell apoptosis (34).

Spermatozoa may be most susceptible to damage in the pre-meiotic stage at which chromatin remains mostly unstable due to the ongoing processes of histone modifications and hyper acetylation. This unstable chromatin may get easily affected by increased genital heat and hinder sperm chromatin condensation (37).

Table 1.1: Potential negative impact of various lifestyle factors on female and male endocrine parameters of reproductive system (38).

Lifestyle Factor	Semen Parameters	Endocrine Parameters	Proposed Mechanisms
Alcohol Consumption	↑ Seminal leukocytes SDF ↓ Concentration Motility Viability Morphology	↑ LH FSH Prolactin Estrogen ↓ Testosterone Progesterone	• Impaired spermatogenesis and steroidogenesis • Spermatogenic arrest • Impaired Leydig cell • Apoptosis • Testicular atrophy and OS
Tobacco Consumption	↑ Seminal leukocytes SDF ↓ Concentration Motility Viability Morphology	↓ Testosterone	• Impaired spermatogenesis and steroidogenesis • Testicular OS • Hypoxia
Cannabis, Opioids and Anabolic Steroids	↓ Concentration Motility Sperm functions	↓ LH Testosterone	• Impaired HPT axis and spermatogenesis
Caffeine	• No significant impact confirmed • May increase sperm motility • May increase SDF	• May increase testosterone • May decrease LH and FSH	Not determined

FSH, follicle-stimulating hormone; HPT axis, hypothalamic–pituitary–testicular axis; LH, luteinizing hormone; OS, oxidative stress; SDF, sperm DNA fragmentation

1.3 Seminal Fluid Analysis

The simplest method of evaluating male infertility is semen analysis, or SA. At the moment, the standards outlined in the World Health Organization's(WHO) laboratory manual for the examination and processing of human semen are used to evaluate semen parameters (WHO 2010) (39); (40).

To improve the effectiveness and calibre of laboratory work, semen analysis evaluates the descriptive characteristics of ejaculates primarily obtained by masturbation in a suitable chronological order (41). The basic information on which physicians base their initial diagnosis come from a male infertile patient's sperm test (42). Even though, standard sperm concentration, motility, and morphology measurements cannot cover the broad range of biological properties that the spermatozoon exhibits as a highly specialized cell, they do reveal subtle defects (43). However, there remain several limitations associated with the conventional semen analysis in the assessment of male infertility (44). There have been significant advances with the incorporation of recent developments in semen examination techniques, methods of sperm preparation and cryopreservation, and new technologies to improve quality control and assurance (45); (46). Recent scientific advances in the understanding of sperm DNA fragmentation (SDF), seminal oxidative stress (OS), and reactive oxygen species (ROS) testing have shed additional light on the prognosis of reproductive outcomes in terms of natural conception and assisted reproductive technology (ART) (47); (48).

1.4 Oxidative stress

Oxidative stress(OS) is defined as a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defences, Sperm cells are vulnerable to ROS because of the abundance of polyunsaturated fatty acids in their plasma membrane and cytoplasm (49). Intracellular ROS

concentrations are determined by the balance between the rates of ROS production and their rates of clearance by various antioxidant defines mechanisms. At normal physiological levels, ROS regulate intracellular signalling cascades, thus mediating essential physiological mechanisms such as sperm maturation, hyper activation, capacitation, acrosome reaction, as well as fertilization (50).

Widely appearing literature data combining the topic of oxidative stress (OS) with the problem of infertility constitute an evidence of a never-ending interest in the subject (51). Increased levels of reactive oxygen species (ROS) harm nuclear and mitochondrial DNA, raise the risk of male infertility, impair sperm motility, and may even be the root cause of birth abnormalities in offspring (52). OS is characterized by an imbalance between increased sperm injury caused by reactive oxygen species (ROS) and the inability of anti- oxidative agents to remove these (53).

World Health Organization recommends a seminogram in male fertility evaluation, which is a general examination of semen; however, this does not always enable the determination of the cause of infertility and spontaneous abortions and pregnancy losses after in vitro procedures, after intracytoplasmic sperm injection (ICSI). The quality of sperm and oocytes plays a decisive role in the ICSI fertilization, in which OS may participate. It has been demonstrated that a 25% increase in ROS production in seminal fluid activates cellular response, OS induction, and apoptosis in the p53-dependent pathway associated with inhibition of natural antioxidants, such as GSH, superoxide dismutase (SOD), catalase (CAT), or glutathione peroxidase (GPx). Increasing OS, co-accompanying other overlapping factors, reduces the ability of sperm to reproduce (54).

1.4.1. Reactive Oxygen Species (ROS)

In mammalian cells, both enzymatic and non-enzymatic processes can produce reactive oxygen species (ROS) (55). The word reactive oxygen species (ROS) involves the reduced form of oxygen and its reaction products with other molecules. Reactive oxygen species (ROS) are highly reactive molecules that are formed by the metabolism of oxygen. These may be free radicals or non-radicals. Free radicals are molecules that contain at least one unpaired valence electron at their outer shell, making them highly reactive and short lived (56). The most important ROS radicals are: superoxide anion radical (O_2^-), hydroxyl radical (OH^-), alkyl radical ($RO^{\cdot}$), lipid peroxide radical ($ROO^{\cdot}$), and hydro peroxy radical ($HOO^{\cdot}$). The non-radicals ROS are: hydrogen peroxide (H_2O_2), singlet oxygen (O_2), ozone (O_3), organic peroxide (ROOH), and hypo-chlorous acid (HOCl) (57). Both reactive oxygen species and reactive nitrogen species, when present in physiological amount, have important roles in normal cellular functions such as fighting against infection, regulating different intercellular signalling pathways, facilitating normal maturation and fertilization in reproductive systems (58). However when ROS are present at a high concentration, overwhelming antioxidant defines system, oxidative stress (OS) results that lead to cellular dysfunction by lipid peroxidation, protein and DNA damages (59). Owing to these damaging effects on the cells, oxidative stress is associated with many pathological conditions including infertility (60).

Reactive oxygen species found in seminal plasma originates from various endogenous and exogenous sources, The two most important sources of ROS production in males are Leukocytes and immature spermatozoa (61). Leukocytes particularly neutrophils and macrophages are strongly associated with excessive

ROS production leading to sperm dysfunction (62). It is not known at which point the per oxidative damage to spermatozoa occurs, whether within semen (during the time required for liquefaction), in the epididymis (where spermatozoa are stored before ejaculation), or in the testis. By altering membrane integrity, ROS may impair sperm motility and morphology and can lead to sperm cell death (63)figure (1.4).

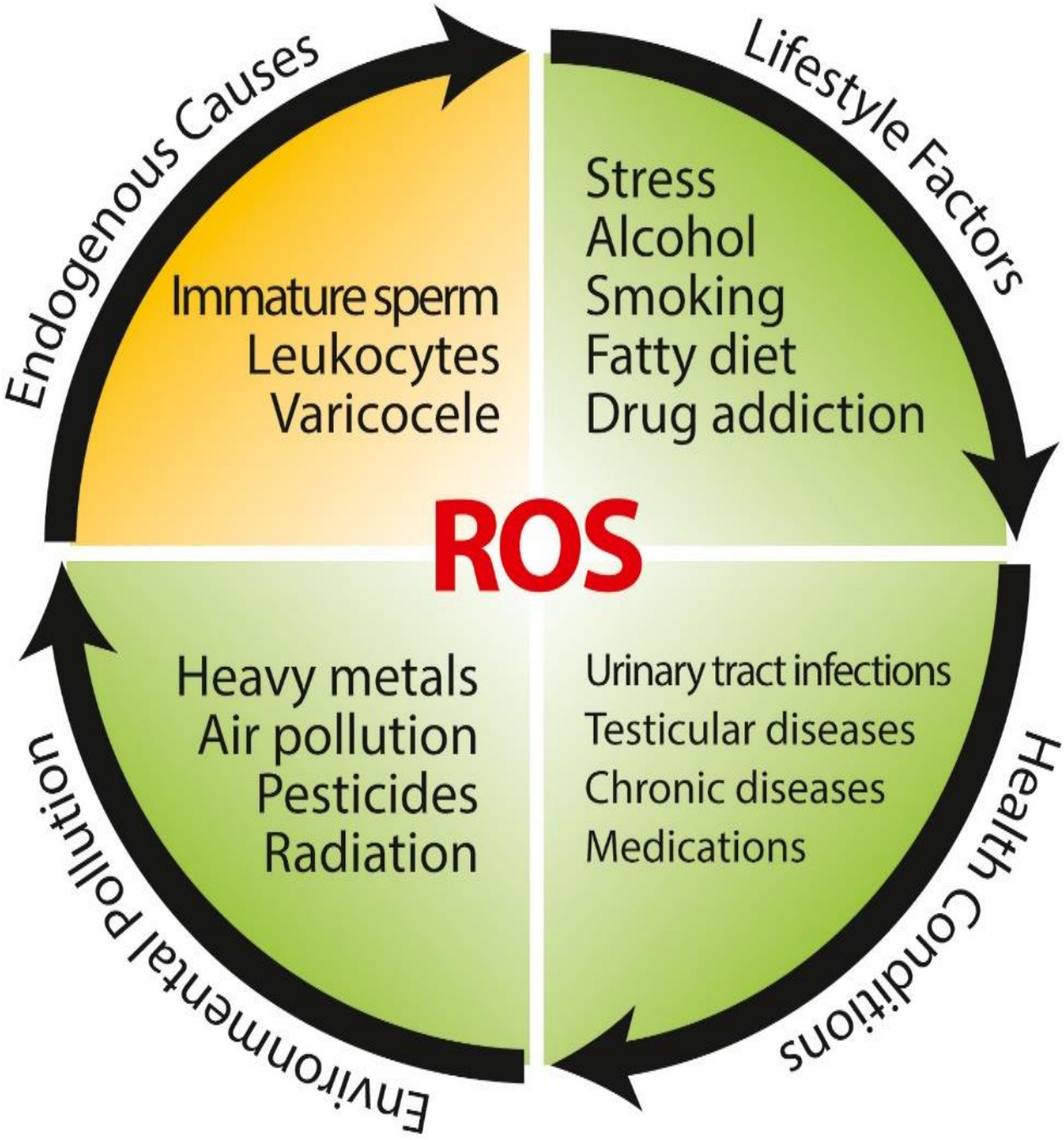


Figure (1.3) Endogenous and exogenous sources of ROS (64).

Antioxidants are chemicals that lessen or prevent the effects of free radicals they donate an electron to free radicals, thereby reducing their reactivity. Antioxidants are highly concentrated in seminal plasma, protecting gametes from ROS. The potential anti-ROS enzymes superoxide dismutase (SOD), catalase,

glutathione peroxidase (GSH-Px), low molecular weight compounds (tocopherol, -carotene, ascorbate, and urate), and transition metal chelators make up the total antioxidant capacity of seminal plasma (transferrin, lactoferrin, ceruloplasmin) (65). Antioxidant defines mechanisms are built into cells, controlling pro-oxidant levels and shielding them from the harmful effects of free radicals. These defences are categorized as follows:

- Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). SOD neutralizes O_2^-, converting it to H_2O_2, which is then decomposed to O_2 or alcohols by CAT and GPx, respectively, with the synthesis of water (66).
- Non-enzymatic antioxidants include vitamins (A, C and E), coenzymes Q10, glutathione, β-carotene, selenium and zinc which act as cofactors for many antioxidant enzymes (67). Redox proteins, on the other hand, are structurally characterized by the presence of catalytic sites which can accept or donate electrons (68).The ability to mediate the electron transfer makes them key proteins in redox reactions occurring in several biological processes. A classic example of a redox protein is thioredoxin (Trx) (69). Zinc (Zn) is necessary for the normal function of the male reproductive system and spermatozoa , Oxidative stress (OS) induced by reactive oxygen species is likely as the main mechanism of zinc deficiency which is associated with sperm DNA fragmentation, decrease in sperm membrane integrity, apoptosis, depletion of antioxidants, and consequently poor sperm quality and male infertility (70).

1.4.2 Effects of ROS on Different Sperm Functions

The effects of the ROS on the Sperm state are Functions and are divided into;

1.4.3.1. Physiological Effect

Spermatozoa mature in the epididymis, where changes to the cell membrane, protein rearrangements on the cell surface, and enzymatic and nuclear remodeling all occur (71). Cellular signal transduction mechanisms that control this crucial stage of sperm development are influenced by ROS concentrations (72). The mammalian spermatozoon's chromosomal DNA is tightly packed because its histones have been replaced by smaller-sized protamines. To ensure chromatin stability, protamine cysteine residues form intra- and intermolecular disulfide linkages. ROS may aid in the creation of disulfide bonds, ensuring chromatin integrity and shielding DNA from harm. In order to protect mitochondria from proteolytic degradation, peroxides may also help the proper formation of the mitochondrial capsule, which is made up of protein network rich in disulfide bonds (73); (74).

Hyper activation is a particular state of sperm motility characterized by high amplitude, increased and asymmetric flagellar movement, elevated side-to-side, sperm head displacement, along with non-linear motility (75) . It is considered to be a part of capacitation and is required for successful sperm penetration of the zonapellucida and fertilization. Reactive oxygen species has positive impacts on the hyper activation processes in spermatozoa (76). The initiation process of capacitation and hyper activation is induced by the influx of calcium (Ca^{2+}) and bicarbonate (HCO_3^-), probably by the inactivation of an ATP-dependent Ca^{2+} - regulatory channel (plasma membrane Ca_2+ - ATPase, PMCa) and alkalization of the cytosol. Calcium ions and ROS, specifically O^{2-}, lead to the activation of adenylate cyclase, generating Cyclic Adenosine Monophosphate (cAMP) Figure (1.4).

cAMP via Protein Kinase A (PKA) activation triggers NADPH oxidase and thereby stimulates greater ROS generation. Protein tyrosine kinase (PTK) also phosphorylates serine and tyrosine residues, which can also activate protein

tyrosine kinase (PTK). Consequently, PTK triggers phosphorylation of tyrosine residues in the fibrous sheath around the axoneme and the cytoskeleton of the sperm flagellum. ROS, especially H_2O_2, elevate tyrosine phosphorylation by inducing PTK and inhibiting phosphor tyrosine phosphatase (PTPase), which leads to de-phosphorylation of tyrosine residues. The final step in the process of hyper activation is presumably increased tyrosine phosphorylation (71). O^{2-} has been observed to be the major ROS contributor to this ameliorating effect (77), (78).

The ultimate functional process in spermatozoal maturation needed for making the sperm competent to fertilize an ovum is capacitation. The established molecular pathway by which ROS facilitates capacitation is by triggering intracellular cAMP levels inducing downstream PKA, which in turn phosphorylates Mitogen-activated protein/extracellular signal-regulated kinase kinase MEK (extracellular signal regulated kinase)-like proteins, threonine-glutamate-tyrosine, and fibrous sheath proteins (79); (80). These signalling cascades bring about final capacitation of the sperm rendering it totally prepared for the acrosome reaction (77); (81).

Reaction to ensure fertilization, the hyper activated spermatozoon must pass across the cumulus oophorous, bind to the zonapellucida of the oocyte and create a pore in its extracellular matrix via exocytotic release of proteolytic enzymes (73) . These acrosome reactions are mediated through phosphorylation of tyrosine proteins, Ca^{+2} influx resulting in an intracellular rise in cAMP and PKA, thereby enabling the spermatozoon to penetrate and fuse with oocyte. Reactive oxygen species has been observed to facilitate actions on the zonapellucida of the spermatozoon by various means including phosphorylation of three relevant plasma membrane proteins (71); (72).

After helping the metabolic cascades of spermatozoa capacitation and acrosome reactions, reactive oxygen species seem to improve membrane fluidity necessary

for effective sperm-oocyte union. By preventing protein tyrosine phosphatase activity throughout capacitation, ROS keeps phospholipase A2 from losing its active state. This allows phospholipase A2 to cleave the secondary fatty acid from the membrane phospholipid tri-glycerol and improve membrane fluidity (82).

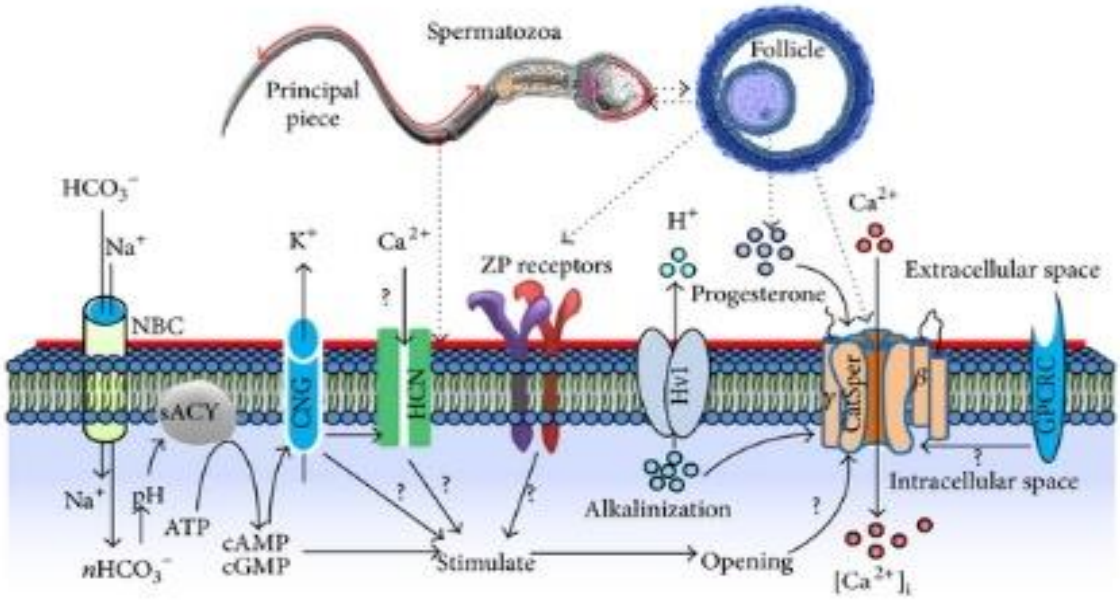


Figure (1.4) Calcium Influx and Male Fertility in the Context of the Sperm Proteome

1.4.3.2 Pathological Effect

The plasma membrane of the sperm cell contains significant quantities of lipids, primarily in the form of polyunsaturated fatty acids (PUFAs) with unconjugated double bonds between their methylene groups. The hydrogen atom is extremely vulnerable to oxidative damage because the double bond close to the methylene group weakens the connection between the methyl carbon and hydrogen. Lipid peroxidation (LPO) is the end outcome of a series of processes that are triggered as the intracellular ROS levels rise uncontrollably (LPO) (83). In which almost 60% of the membrane fatty acids are lost, diminishing its fluidity, enhancing non-specific permeability to ions, and also inhibiting the actions of

membrane receptors and enzymes. Reactive oxygen species are also responsible for reduced sperm motility by inhibiting energy generation, via LPO and importantly mitochondrial DNA (mtDNA) mutations damage to at least one of the 13 genes coding for the electron transport chain transporter system in the mitochondria will reduce ATP production and induce intracellular ROS production. Reactive oxygen species may reduce sperm motility also by oxidation of a thiol group in glyceraldehyde-3-phosphate dehydrogenase (GAPDH) Figure (1.5) which is a glycolytic enzyme, or deletion of adenine and pyridine nucleotides by LPO (77). Reactive oxygen species are capable of disrupting the inner and outer mitochondrial membranes releasing cytochrome C. This cytochrome C in turn activates the apoptotic caspases (77) (79). This mechanism of induction of apoptosis in the spermatozoa by ROS is evident in infertile men, as high levels of cytochrome C have been found in the seminal plasma of infertile men, which is an indicator of severe mitochondrial damage (77) (84).

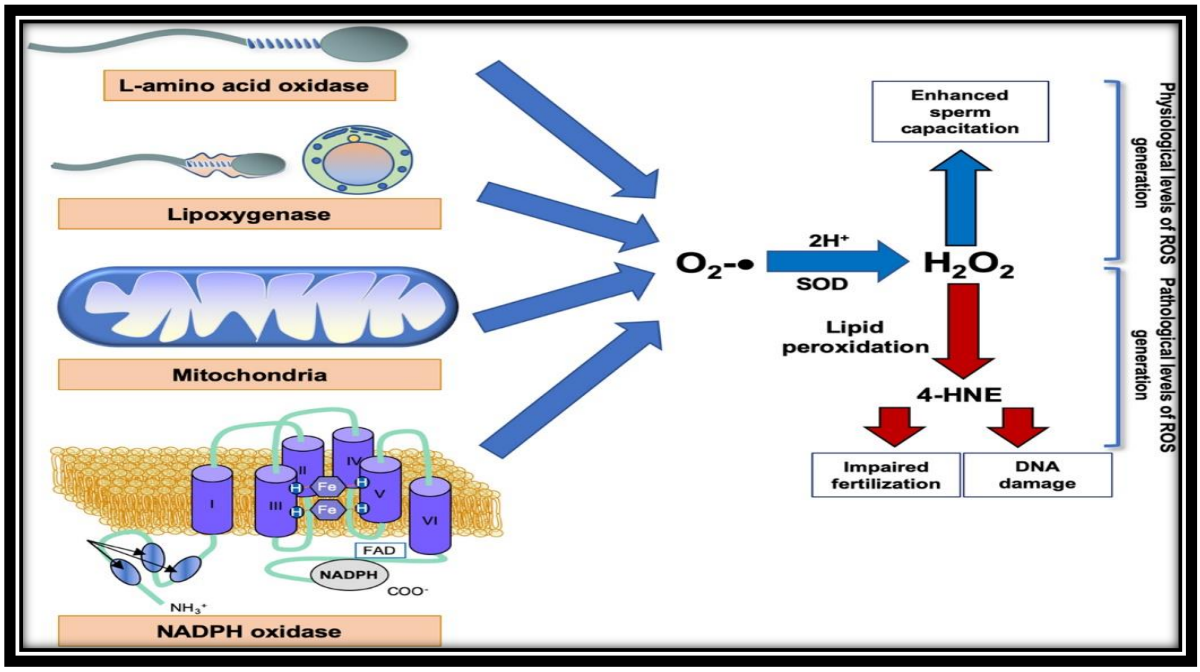


Figure (1.5) Contradictory role of ROS in spermatogenesis (85)

1.4.4. ROS and Male Infertility

Male oxidative stress infertility, or MOSI (Male Oxidative Stress Infertility), is a term that has recently been proposed to describe infertile patients with aberrant semen characteristics and oxidative stress (86), (87). Polyunsaturated fatty acids (PUFAs), which are abundant in the sperm membrane, help to maintain the fluidity of the membrane, which is necessary for sperm-oocyte union. However, because they contain carbon-carbon double bonds, these lipids are extremely vulnerable to oxidative damage. Actually, ROS stimulates the production of lipid peroxide radicals, which then interact with other molecules to cause a self-replicating chain reaction that amplifies lipid peroxidation (88). Since the mitochondrial membrane is also damaged by ROS, motility is the first to be affected by lipid peroxidation, causing a reduction in mitochondrial membrane potential and defects in the sperm mid-piece and axonal region (89). Protein oxidation of the α-central carbon generates radical amino acids and induces the cleavage of peptide skeletons. Furthermore, the SH-rich side chains of cysteine and methionine are prone to be oxidized with generation of disulphides and methionine sulphoxide, respectively. In the same way, proline, arginine, lysine and threonine are oxidized, resulting in the formation of aldehydes and ketones, which indicate the protein oxidation status (90).

These modifications alter the protein structure and their function, with repercussion on spermatogenesis and fertility. Oxidative stress can have deleterious effects on DNA integrity and an increased rate of sperm DNA fragmentation has been reported in infertile patients having high levels of ROS (91). During spermatogenesis, genomic DNA is folded around protamine, which enforce a compact state through the formation of disulphide bonds. Therefore, protamine oxidation leads to a lesser degree of compaction, making sperm DNA more susceptible to ROS-mediated oxidation. In addition, reaction between

deoxyribose sugars and ROS disrupts the DNA strands, while oxidation of purine and pyrimidine bases alters the normal DNA reading, leading to a higher mutation rate (92). Since spermatozoa lack base repair mechanisms, activation of the apoptotic cascade occurs in case of excessive DNA damage, leading to reduced sperm concentration and male infertility (88).

1.5 Heat shock protein (HSPs)

1.5.1 definition

Heat shock protein (HSPs): are highly maintained polypeptides that can live in nearly any cell. Their molecular weights were used to categorize them, and one of their most crucial functions is that of molecular chaperones, which actively contribute to maintaining protein homeostasis and cell viability (93); (94). Heat shock proteins defind the body under various environmental stress conditions and are rapidly synthesized in response to stress (95). HSPs function as molecular chaperones by combining denatured proteins, preventing misfolding or aggregation, and assisting in refolding following resumption of the normal status under stress conditions; they are associated with protein folding (96). HSP70 plays an essential role in normal cell function and is one of the most sensitive proteins, which is synthesized under stress conditions (95). Heat shock protein 70 is a member of HSPs family, which are spread in all living organisms, whether eukaryotic or prokaryotic (97); (98). Which in addition to working as molecular chaperones, have immune functions (99). But its main role is folding the proteins and prevent the improper assembly of proteins (100). Hsp70 proteins can act to protect cells from thermal or oxidative stress. These stresses normally act to damage proteins, causing partial unfolding and possible aggregation. By temporarily binding to hydrophobic residues exposed by stress, Hsp70 prevents these partially denatured proteins from aggregating, and inhibits them from

refolding; Hsp70 seems to be able to participate in disposal of damaged or defective proteins (101).

1.5.2 Discovery of heat shock protein 70

In the 1960s, Ferruccio Ritossa made the discovery of heat shock after a lab technician unintentionally increased the temperature at which Drosophila were incubating (fruit flies). Ritossa discovered a "puffing pattern" on the chromosomes when he looked for enhanced gene transcription of an unidentified protein (102) .

1.5.3 Structure of heat shock protein 70

Figure (1.6) shaow the Structure of heat shock protein 70

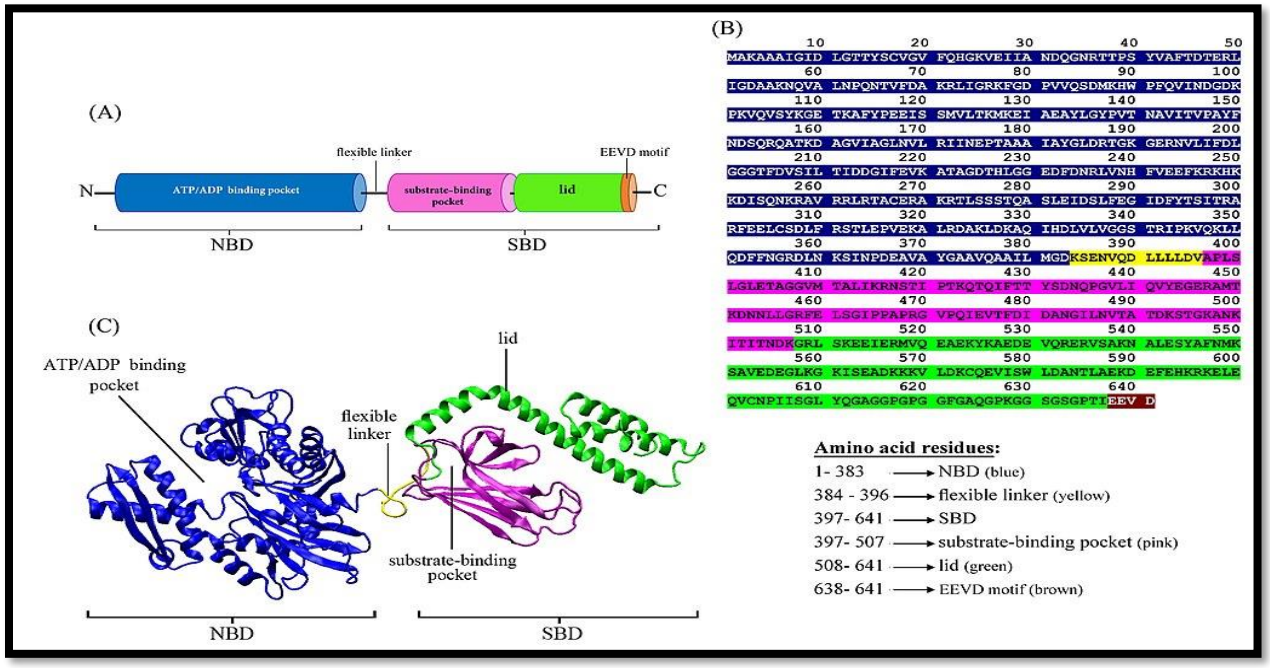


Figure (1.5) the Structure of Heat shock protein HSP70 (105)

A- The Hsp70s schematic domains. The Hsp70s consist of two high conserved functional domains including an NBD (N-terminal nucleotide-binding domain) and a C-terminal substrate-binding domain (SBD), also an EEVD motif at C-terminal. The NBD contains the ATP/ADP pocket that binds and The SBD contains a substrate binding pocket that interacts with extended polypeptides as substrate, a α helical subdomain from the C-terminal side of SBD forms a flexible lid. EEVD motif participates in binding to co-chaperones and other HSPs. (figure 1.5)
B- The complete amino acid sequence of human Hsp70 (UniProtKB identifier: P0DMV8) is a major stress-inducible member of the Hsp70 family.
C- Secondary structures of Hsp70 virtualized using VMD 1.9.1 software. Hsp70, heat shock protein 70 kDa; NBD, N-terminal nucleotide-binding domain (103).

1.5.4 The major functional domains of heat shock protein 70

- N-terminal ATPase domain – binds ATP (Adenosine triphosphate) and hydrolyzes it to ADP (Adenosine diphosphate). The NBD (nucleotide binding domain) consists of two lobes with a deep cleft between them, at the bottom of which nucleotide (ATP and ADP) binds. The exchange of ATP and ADP leads to conformational changes in the other two domains.

- Substrate binding domain – is composed of a 15 kDa β sheet subdomain and a 10 kDa helical subdomain. The β sheet subdomain consists of stranded β sheets with upward protruding loops, as a typical β barrel, which enclose the peptide backbone of the substrate. SBD contains a groove with an affinity for neutral, hydrophobic amino acid residues. The groove is long enough to interact with peptides up to seven residues in length.

- C- Terminal domain – rich in alpha helical structure acts as a 'lid' for the substrate-binding domain. The helical subdomain consists of five helices, with two helices packed against two sides of the β sheet subdomain, stabilizing the inner structure. In addition, one of the helix forms a salt bridge and several hydrogen bonds to the outer Loops, thereby closing the substrate-binding pocket like a lid. Three helices in this domain form another hydrophobic core, which may be stabilization of the "lid". When an Hsp70 protein is ATP bound, the lid is open and peptides bind and release relatively rapidly. When Hsp70 proteins are ADP bound, the lid is closed, and peptides are tightly bound to the substrate-binding domain (104).

1.5.5 Function and Regulation of heat shock protein 70

The function of Hsp70 in both (re) folding and degradation of misfolded client protein Figure (1.6)

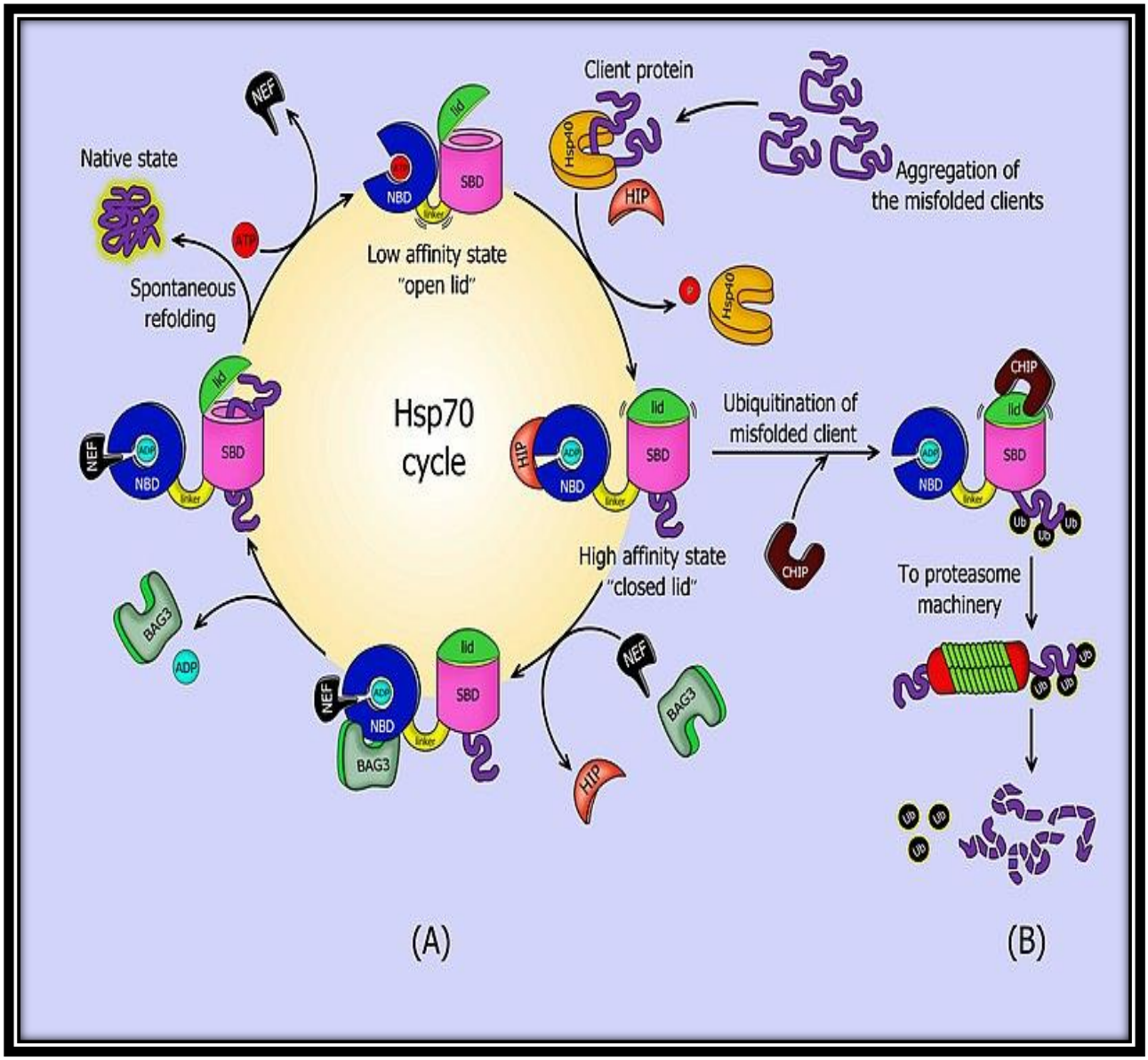


Figure 1.6: Function and Regulation of HSP70 (103)

(a) Schematic of the Hsp70 ATP–ADP cycle for (re) folding of client protein causes a conformational change of the chaperone, ATP hydrolysis, and exchange.
(b) Hsp70–CHIP complex promotes client protein ubiquitination and proteasomal degradation. CHIP interacts with the TPR domain of Hsp70 and acts as a ubiquitin ligase for clients. CHIP, chromatin immunoprecipitation; Hsp70, heat shock protein 70 kDa; TPR, tetratricopeptide-repeat domain (103).

The heat shock protein 70 system interacts with extended peptide segments of proteins as well as partially folded proteins to cause aggregation of proteins in key pathways to downregulate activity (103).

Without interacting with a substrate peptide, Hsp70 is usually in an ATP bound state. Hsp70 by itself is characterized by a very weak ATPase activity, such that spontaneous hydrolysis will not occur for many minutes. As newly synthesized proteins emerge from the ribosomes, the substrate-binding domain of Hsp70 recognizes sequences of hydrophobic amino acid residues, and interacts with them. This spontaneous interaction is reversible, and in the ATP bound state Hsp70 may relatively freely bind and release peptides. However, the presence of a peptide in the binding domain stimulates the ATPase activity of Hsp70, increasing its normally slow rate of ATP hydrolysis. When ATP is hydrolyzed to ADP the binding pocket of Hsp70 closes, tightly binding the now-trapped peptide chain. Further speeding ATP hydrolysis are the so-called J-domain cochaperones: primarily Hsp40 in eukaryotes, and DnaJ in prokaryotes. These cochaperones dramatically increase the ATPase activity of Hsp70 in the presence of interacting peptides.

By binding tightly to partially synthesize peptide sequences (incomplete proteins), Hsp70 prevents them from aggregating and being rendered nonfunctional. Once the entire protein is synthesized, a nucleotide exchange factor (prokaryotic GrpE, eukaryotic BAG1 and HspBP1 are among those which have been identified) stimulates the release of ADP and binding of fresh ATP, opening the binding pocket. The protein is then free to fold on its own, or to be transferred to other chaperones for further processing (105).

1.6. Galectin-3 binding protein (GAL-3BP)

1.6.1 Definition

A family of proteins known as galectins binds carbohydrates. They have a distinctively conserved peptide sequence in their carbohydrate recognition domains (CRDs), which have a strong affinity for glycoconjugates containing N-acetyllactosamine (LacNAc; Gal1,4GlcNAc) (106).One of the members is galectin-3 with a size of about 31 kDa. Its C-terminal is unique to other family members in linking to an atypical N-terminal aggregating domain (NAD). The domain causes galectin-3 pentamerization enabling multivalent binding or lattice formation of glycoprotein ligands (107). Galectin-3 can be found on cell surface or in vesicles in extracellular space (108).

1.6.2 Discovery of Galectin-3 binding protein

Galectin-3 binding protein has been detected in the cytoplasm, nucleus as well as in the extracellular space (109). In 1994, galectins were defined as a family of proteins sharing conserved motifs for such core sequences, as well as the ability to specifically bind galactosidase sugars, in vertebrate's galectins were found in a variety of tissues and cells such as skin, muscle, brain, intestine, liver, kidney, placenta, cultured fibroblast and many tumour cells. A number of observations indicated their possible involvement in a variety of important phenomena occurring in multicellular animals (i.e., development, differentiation, morphogenesis, immunity, apoptosis, etc.) (110).

1.6.3 Structure of Galectin-3 binding protein

Galectin-3 binding protein is the unique chimera-like galectin in the family having an extra-long and flexible N-terminal domain consisting of 100–150 amino acid residues figure(1.7) according to species of origin, made up of repetitive

sequence of nine amino (111).This means that GAL-3BP consists of carbohydrate recognition and collagen-like domains, which makes it able to interact with a wide array of extracellular matrix proteins, carbohydrates (N-acetyllactosamine), and glycosylated molecules, such as cell surface receptors, extracellular receptor and glycosylated proteins of the matrix, including lamina, fibronectin, and tenascin (112).

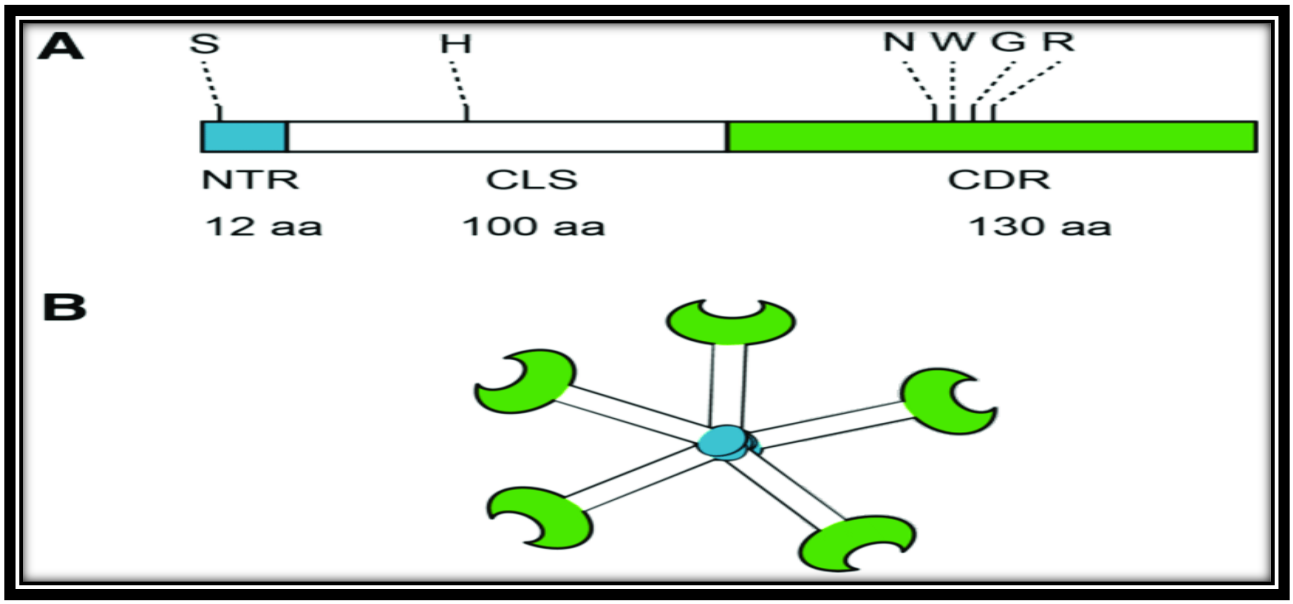


Figure (1.7) Structure of Galectin-3BP (113)**.**

(A) Galectin-3 protein structure consists of N Terminal Domain (NTD), which has a N Terminal Region of 12 amino acids (aa) and contains serine 6 (S) phosphorylation site. The carbohydrate recognition domain (CRD) 130 aa comprise the C-terminal and contains the NWGR motif;

(B) Pentameric structure of Galectin-3.

1.6.4. Function and Regulation of Galectin-3 binding protein

GAL-3BP enters in many pathological processes, including inflammation, tumour growth, and fibrosis (114). Recent research revealed that this protein is associated with several steps of invasion and metastasis, like angiogenesis, cell-matrix interaction, dissemination through blood flow and extravasation (115).

GAL-3BP is expressed widely in epithelial and immune cells and its expression is correlated with cancer aggressiveness and metastasis. Moreover, enhanced production and release of GAL-3BP have been demonstrated in a number of inflammatory and fibrotic conditions including chronic pancreatitis, cirrhosis and lung fibrosis, as well as in patients with heart failure (116) .

CHAPTER TWO

SUBJECTS, MATERIALS AND METHODS

2.1. Subjects

A case control study was conducted on 119 semen samples, 31men with (asthenozoospermia), 28 men with (oligoasthenozoospermia), while another 60 healthy controls were diagnosed, semen samples were collected from Iraqi patients who attended infertility clinics for infertility diagnosis and assisted reproductive technologies in Karbala for the period between November 2021 and May 2022.

The study was conducted in accordance with the ethical standards set forth in the Declaration of Helsinki. Prior to sampling, it was performed with the patient's oral and analytical consent. The local ethics committee checked and approved the study protocol, subject information, and permission form pursuant to Document 3326 (containing the number and date 5/12/2021) for this approval.

The patient was instructed to collect his semen sample in a dry, sterile and wide-mouthed plastic container. The age of patients was ranging from 20 to 40 years old. The following information was obtained from patients and labelled with precise details such as (name, age, height, weight, body mass index, smoking, period and type of fertility, abstinence period, time of sample collection), (appendix).

After liquefaction of semen sample, the examination was carried out according to the World Health Organization 2010. After that, every semen sample was taken divided into three parts after examination. The sample was placed in a centrifuge at a constant speed and time (3000 rpm for 10 min) and the supernatant is separated from the fail, and placed Supernatant in three Eppendorf tubes to measure the total antioxidant, state Galectin-3 Binding Protein and Heat Shock Protein 70 of seminal plasma by Elisa.

2.2. Study Design:

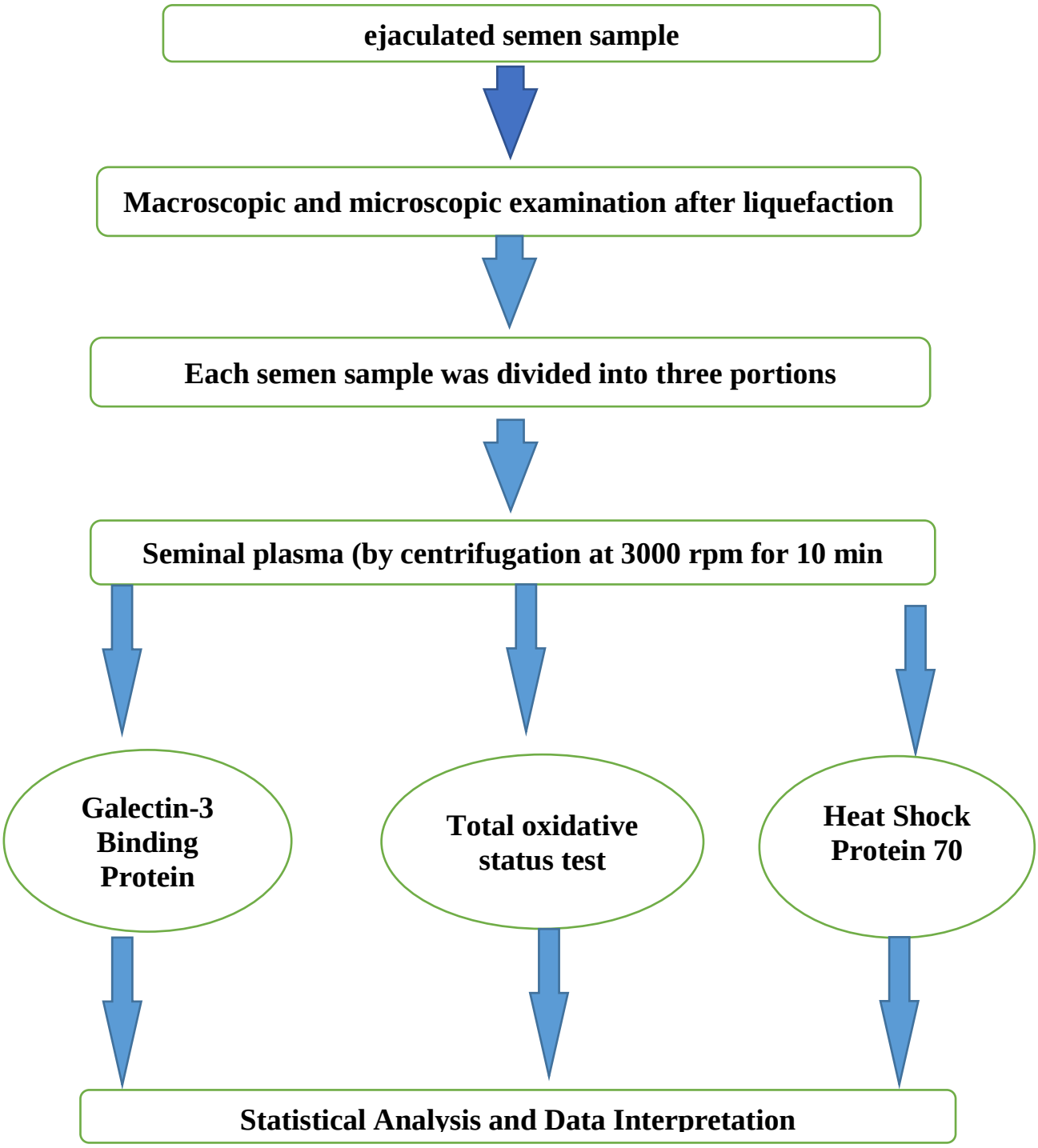


Figure 2.1: Study analysis.

2.3. Materials, Equipment's and Devices

The following Materials, Equipment's and Devices were used in the current study as shown in the table (2.1).

Table (2.1): The table shows Materials, Equipment's and Devices that used in this study.

Materials, Equipment's and Devices	**Company , Origin**
Distilled water	Germany
Centrifuge	Germany / Hettich
Eppendorf tube	Germany
Gloves	China
Incubator	Germany / Memmert
Litmus paper (PH indicator rolls)	USA
Micro- pipette tips	Japan / nexty
Refrigerator	China / biobase
Slides and cover slip China	China / Sail brand
ELISA system (Wisher, Printer, Reader)	USA/ meridian
Human Galectin-3 ELISA Kit	China / Sun Long Biotech Co
Human heat shock protein ELISA Kit	China / Sun Long Biotech Co
Human total anti-oxidant ELISA Kit	China / Sun Long Biotech Co

2.4. Seminal Fluid Analysis

2.4.1 Semen Collection

Samples were collected in sterile containers from patient by masturbation method after 2 to 7 days' abstinence. The container in which the seminal fluid sample was collected must be labelled with the following information: name, age, abstinence period, and time of sample collection. The specimens were placed in an incubator at 37 ºC for 30-60 minutes to allow liquefaction. The liquefied semen is carefully mixed for few seconds, and then the specimen was examined by macroscopic and microscopic examination as recommended by WHO 2010 guidelines (117).

2.4.2. Macroscopic Examination

2.4.2.1 Volume

A graduated cylinder with a conical base was used to determine the volume of the semen sample. The normal volume should be equal or more than 2 mL (WHO, 2010).

2.4.2.2 Appearance

Within one hour of ejaculation simple examination was performed on the semen sample at room temperature. A normal semen sample has a homogenous grey-opalescent appearance. If the sperm concentration is very low, they may appear less opaque. When red blood cells are present it may appear red-brown colour and in a patient with jaundice or taking some vitamins or drugs it appears yellow (WHO, 2010).

2.4.2.3 Liquefaction Time

Normal semen sample liquefied within 60 minutes at 37 ºC, although this usually occurs within a period of 15 minutes. Some samples may not liquefy, so they were induced to liquefy by mechanical mixing or addition of an equal volume of culture medium followed by repeated pipetting. Before microscopic examination the sample was well mixed in the original container (WHO, 2010).

2.4.2.4 PH

PH litmus paper ranging between 6 to 14 was used to determine the pH of the semen. Immersing the litmus paper in the semen sample and reading it after 30 seconds, when the pH of the semen is slightly alkaline ranging between 7.2 to 8.0 it is conside red normal (WHO, 2010).

2.4.2.5. Viscosity

The semen sample's viscosity can be determined by carefully aspirating it into a wide-bore pasture pipette, to allow the semen to drop by gravity and to observe the length of any thread. In small discrete drops, a normal sample leaves the pipette. If the drop was forming a thread more than 2 cm long, viscosity was abnormal (WHO, 2010).

2.4.3. Microscopic Examination

Microscopic examination of semen sample includes sperm concentration, motility, morphology in addition to non-sperm cellular components like, leukocyte concentration and immature germ cells. At the first, the semen sample mix well by pasture pipette then take 10µL of liquefied semen and put on a clean glass slide and covered with 22 × 22 mm cover slip. The volume of semen and the dimension of the cover slip are standardized so that the analyses are always carried out in a preparation with fixed depth allows full expression of the rotating movement of normal spermatozoa. The preparation is then examined under an ordinary light microscope at a magnification of 40 X, which is recommended for all examinations of stainless preparations of fresh semen sample (WHO, 2010).

2.4.4.1. Sperm Concentration

Referred to estimation of how many million sperm are present in each millilitre of semen. The mean number of spermatozoa in five random microscopically a field was multiplied by a factor of one million to get the sperm concentration per millilitre (ml). By multiplying concentration of sperm by semen volume getting total sperm count. The

lower reference limit for sperm concentration is 20 × 10^6 spermatozoa per mL and the lower reference limit for total sperm number is 40 × 10^6 spermatozoa per ejaculate (WHO, 2010).

- Sperm concentration (million/ml) = number of sperm × 10^6.
- Total sperm count (million/ejaculate) = concentration of Sperm × volume.

2.4.3.2 Sperm Motility

Means the percentage of sperm that are moving. The freshly made; wet preparation is left to stabilize for approximately one minute. The microscopic field is scanned systematically and the motility of each spermatozoon measured according to WHO (2010) whether it shows:

A: (rapid progressive motility) sperms are those which swim forward rapid in a straight line

B: (slow or sluggish progressive motility) sperms swim forward, but either in a bent or warped line or slowly (slow linear or nonlinear motility) It must be the sum of (A & B > 32%).

C: non progressive motility.

D: immotility.

*Sperm motility (%) = No. of motile sperm / total No. of sperms

2.4.3.3 Sperm Morphology

This referred to the percentage of morphological normal sperms which considered normal when equal 14% or more according to (WHO, 2010). For examination of morphologically normal sperms, slides are using that previously used for determination sperm motility and calculating the ratio according to this formula

*Normal sperm morphology (%) = No.of normal sperms / total No. of sperms (normal and abnormal)

3.4.3.4 Round Cells

The round cell used to describe all cells other spermatozoa present in ejaculate which include: leukocytes, epithelial cells from the genitourinary tract, spermatogenic cells and others (WHO, 2010). To estimation the number of round cells in the semen samples counting their mean number in 10 random microscopic fields and multiplied by a factor of 1 million. The number of round cells value were counted using HPF method. The semen sample with (5round cells/ HPF) was considered normal according to WHO (2010).

2.4.3.5 Sperm Agglutination

The sticking of motile spermatozoa to each other is known as Agglutination as head to head or mid piece to mid piece or tail to tail, or mixed, e.g. mid piece to tail. The aggregation is different from agglutination that term is used to describe the adhering of motile or immotile spermatozoa to mucus threads or to cells other than spermatozoa, or to debris (WHO, 2010).

2.5. Total anti-Oxidant Status Test & heat shock protein & Galectin 3 binding protein

2.5.1 Principal Elisa.

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in the kit has been pre-coated with an antibody specific to (TAOS) (Hsp70) (GAL-3BP) figure (2.2). Standards or samples are added to the micro-ELISA plate wells and combined with the specific antibody. Then, an Avid in-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human (TAOS) or (Hsp70) or (GAL-3BP) and Avid in-HRP conjugate will appear blue in colour. The enzyme-substrate reaction is terminated by the addition of stop solution and the colour turns yellow. The optical density (OD) is measured spectrophotometric ally at a wavelength of 450 nm. The OD value is proportional to the concentration of Human. (TAOS) & (Hsp70) & (GAL-3BP) (Sun Long Biotech, 2016).

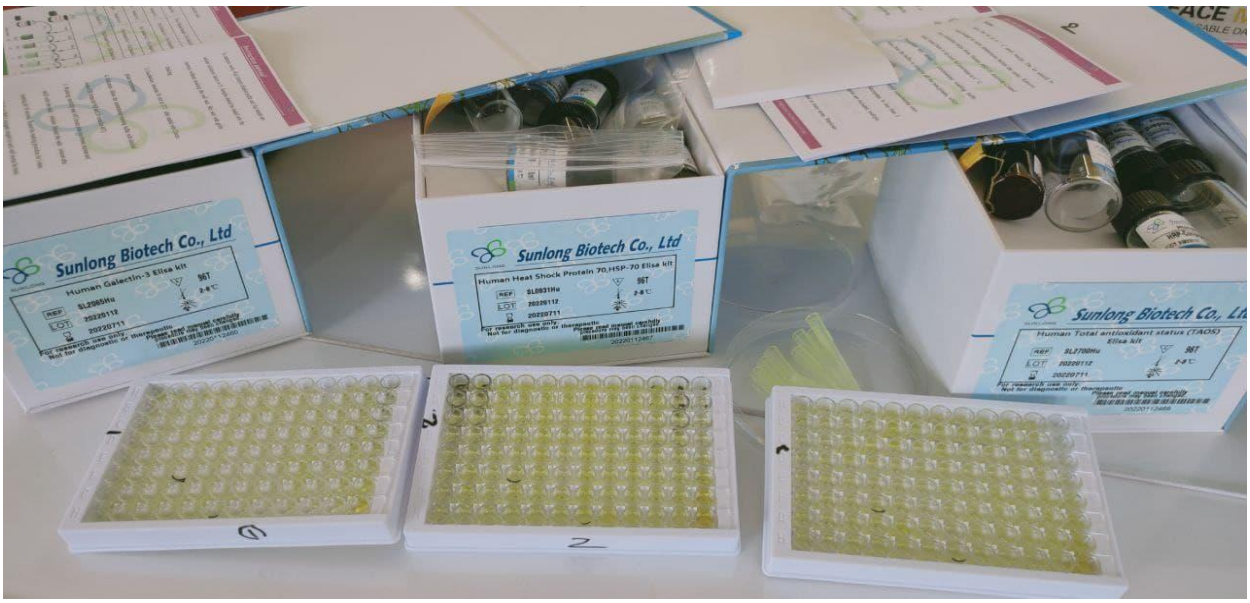


Figure 2.2: kits to (TAOS) , (Hsp70) ,(GAL-3BP)

2.5.2 Reagents of ELISA:

All chemical reagents that used for determination of Serum Galectin-3 Level are listed below table (2-2):

Table (2-2): All chemical reagents that used for determination of Serum Galectin-3 Level are list below

Reagent	Specifications	Quantity
Chromogen Solution A	1 bottle	6ml
Chromogen Solution B	1 bottle	6ml
Closure plate membrane	2	-
HRP-Conjugate reagent	1 bottle	6ml
Micro Elisa strip plate	1	96 wells
Sample diluent	1 bottle	6ml
Standard diluent	1 bottle	1.5ml
Standard：27ng/ml	1 bottle	0.5ml
Stop Solution	1 bottle	6ml
Wash solution	1 bottle	（20ml×30 fold）

2.5.3 Reagent Preparation:

All reagents and required number of strips were allowed to reach room temperature prior to use.

2.5.4 Wash Solution:

Twenty mil of concentrated wash buffer were diluted in 580 ml distilled water. The unused working wash solution was put back at 4°C. The diluted working wash solution is stable for two weeks at room temperature.

2.5.5 Procedure of ELISA:

1- 50µl of series standard were added to standard well, and 10µl of sample and 40µl sample dilution buffer was added to each sample wells.

2- Incubate for 30 min at 37℃.

3- Aspirated and washed five times with diluted working wash solution.

4- 50µl of HRP-Conjugate reagent was added to each well.

5- Incubate for 30 min at 37℃.

6- Aspirated and washed five times with diluted working wash solution.

7-50µl of Chromogen Solution A and Chromogen Solution B were added to each well, evaded the light preservation for 15 min at 37℃.

8-50µl of Stop Solution was added to each well. The absorption was read at 450nm.

9-The results were Calculated.

2.5.6 Calculation of concentration:

- **Calculate galectin-3 binding**

The standard density was taken as the horizontal, the OD value of the vertical, draw the standard curve on graph paper, find out the corresponding density according to the OD value of the sample by the sample curve, multiplied by the dilution multiplier, or calculate the straight line regression equation of the standard curve with the standard density and OD value, with The OD value of the sample in the equation, calculate the density of the sample.

- **Standard concentrations were prepared as follows;**

The sample was diluted and added to the standard: 10 standard wells were placed on ELISA coated plates, 100 µl standard was added to the first and second wells, then the 50 µl standard dilution was added to the first and second wells, mixing; 100 µl was taken out from the first and second well and then added to the third and fourth well separately. Then a standard dilution of 50 µl is added to the third and fourth well, blending; Then 50 µl was taken out from the third and fourth well, then 50 µl was added to the fifth and sixth well, then a standard dilution of 50 µl was added to the fifth and sixth well, and they were mixed; 50 µl were taken out from the fifth and sixth well and added to the seventh and eighth well, then a standard dilution of 50 µl was added to the seventh and eighth well, and they were mixed; 50 µl taken out from the seventh and eighth well and added to the ninth and tenth well, the standard dilution of 50 µl was added to the ninth and tenth well, mixed, and 50 µl was taken out from the ninth and tenth well (the 50 µl sample was added to each well after dilution, (density: 48 ng/ L 36 ng/L, 18 ng/L, 9 ng/L, 4.5 ng/L)

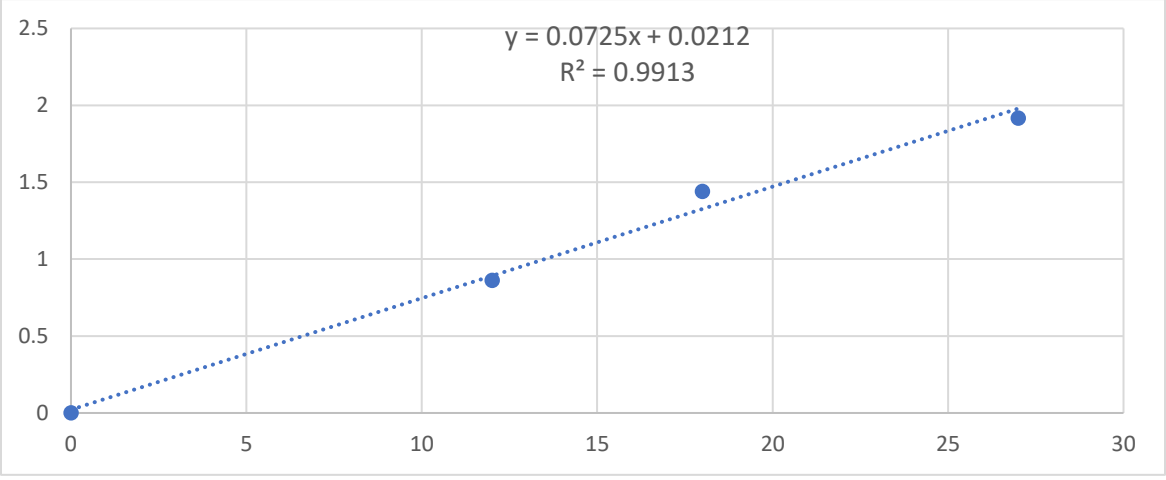


Figure 2-3: Calibration curve of galectin-3 (ng/L)

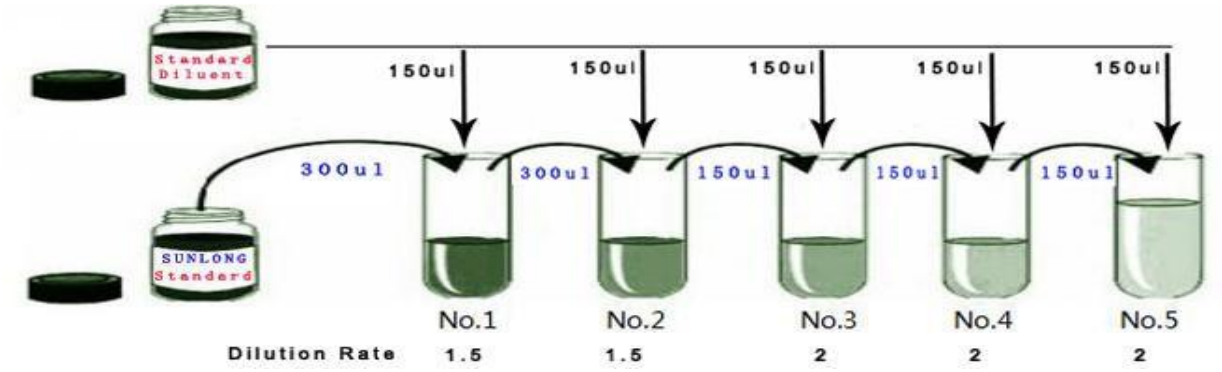
- **Calculate heat shock protein 70**

Known concentrations of Human HSP-70 Standard and its corresponding reading OD is plotted on the log scale (x-axis) and the log scale (y-axis) respectively. The concentration of Human HSP-70 in sample is determined by plotting the sample's O.D. on the Y-axis. The original concentration is calculated by multiplying the dilution factor.

Standard concentrations were prepared as follows;

The standard was diluted with micro tubes first, then a 50ul pipette from each tube into a well micro plate, each tube using two wells, ten wells total.

36 ng/ml	Standard No.1	300µl Original Standard + 150µl Standard diluents
24 ng/ml	Standard No.2	300µl Standard No.1 + 150µl Standard diluents
12 ng/ml	Standard No.3	150µl Standard No.2 + 150µl Standard diluent
6 ng/ml	Standard No.4	150µl Standard No.3 + 150µl Standard diluent
3 ng/ml	Standard No.5	150µl Standard No.4 + 150µl Standard diluent



54 ng/ml	36 ng/ml	24 ng/ml	12 ng/ml	6 ng/ml	3 ng/ml

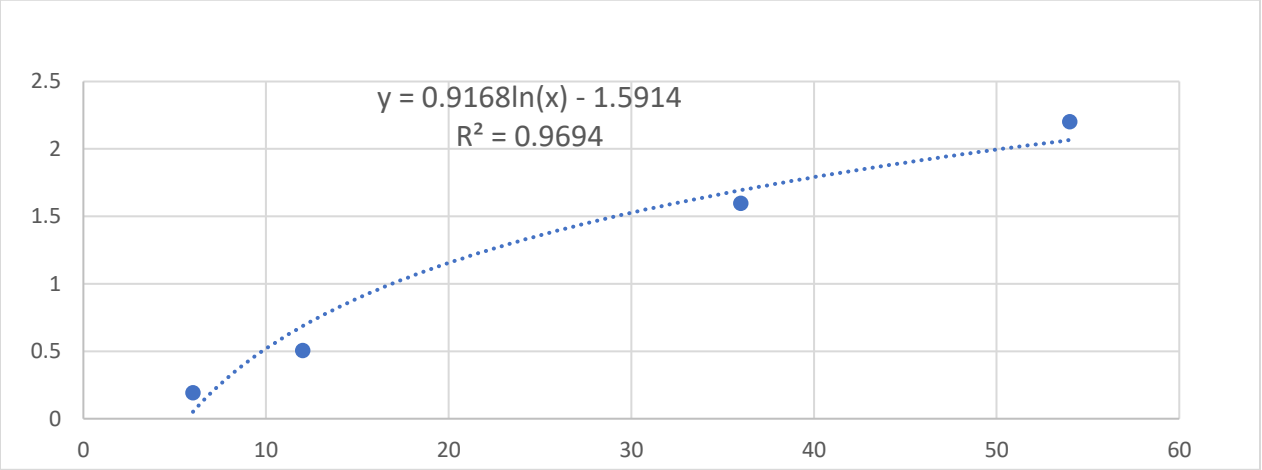


Figure 2-4: Calibration curve of heat shock protein (ng/ml)

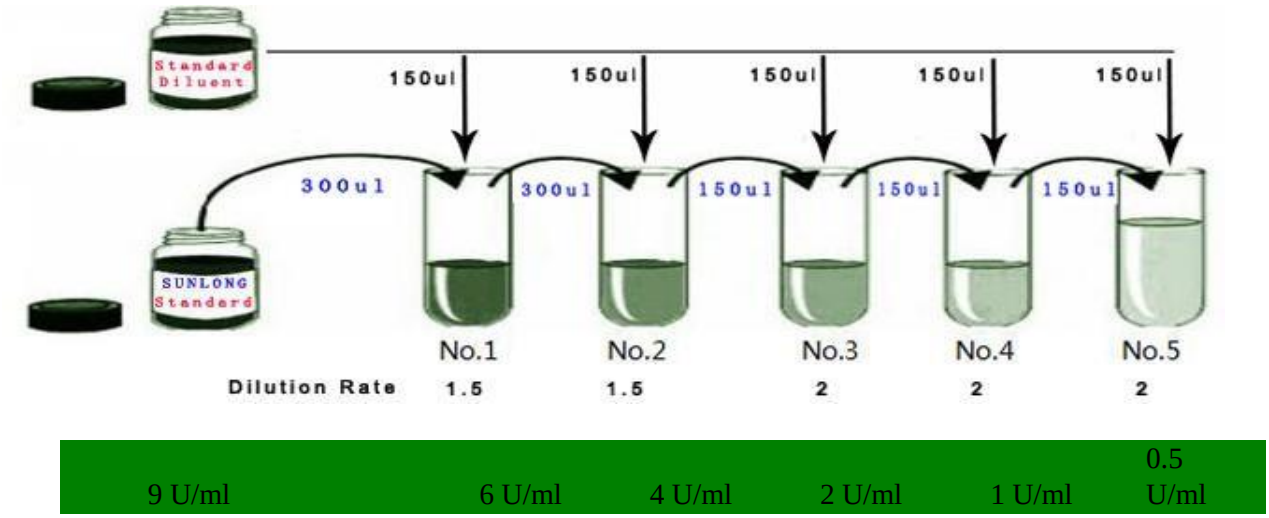
- **Calculation total anti-oxidant**

Known concentrations of Human total anti-oxidant Standard and its corresponding reading OD is plotted on the log scale (x-axis) and the log scale (y-axis) respectively. The concentration of Human total anti-oxidant in sample is determined by plotting the sample's O.D. on the Y-axis. The original concentration is calculated by multiplying the dilution factor.

Standard concentrations were prepared as follows;

The standard was diluted with micro tubes first, then a 50ul pipette from each tube into a well microplate, each tube using two wells, ten wells total.

6 U/ml	Standard No.1	300µl Original Standard + 150µl Standard diluents
4 U/ml	Standard No.2	300µl Standard No.1 + 150µl Standard diluents
2 U/ml	Standard No.3	150µl Standard No.2 + 150µl Standard diluent
1 U/ml	Standard No.4	150µl Standard No.3 + 150µl Standard diluent
0.5 U/ml	Standard No.5	150µl Standard No.4 + 150µl Standard diluent



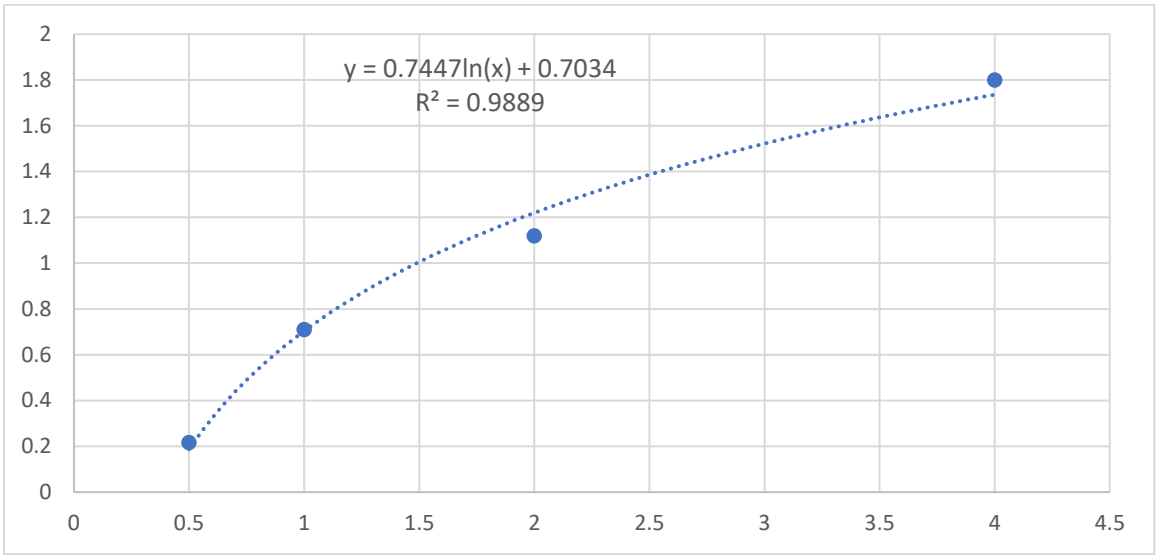


Figure 2-5: Calibration curve of total anti-oxidant (U/ml)

2.6 Excluded criteria

Samples from people over 40 years old and less than 20 years old, as well as people who were taking drugs containing steroids, chemotherapy, cimetidine, phenytoin, spironolactone, sulfasalazine, vitamins, patients with diabetes, and endocrinologists, were also excluded. Exclude people who have had previous surgery for varicose veins or who have had mumps previously.

2.7 Statistical analysis

Information from the questionnaire from all participants was entered a data sheet and were assigned a serial identifier number. Multiple entry was used to avoid errors. The data analysis for this work wear generated using The Statistical Package for the Social Sciences software, version 28.0 (IBM, SPSS, Chicago, Illinois, USA). Descriptive statistics was performed on the participants' data of each group. Values were illustrated by Mean ±SD for continuous variable. The distribution of the data was checked using Shapiro test. The association between the analysed factors was estimated using odds ratios (ORs) and 95% Confidence Interval Range which calculated by a nova conditional logistic regression.

Significant differences in categorical variables among the parameters were confirmed through analytical statistical tests. Results of all hypothesis tests with p-values <0.05 (two-side) were considered to be statistically significant.

CHAPTER THREE

RESULTS AND DISCUSSION

3. Results and Discussion

3.1 Demographic and clinical characteristics:

The study was conducted on 119 men, and they were classified into two groups, 59 men with infertility, as a group of patients divided into two cases. The first cases 28 samples (Asthenozoospermea) and the second cases 31 samples (oligoasthenozoospermia), and 60 fertile men.

The clinical demographic characteristics and laboratory parameters of the patient group were summarized in Figure (3.1), (3.2), (3.3).

In the current study, smoking was observed in 37(62.7%) infertile patients. And 22(37.3%) non-smokers are infertile. smoking is a well-known risk factor for inferior male fertility. In infertile men, a cigarette smoking is associated with adverse effects on sperm density, vitality and motility and morphology, as well as the effect on semen volume, according to a study (Zhang *et al*.,2016) (118).

In the present study, in regard to the type of infertility, there were 46(77.9 %) men with primary infertility and 13(22.1%) men with secondary infertility. Based on the observation of (Alshahrani *et al*., 2014) (119).the rate of primary infertility was (79.6%) and that of secondary infertility was (20.4%) and according to (Öztekin *et al*., 2019), the rate or primary infertility was (77.3%) and that of secondary infertility was (22.7%); therefore, the observation in this study is in accordance with previous reports that most males who seek medical advice had primary infertility (120).

In the current study, the effect of the type of work on the patient's condition appeared, as it was noted that 12 (20%) sterile who were working in office work, 17 (28%) sterile were soldiers, and 30 (51%) were sterile among the people who practiced physical labour as workers (Municipality, load motorists and furnace workers) based on the observation of (Epstein *et al*., 2006) (121). Occupational hazards negative it is associated with semen quality and is considered a major contributor to male infertility, As stated in a study (Hamerezaee *et al*., 2018) (122).

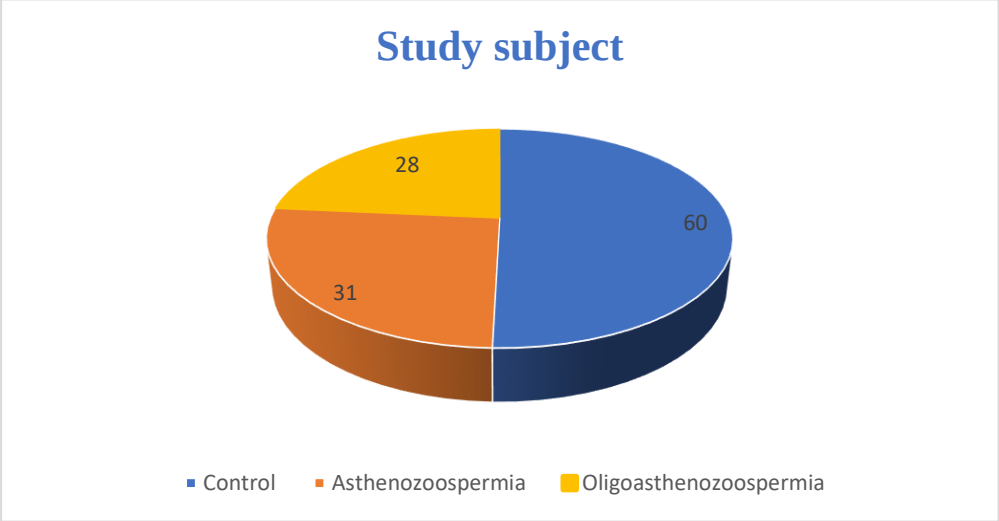


Figure 3.1: Descriptive of the Demographic and of the study population (n= 119)

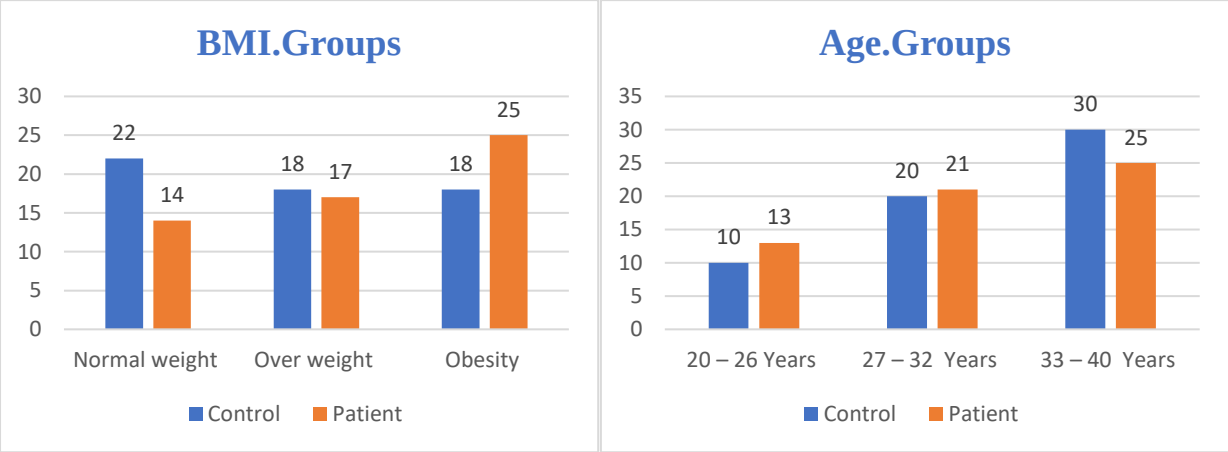


Figure 3.2: Distribution of the cases study according to the colleges, and the (Age & BMI) groups

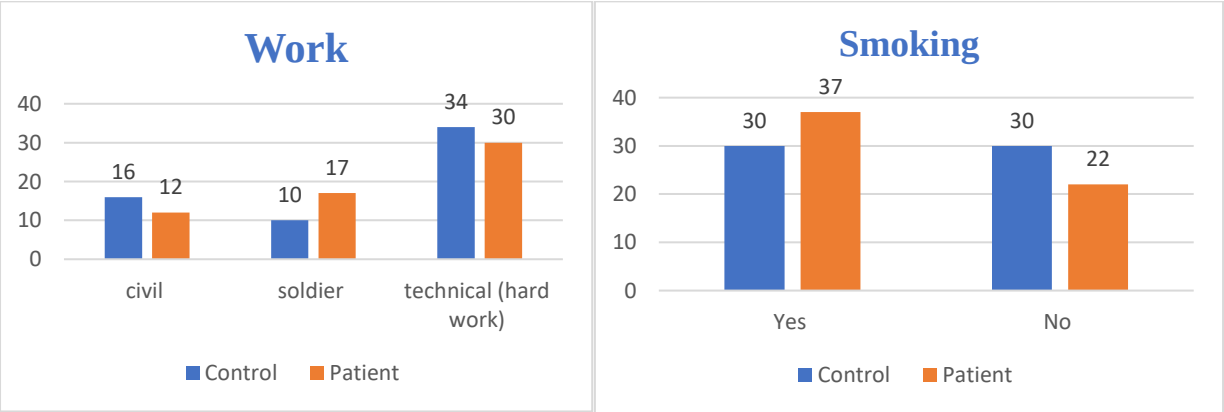


Figure 3.3: Distribution of the cases study according to the colleges, and the (Work & Smoking)

3.2: Results of the analysis of basic semen:

Table (3.1) shows the analysis of basic semen characteristics, such as concentration and mobility. There was a significant difference in these variables. The concentration, Progressive cell and non-progressive cell of semen in the infertile men patients' groups were lower than control groups. The mean±SD level of the concentration was (29.79±18.94 M/ml) and (63.62±28.09 M/ml) in patient & control respectively. The present of the Progressive cell was (10.4051±8.47) and (47.67±12.03) while the non-progressive cell, were (20.84±14.76) in patients and (27.26±6.54) in control group. On the other hand, the non-moving sperm, were increased markedly in the infertile men patients' groups compared to control group, the mean of the percentage in patients were (68.55±18.72) and in control was only (22.89±10.77). The present of the Progressive cell was (10.4051±8.47) and (47.67±12.03) while the semen Volume, were (4.68±1.92) in patients and (3.12±0.76) in control group

Table 3.1: Results of the analysis of basic semen characteristics.

Variable	Control(N=60) Mean± SD	Patient(N=59) Mean ±SD	P value
Concentration (M/ml)	63.62±28.09	29.79±18.94	**<0.001[S]**
Progressive cell (%)	47.67±12.03	10.4051±8.47	**<0.001[S]**
Non-progressive cell (%)	27.26±6.54	20.84±14.76	**<0.001[S]**
Non-moving sperm (%)	22.89±10.77	68.55±18.72	**<0.001[S]**
Volume(ml)	3.12±0.76	4.68±1.92	**<0.001[S]**
T-test was *: significant at p ≤ 0.01			

N: number of cases; SD: standard deviation; S: significant

A standard semen analysis is the first test prescribed by a clinician to assess male factor infertility in a couple. The lower reference values provided by the WHO are used to guide the diagnosis and treatment of the male partner. Diagnosis of normozoospermia may push the investigation towards the female partner as the primary cause of infertility, while a semen sample with altered sperm parameters

suggests male factor as a putative cause for infertility and guide towards more advanced investigations, genetic testing and treatments (Wang *et al*., 2018) (123). Furthermore, morphological abnormalities such as globozoospermia, macrocephaly, multiple tail defects and decapitated sperm syndrome are indicative of genetic disorders, As stated in a study (Gatimel *et al*., 2017) (124). In the present study, sperm concentrations and motility in fertile men were more than the concentrations and motility of infertile men, which agreed with reference values provided by the recommended by WHO guidelines (2010) (117). This study showed that there is an increase in semen volume in infertile male patients, and this result was consistent with the results of some other studies such as (Mustafa *et al*., 2019) (125); (Abdulrahman *et al*., 2019) (126).

3.3. Sociodemographic Factors of Infertile Men Enrolled in The Present Study

The results in table (3.2): The Mean±SD age of people in the fertility and infertility groups was (32.2±5.76) and (31.07±5.59) years, respectively. There were no statistically significant differences between infertile men who suffered from (Oligoasthenozoospermia and Asthenozoospermia) compared with men fertile.

The Mean±SD BMI of people in the fertility and infertility groups was (27.52 ± 5.10) and (29.25 ± 5.75), respectively; there was a slight increase in the mean mass index but no significant differences between infertile men.

The current study included 59 infertile men. The mean age of all enrolled infertile men was (31.07±5.59) years with an age range of 20 to 40 years. Most of enrolled men were between 20 to 40 years as they accounted for 77 (80.2 %). This reflects that, many infertile couples quit seeking infertility treatment with time; and this gives an important hint that infertility treatment with time-frame plan is a priority and postponing an indicated modality of treatment is actually against the couple’s benefit.

Table 3.2: difference between Sociodemographic Factors of Infertile Men & Control groups

Parameter	Control (N=60) Mean±SD	Patient(N=59) Mean±SD	P value
Age(year)	32.2±5.76	31.07±5.59	0.27[NS]
BMI(kg/m2)	27.52±5.10	29.25±5.75	0.139[NS]
T-test was *: significant at p ≤ 0.01			

N: number of cases; SD: standard deviation; S: significant; NS= Non significant

Semen parameters have different susceptibility to be altered in response to change in BMI. It is also expected to be affected by hormonal changes in response to higher weight and BMI. Thus, factors affecting fertility such as hormonal changes, varicocele diabetes, and other fertility-affecting factors were excluded from this study to evaluate semen parameters with BMI away from other factors that may contribute to semen parameters changes.

In a previous study (Öztekin *et al*., 2019) the mean BMI was 26.6 ± 4.1 kg/m2 (127) .while a study conducted by (Punab *et al*., 2017) reveals that the mean BMI was (26.7 ± 4.6 kg/m2) (128).Thus, the average BMI in the current study is slightly higher than that reported by the previous authors. In addition to poor semen quality, fertility in obese men may be affected by endocrinopathy, aromatic activity, psychological and thermal effects, sleep apnea, leptin and minor toxins according to the study (Katib ., 2015) (129) .

3.4 Difference between the level of biomarkers in the patient and control groups

The results in table (3.3) indicated that there was a significant difference in the Mean±SD level of (TAOS) and (Hsp70) in patient's groups compared to control group.

The Mean±SD level of TAOS was (1.98±0.20) U/ml in patients' groups while it was (3.53±0.64) U/ml in control group. On the other hand, The Hsp70 Mean±SD level was (47.66±7.10) ng/L in patients' groups while it was (40.17±1.94) ng/L in

healthy control. and GAL-3BP Mean±SD level was (28.00±8.21) ng/L in patients' group and (26.87±5.73) ng/L in healthy control.

Table 3.3: the results of seminal plasma of biomarkers in the fertile and infertile groups

Parameter	Control (N=60) Mean±SD	Patient(N=59) Mean±SD	P value
Total anti-oxidant U/ml	3.53±0.64	1.98±0.20	**<0.001[S]**
Heat shock protein 70 (ng/L)	40.17±1.94	47.66±7.10	**<0.001[S]**
Galectin 3 binding protein (ng/L)	26.87±5.73	28.00±8.21	0.439[NS]
Studded T-test was *: significant at p ≤ 0.01			

N: number of cases; SD: standard deviation; S: significant; NS= Non significant

- **Total anti-oxidant**

Spermatozoa are vulnerable Oxidative stress due to their high content of polyunsaturated fatty acids, deficiencies in intracellular antioxidant enzymes, and limited DNA repair ability. In particular, the high content of polyunsaturated fatty acids in the sperm plasma membrane regulates membrane fluidity but also represents a preferential substrate for Reactive oxygen species attack. In fact, lipid peroxidation of the sperm plasma membrane polyunsaturated fatty acids has been the first oxidative damage recognized in male infertility, highly reactive lipid aldehydes produced by peroxidation form adducts with proteins and DNA and induce sperm mitochondrial dysfunction through binding to electron transport chain proteins. This reinforces the production of mitochondrial Reactive oxygen species and compromises both the competence and the DNA integrity of the spermatozoa as stated in a study, (Aitken *et al*., 2020) (130). Thus, it causes mutations in the sperm genome, as stated by a study (Aitken *et al*., 2020) (131).

Nowicka-Bauer, K.; Nixon 2020 reported the association of 4-hydroxynonenal-protein adducts generation with loss of membrane integrity,

motility, and decreased fertility when the comparison was made between fertile men (amino sperm males) and infertile men (132).

Significant higher levels of Total anti-oxidant were observed in this study in men with control compared to men with infertility and This result is supported by a study (Yurchuk *et al*., 2021) (133).

The conclude from previous studies and the results of this study that the lack of antioxidants in seminal plasma is directly related to a defect in the function of sperm, its movement, Concentration, and thus a defect in the process of fertilization ability and leads to be a cause of infertility.

- **Heat shock proteins 70**

The higher values of Hsp70 could be due to their protective function of mitigating oxidative stress associated with cells. Hsp70 exist in the plasma in relatively low concentrations but their concentration could increase exponentially due to oxidative stress beyond the physiological limit. Therefore, their increase could be targeted toward preventing sperm cell deformation or death by free radicals or reactive oxygen species as stated in a study of (Aworu *et al*., 2022) (134).

Significant higher levels of Hsp70 were observed in this study in men with infertility compared to men with normozoospermia, and this result was confirmed by studies (Ferlin *et al*., 2010) (135) ; (Agarwal *et al*., 2012) (136).

The conclude from previous studies and the results of this study that the lack of antioxidants in seminal plasma is offset by an increase in oxidative stress, and since Hsp70 are proteins that attempt to repair the imbalance and damage caused by free radicals on the cell surface and its content, So the results of Hsp70 found at a higher level in the semen of infertile men from the semen of fertile men.

- **Galectin 3 binding protein**

In the male reproductive system, it is known that GAL-3BP is a secretory lectin in human seminal plasma. In the initial studies, it was demonstrated that there is a strong GAL-3BP immunoreactivity in the acrosomal region of ejaculated and capacitated spermatozoa. This immunoreactivity was much stronger than that of epididymal spermatozoa. Therefore, it was tried to find a source of GAL-3BP to release in seminal plasma and it was found GAL-3BP can originate from seminal plasma-derived extracellular vesicles. Those GAL-3BP molecules in the next step means during post-testicular maturation can be transferred to the sperm surface. They play an important role later and in the time of fertilization for strong binding between spermatozoa and zona pellucida (137). In this study, it is found that a slight increase in the GAL-3BP value of infertile men than in fertile men but it's not Significant, and this result was confirmed by study (Davalieva K *et al*., 2012) (138). But the other study found increase in the GALBP-3 have been observed in men with normal azoospermia, by study (Giacomini *et al*., 2015) (139).

Through the above studies, and through the results of this study, the conclude the results of GALBP-3 in semen is of no clinical significance to infertile men and fertile men.

3.5 difference between biomarker Mean±SD level in Control groups & Oligoasthenozoospermia & Asthenozoospermea

The results in table (3.4) By comparing the mean Level of (TAOS) with Study groups, the test revealed a significant difference in Mean±SD of (TAOS) ($P \leq 0.001$).by using Post hoc tests revealed that control males group was significantly higher than in other groups. Also, there was a significant difference in Mean±SD Level of (TAOS) with study groups, the test revealed a significant difference in Mean±SD (Hsp70) ($P \leq 0.001$).by using Post hoc tests revealed that Oligoasthenozoospermia group was significantly higher than in other groups (50.02±7.39) ng/L. lastly, no significant difference in Mean±SD level (GAL-3BP).

Table 3.4: Difference between mean of Lab finding in Asthenozoospermea, Oligoasthenozoospermia & Control groups

Parameter	Control Mean±SD	Asthenozoospermea Mean±SD	Oligoasthenozoospermia Mean±SD	P Value
TAOS (U/ml)	3.53±0.64*	2.07±0.20	1.88±0.15	**<0.001[S]**
Hsp70 (ng/L)	40.17±1.94	45.52±6.21	50.02±7.39*	**<0.001[S]**
GAL-3BP (ng/L)	26.87±5.74	28.60±9.50	28.00±6.64	0.855[NS]
ANOVA was *: significant at p ≤ 0.01				

N: number of cases; SD: standard deviation; S: significant; NS= Non significant

- **Total anti-oxidant**

Decrease in antioxidant levels and an increase in oxidative damage could induce oxidative stress and causing sperm abnormalities impairing their potential for reproduction is considered one of the major sperm-damaging factors, As stated in studies (Martin-Hidalgo *et al*., 2019) (140); (Cho *et al*., 2018) (141); (Panner Selvam, M.K.; Agarwal 2018) (142). Oxidative stress affects their activity, damages DNA structure, accelerates apoptosis, all of which subsequently reduces their numbers, impairs movement and the development of normal morphology, and impairs function. This leads to disturbances in fertility or disruption of fetal development, As stated in a study (Walczak–Jedrzejowska *et al*., 2013) (143).

This is what this found in the study through the results, it was observed that the level of TAOS decreased in infertile men. When the comparison was made between men who have fertility (normozoospermic males) and men who suffer from (Oligoasthenozoospermia & Asthenozoospermea).

Where significantly higher levels of (TAOS) were observed in this study in men with control males compared to men with Oligoasthenozoospermia, and this result was confirmed by studies (Alahmar *et al*., 2021) (144); (Abdurrahman *et al*., 2019) (145).

On the other side Significantly higher levels of TAOS the observed in this study in men with control males compared to men with Asthenozoospermea, and this result was confirmed by studies (Abdulrahman *et al*., 2019) (145); (Palani *et al*., 2019) (146).

Significantly higher levels of TAOS were observed in study in men with Asthenozoospermea compared to men with Oligoasthenozoospermia, and this result was confirmed by study (Mehrotra et al., 2013) (158).

But in a study (Aworu et al., 2022) found low TAOS have been observed in men with asthenozoospermea (159).

The conclude from previous studies and the results of this study that the lack of antioxidants in seminal plasma affects the cell membrane through free radicals and thus affects its contents such as mitochondria, which produce forms of damage to the cell through cell shape, number and movement and thus leads to pathological conditions such as Oligoasthenozoospermia & Asthenozoospermea.

- **Heat shock proteins 70**

The higher values of Hsp70 could be due to their protective function of mitigating oxidative stress or distress associated with cells. Hsp70 exist in the plasma in relatively low concentrations but their concentration could increase exponentially due to oxidative stress beyond the physiological limit. Therefore, their increase could be targeted toward preventing sperm cell deformation or death by free radicals or reactive oxygen species, The Heat shock protein including Hsp70 are known to conserve and protect cells such as spermatozoa that are prone to lipid peroxidation and degradation, Sperm proteins were altered in individuals with abnormal sperm cell morphologies, was confirmed by studies (Krisfalusi M *et al*., 2009) (147); (Aworu *et al*., 2022) (148).

And this is what that found in this study through the results, it was observed that the level of Hsp70 increased in infertile men. When the comparison was made between men who have fertility (normozoospermic males) and men who suffer from (Oligoasthenozoospermia & Asthenozoospermea). The Significantly low

levels of Hsp70 were observed in our study in men with normozoospermic males compared to men with Oligoasthenozoospermia, and this result was confirmed by studies (Ferlin *et al*., 2010) (149); (Waheed MM *et al*., 2013) (150).

But in a study (Cedenho *et al*., 2006) it was found that the level of Hsp70 is higher in men with normozoospermic (151).

On the other side Significantly low levels of Hsp70 were observed in our study in men with normozoospermic males compared to men with men Asthenozoospermia, and this result was confirmed by studies (Wu *et al*., 2019) (152); (Bracke *et al*., 2018) (153).

Significantly low levels of Hsp70 were observed in our study in men with Asthenozoospermea compared to men with Oligoasthenozoospermia, and this result was confirmed by study (Aworu et al., 2022) (151).

But in a study (Aworu et al., 2022) found High levels of Hsp70 have been observed in men with Asthenozoospermea (159).

That conclude from previous studies and the results of this study the increase in Hsp70 for the two groups (Oligoasthenozoospermia & Asthenozoospermea) is an increase to prevent and curb the damage resulting from a lack of antioxidants that leads to cell deformation, and since the Hsp70 is a protein that tries to prevent abnormalities in cells, so we see it at a higher level than it is for the fluid The sperm that contains more antioxidants, It was also studied(Arnos *et al*., 2018) (101).

- **Galectin 3 binding protein**

In the male reproductive system, GAL-3BP expression has been previously determined in the entire male reproductive system. It has been found in human seminal plasma (Fung KY *et al*., 2004) (154); (Tsuruya S *et al*., 2006) (155). prostate secretions, and prostate cancer cells (Bair EL *et al*., 2006) (156). in relation to the multiple functions of GAL-3BP in Human semen, GAL-3BP has been reported to be associated with semen liquefaction, sperm motility,

angiogenesis in the female reproductive system, and finally in the form of a Pro-inflammatory factor (Kovak MR *et al*., 2014) (157).

In this study, the found a slight increase in the GAL-3BP value of infertile men (Oligoasthenozoospermia) than in fertile (normozoospermic) but not Significantly, and this result was confirmed by study (Davalieva K *et al*., 2012) (138).

But in a study (Giacomini *et al*., 2015) found increase in the GAL-3BP have been observed in men with control male (139) .

On the other side in this study, the found a slight increase in the GAL-3BP value of Asthenozoospermea than in control male but not Significantly, and this result was confirmed by study (Davalieva K *et al*., 2012) (138).

In this study, we found a slight increase in the GAL-3BP value of Asthenozoospermea men than in Oligoasthenozoospermia men but not Significantly, and this result was confirmed by studies (Giacomini et al., 2015) (138).

Through the results of this study and through previous studies, the conclude that the absence of clinically significant results for the GAL-3BP, because the protein is not affected by cases (Oligoasthenozoospermia & Asthenozoospermea) except in a very simple way, but its quality is necessary for the process of adhesion that occurs in fertilization, according to the above studies.

3. 6 Difference between biomarker in Oligoasthenozoospermia & Asthenozoospermea groups with semen viscosity characteristic

The study was also included the examination of semen viscosity characteristics of the Asthenozoospermea & Oligoasthenozoospermia groups on the biomarkers. Patients were divided into three groups based on the semen viscosity (low, moderate and high viscosity).

Table (3.5) the comparison of biomarkers levels between groups which were statistically significant in TAOS and Hsp70, (3.14±0.89, 2.02±0.24, 1.99±0.19)

U/ml for TAOS levels and (41.89±4.54, 47.04±6.84, 48.75±8.62) ng/L for Hsp70 respectively, P value ≤0.001.

Hyper viscosity of liquefied semen is a biophysical alteration of an ejaculate whose biochemical etiology is scarcely known, despite the different studies that have been carried out on this topic, Such as (Overstreet JW *et al*., 1980) (158); (Issa Layali *et al*., 2015) (159).

Table 3.5: difference between biomarker mean level in Asthenozoospermea & Oligoasthenozoospermia groups in term semen Viscosity characteristic

Biochemical parameters	Viscosity (N=119)			P value
	Low N=79	Moderate N=22	High N=18	
Total antioxidant (U/ml)	3.14±0.89*	2.02±0.24	1.99±0.19	**<0.001[S]**
Heat shock protein70 (ng/L)	41.89±4.54	47.04±6.84	48.75±8.62*	**<0.001[S]**
Galectin 3 binding protein (ng/L)	26.96±6.06	26.28±7.12	26.18±10.51	0.624[NS]
ANOVA was *: significant at p ≤ 0.01				

N: number of cases; SD: standard deviation; S: significant; NS= Non significant

- **Total antioxidant**

The viscosity of semen is an essential parameter for achieving fertilization. Viscosity increases frequently in infertile patients. However, the mechanism by which viscosity causes infertility is still poorly understood. Since increased blood viscosity is associated with diseases caused by oxidative stress, it can be hypothesized that there is a relationship between semen viscosity and oxidative stress in male sterility, as I showed in study (Schallmoser *et al*., 2021)(160) . Therefore, this systematic review aims to investigate the relationship between hyper viscid semen and oxidative stress.

This study has demonstrated a significant relationship low TAOS in semen with increased viscosity. Possible negative oxidative stress effect on low Sperm motility cannot be ruled out. This indicates that Antioxidant therapy may be beneficial for

patients ,showing abnormal semen consistency to protect sperm cells by oxidative damage and to improve their functional properties, this result was confirmed by studies (Siciliano *et al*., 2001) (161); (Harchegani *et al*., 2019) (162).

- **Heat shock protein70**

This study has a significant relationship high Hsp70 in semen with increased viscosity. The decrease in the level of TAOS in the samples of infertile men increases the viscosity in the semen, and since the correlation of the amount of antioxidants is inversely related to the Hsp70, see an increase in the amount of protein with the viscosity (Siciliano *et al*., 2001) (161); (Harchegani *et al*., 2019) (162).

- **Galectin 3 binding protein**

This GAL-3BP in semen in the study slight increased viscosity to fertile men, but no significant, and this result was confirmed by study (Giacomini *et al*., 2015) the slight increase that occurred is not clinically significant (139).

3.7 Pearson's correlation coefficient (r) and linear regression analysis

The strength of the relationship was interpreted in line with the scheme: $|r| \geq 0.9$-very strong relationship. $0.7 \leq |r| < 0.9$-strong relationship, $0.5 \leq |r| < 0.7$ moderately strong relationship,$0.3 \leq |r| < 0.5$-weak relationship, and $|r| < 0.3$-very weak relationship (negligible). Scheme of interpretation according to (Hinkle *et al*) (163).

Table (3-6) shows correlation between biomarkers and independent variables including (basic semen characteristics among the infertile men patients group. Total anti-oxidant levels showed a positive significant correlation with the Concentration, Progressive cell and non-progressive cell [r =0.4, 0.8 and 0.3 respectively] P value ≤0.001, and negative significant correlation with the sample volume and Non-moving sperm cell% [r =-0.4, -0.7 respectively] P value ≤0.001.

Furthermore, a weakly significant positive-correlation was demonstrated between Heat shock protein levels with sample volume and Non-moving sperm cell% [r =0.4, 0.51 respectively], while the Concentration, Progressive cell and Non-progressive cell demonstrated a weak negative significant correlation [r =0.3, -0 .5 and -0.23 respectively] P value ≤0.001. No significant Correlations were found with the Galectin 3 binding protein levels.

Table (3-6): Correlations of the Biochemical parameters independent variables

Variables	Total antioxidant	Galectin 3 binding protein	Heat shock protein 70
Sample Volume	r= -0.4 p= **<0.001[S]**	r= 0.03 p= 0.748[NS]	r= 0.4 **p= <0.001[S]**
Concentration	r= 0.4 p= **<0.001[S]**	r= -0.5 p= 0.613[NS]	r= -0.3 **p= <0.001[S]**
Progressive cell	r= 0.8 p= **<0.001[S]**	r= -0.1 p= 0.844[NS]	r= -0.5 **p= <0.001[S]**
Non-progressive cell	r= 0.3 p= **0.018[S]**	r= -0.1 p= 0.163[NS]	r= -0.2 **p= 0.012[S]**
Nonmoving sperm	r= -0.7 p= **<0.001[S]**	r= -0.1 p= 0.470[NS]	r= 0.5 **p= <0.001[S]**

r: Pearson's correlation coefficient; S: significant; NS= Non significant

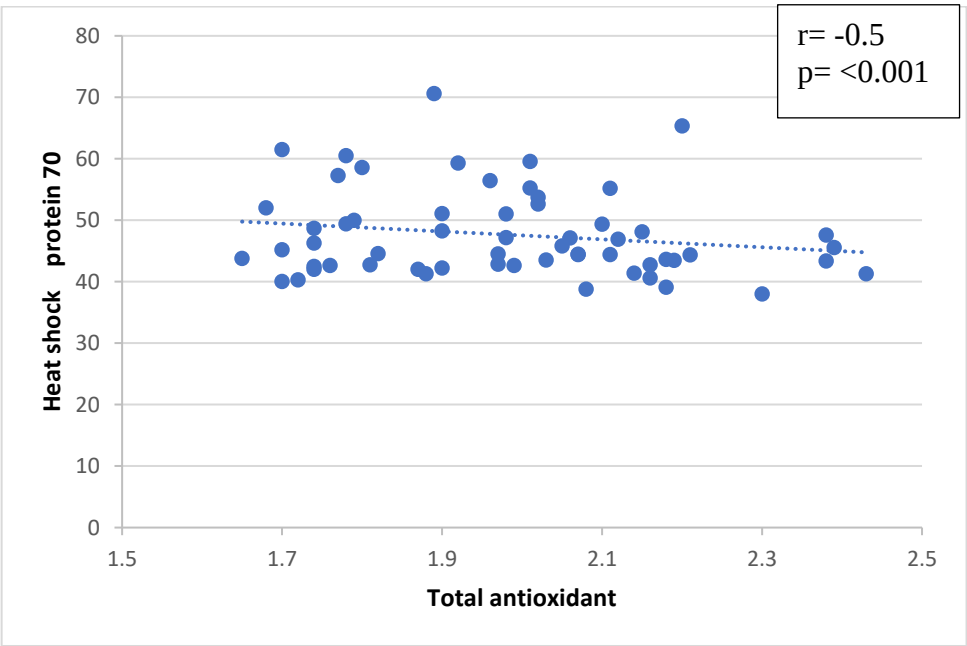


Figure (3-1) Simple linear regression Between Total anti-oxidant & Heat shock protein 70 levels of infertile men patients group

- **Total antioxidant**

Significantly higher levels of TAOS were observed in this study in men with control compared to men with infertility, suggesting a significant relationship between TAOS in the seminal plasma and the integrity of the cell membrane, viability, or sperm morphology, while other studies also report a positive correlation with sperm motility (Moraes; Meyers 2018) (164).

The results of the value of the TAOS in male semen obtained in this study are consistent with the results of other published. Importantly, this indicator was significant and positively correlated with the parameters of the semen function, such as sperm motility and concentration.

This study results are in agreement with other studies (Pahune *et al*., 2013) (165); (Fazeli *et al*., 2016) (166); (Krzyściak *et al*., 2020)(167) and a negative correlation with sample volume and non-moving sperm, As with studies(Aleksandra *et al*., 2004) (168); (Colagar *et al*., 2009) (169); (Mustafa *et al*., 2019) (125); (Waheed *et al*., 2018) (170) .

- **Heat shock protein 70**

Heat shock protein 70 are a family of housekeeping proteins that are constitutively expressed in cells to regulate various cellular pathways, such as transport, transcription, translation and signal transduction, under stressful cellular conditions, including exposure to heat, hypoxia and oxidative stress, as shown studying (Agarwal *et al*., 2012) (171); (Nixon *et al*., 2017) (172).

The result of Hsp70 significantly negative correlation values observed in total Sperm Concentration, and active motility in infertile males compared to Normospermia fertile males are similar to the work done in (Green KI, Nwachuku EO 2018) (173). and a positively correlation with sample Volume and Non-moving sperm and this result was confirmed by study (Colagar HA *et al*., 2013) (174).

- **Galectin 3 binding protein**

No significant Correlations were found with the Galectin 3 binding protein levels, confirmed by study (Kovak MR et al., 2014) (156).

3.8: Odds ratio

In table (3-7), TAOS and Hsp70 showed a significant association with both Oligoasthenozoospermia and Asthenozoospermea patient groups. Both markers were show to be a risk factor of infertile men patients groups (OR = 2.80 and 8.80) U/ml and (OR = 2.079 and 1.875) ng/L respectively, p value <0.001.

Correlation coefficient was used for determining linear relationships between among biochemical marker in patient's groups compare to control group. The results showed that there was weak non-significant correlation between GAL-3BP in patients compared to control group (OR = 1.000 and 1.017) ng/L. While, TAOS levels and Hsp70 were showed to be significantly negative correlated in infertile men patients group compared to control group (p <0.001).

Table 3.7: The odd ratio between the biomarkers and patients' groups

Variables	Studied Subjects	OR (95% CI)	p value
Total antioxidant (U/ml)	Control	**1[a]**	-
	Oligoasthenozoospermia	2.80 (2.806–2.806)	< **0.001 [S]**
	Asthenozoospermea	8.80 (2.799-2.772)	< **0.001 [S]**
Galectin 3 binding protein (ng/L)	Control	**1[a]**	-
	Oligoasthenozoospermia	1.000 (0.938-1.066)	0.998[NS]
	Asthenozoospermea	1.017(0.956-1.082)	0.593[NS]
Heat shock Protein 70 (ng/L)	Control	**1[a]**	-
	Oligoasthenozoospermia	2.079(1.584-2.730)	< **0.001 [S]**
	Asthenozoospermea	1.875(1.439-2.443)	< **0.001 [S]**

Results are presented [S]; Significant, [NS]; Non significant, OR: Odds Ratio, CI; Confidence Interval, 1[a]; reference category

- **Total anti-oxidant levels**

Decrease in antioxidant levels and an increase in oxidative stress damage could induce oxidative stress and causing sperm abnormalities impairing their potential for reproduction is considered one of the major sperm-damaging factors, as stated in a studies(Martin-Hidalgo *et al*., 2019) (175); (Panner Selvam *et al*., 2018) (176) .

The results of the table (3-9) regarding the severity of TAOS deficiency for infertile patients are in agreement with the above studies (Walczak–Jedrzejowska *et al*., 2013) which aim considered to cause abnormalities in sperm that impair their ability to reproduce. Therefore, this resulted in us a pathological condition represented by it (Oligoasthenozoospermia, Asthenozoospermea) (143).

- **Heat Shock Protein 70**

The Heat Shock Protein 70 level, where the note that its increase in (Oligoasthenozoospermia, Asthenozoospermea) in the case of the case indicates that the increase in the protein is positively associated with an increase in the risk factor of infertility. And there is what was confirmed by the study (Sisti G *et al*., 2015) That aims to increased expression of Hsp70 positively correlates with DNA damage in sperm (177).

- **Galectin 3 binding protein**

As for GAL-3BP, the results of the risk factor for the case of (Oligoasthenozoospermia, Asthenozoospermea) relative to the control group were very weak and not significant, confirmed by the study (Mei S *et al*., 2019) (137).

3.9: Receiver Operating Characteristic Analysis

- **ROC curve and AUC analysis for the H.S.P 70 and G.3.B.P for Patients compared to control group**

Results of the receiver operating curve (ROC) curve and AUC analysis for the Hsp70 and GAL-3BP diagnostic parameters. Hsp70 was shown a good performance for prediction patients compared to control group, data are presented in Figures (3.5) & (3.6) and table (3.8).

For Hsp70 levels: (sensitivity = 81.4%, specificity 90%) at a level = 42.1716, while GAL-3BP levels (sensitivity 49.2%, specificity 73.3%) at a level = 27.9172, the distribution of Hsp70 and GAL-3BP cut-off values was presented in tables (3.9) & (3.10). The p-values of the AUC were <0.05 and statistically significant.

Table (3.8): AUC, optimal threshold, Sensitivity and specificity of proposed marker obtained by the ROC curves for prediction of patients

Test Variable	Heat shock protein 70	GAL-3BP
AUC	89.8%	54%
Sensitivity %	81.4%	49.2%
Specificity %	90%	73.3%
You den index	0.714	0.225
Cut-off points	42.1716	27.9172
CI (95%)	0.841 – 0.955	0.432 – 0.647
PPV	83.33%	64.44%
NPV	66.67%	59.46%
P value	<0.001	0.011

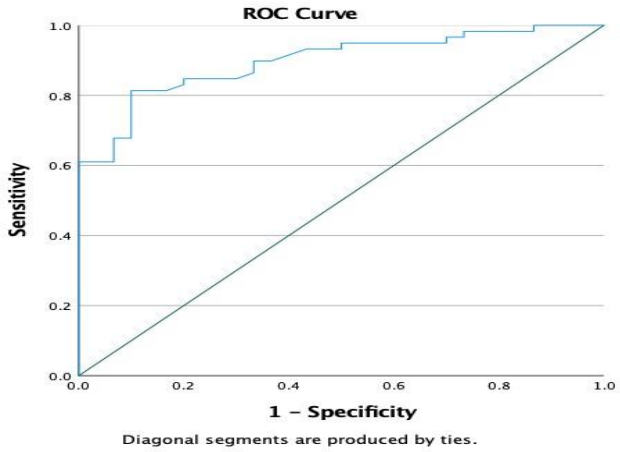


Figure 3.5: Receiver operating characteristics (ROC) curve analysis of Hsp70 levels in Patient and Control, The area under ROC curve: 89.8%. CI 0.841 - 0.955; p value <0.001

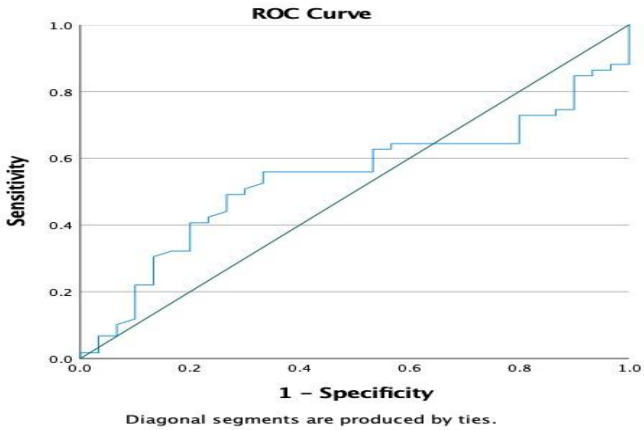


Figure 3.6: Receiver operating characteristics (ROC) curve analysis of GAL- 3 levels in Patient and Control, The area under ROC curve: 54%. CI 0.432 - 0.647; p value 0.45

Table (3.9) Distribution of patients according to the Hsp70 cut off values comparing with control group

Hsp70 cut off	Patients	Control
>42.1716	48	6
<42.1716	11	54
Total	59	60

Table (3.10) Distribution of patients according to the GAL-3BP cut off values comparing with control group

GAL-3BP cut-off	Patients	Control
>27.9172	29	16
<27.9172	30	44
Total	59	60

CHAPTER FOUR

conclusions and Recommendation

4. Conclusions and future work

4.1 Conclusions

- **Total anti-oxidant**

1. TAOS of semen of infertile men enrolled in the current study was less than that reported in fertile men and thus it can be inferred that levels of oxidative stress are higher than normal physiological levels are associated at least partially with male infertility.
2. The highest mean TAOS was observed in Oligoasthenozoospermia and the lowest was reported in Asthenozoospermea.
3. The TAOS levels showed a positive significant correlation with the concentration, progressive cell, and negative significant correlation with the sample volume, semen viscosity, and non-moving sperm cell.
4. TAOS showed a significant association with both Oligoasthenozoospermia and Asthenozoospermea patient groups. A low TAOS level has been shown to be a risk factor for male infertility groups.

- **Heat Shock Protein 70**

1. The Hsp70 of semen of infertile men enrolled in the current study was more than that reported in fertile men and thus it can be inferred that levels of Hsp70 that are higher than normal physiological levels are associated at least partially with male infertility.
2. The highest mean Hsp70 was observed in Oligoasthenozoospermia and the lower was reported in Asthenozoospermea.
3. The Hsp70 showed a negative significant correlation with the concentration, progressive cell, and positive significant correlation with the sample volume, semen viscosity, and non-moving sperm cell.

4. Hsp70 is significantly associated with the two groups of Oligoasthenozoospermia and Asthenozoospermea patients. A high Hsp70 level has been shown to be a risk factor for male infertility groups.

- **Galectin 3 binding protein**

1. The semen GAL-3BP of infertile men enrolled in the current study was slightly more than that reported in fertile men, and therefore it cannot be inferred that GAL-3BP levels as an indication of the presence of infertility.
2. By comparing between the Oligoasthenozoospermia and Asthenozoospermea, was found the GAL-3BP in Asthenozoospermea level to be slightly higher than that of the Oligoasthenozoospermia group, but no significant.
3. The GAL-3BP showed no significant correlation with the concentration, progressive cell, non- progressive cell, sample volume, semen viscosity, and Non-moving sperm cell.
4. No significant association of GAL-3BP with both Oligoasthenozoospermia and Asthenozoospermea patient groups. There is no risk factor for male infertility patient groups.

4.2 Recommendations

From present study, recommended the following:

1. Performing of a larger sample size and multicentre study in order to validate the results of the current study.
2. Conducting an empirical study that evaluates all possible sources and causes of oxidative stress and elevated heat shock proteins to explain exactly relationship between them.
3. In light of the previous data and the results of the current study and because of the lack of the relationship between oxidative stress and Galectin 3 binding protein due to their direct effect on fertility outcomes seems to be necessary to evaluate both marks individually when selecting the appropriate semen sample for assisted reproductive technology process.
4. Conducting an empirical study evaluating all possible sources and reasons for the occurrence of the two cases of (Oligoasthenozoospermia and Asthenozoospermea) and explaining the exact relationship between them.

CHAPTER FIVE

References

References

1. Lee, L.K. and Foo, K.Y., 2014. Recent insights on the significance of transcriptomic and metabolomic analysis of male factor infertility. Clinical biochemistry, 47(10-11), pp.973-982.

2. Agarwal, A., Mulgund, A., Hamada, A. and Chyatte, M.R., 2015. A unique view on male infertility around the globe. Reproductive biology and endocrinology, 13(1), pp.1-9.

3. Ritchie, C. and Ko, E.Y., 2021. Oxidative stress in the pathophysiology of male infertility. Andrologia, 53(1), p.e13581.

4. Adeoye, O., Olawumi, J., Opeyemi, A. and Christiania, O., 2018. Review on the role of glutathione on oxidative stress and infertility. JBRA assisted reproduction, 22(1), p.61.

5. Partyka, A. and Niżański, W., 2022. Advances in storage of poultry semen. Animal Reproduction Science, p.106921.

6. Mei, S., Chen, P., Lee, C.L., Zhao, W., Wang, Y., Lam, K.K., Ho, P.C., Yeung, W.S., Fang, C. and Chiu, P.C., 2019. The role of galectin-3 in spermatozoa-zona pellucida binding and its association with fertilization in vitro. Molecular human reproduction, 25(8), pp.458-470.

7. Lei, T., Blois, S.M., Freitag, N., Bergmann, M., Bhushan, S., Wahle, E., Huang, A.C.C., Chen, H.L., Hartmann, M.F., Wudy, S.A. and Liu, F.T., 2021. Targeted disruption of galectin 3 in mice delays the first wave of spermatogenesis and increases germ cell apoptosis. Cellular and Molecular Life Sciences, 78(7), pp.3621-3635.

8. Ma, F. and Luo, L., 2020. Genome-wide identification of Hsp70/110 genes in rainbow trout and their regulated expression in response to heat stress. PeerJ, 8, p.e10022.

9. Habib, H.N., Khudaier, B.Y. and Hassan, A.F., 2018. Molecular Detection of Polymorphism of Heat Shock Protein 70 (hsp70) in the Semen of Arabi Rams. Basrah Journal of Veterinary Research, 17(3).

10. Hiyama, G., Matsuzaki, M., Mizushima, S., Dohra, H., Ikegami, K., Yoshimura, T., Shiba, K., Inaba, K. and Sasanami, T., 2014. Sperm activation by heat shock protein 70 supports the migration of sperm released from sperm storage tubules in Japanese quail (Coturnix japonica). Reproduction, 147(2), pp.167-178.

11. Tamrakar, S.R. and Bastakoti, R., 2019. Determinants of infertility in couples. Journal of Nepal Health Research Council, 17(1), pp.85-89.

12. Amadi, A.K., 2020. The causes and pattern of presentation of male factor infertility as seen at Kenyatta National Hospital (Doctoral dissertation, University of Nairobi).

13. Zhaku, V., Sh, B., Beadini, N. and Murtezani, B., 2019. Combination of Maca, Korean ginseng extract and antioxidant therapy for male with oligoasthenozoospermia: case study. Journal of Hygienic Engineering and Design, 26(1), pp.28-35.

14. Ghuman, N. and Ramalingam, M., 2018. Male infertility. Obstetrics, Gynaecology & Reproductive Medicine, 28(1), pp.7-14.

15. Akilah Amira, A., Kabel Ahmed, M. and Alharthi Huda, A., 2017. New perspectives in male infertility. GSC Biological and Pharmaceutical Sciences, 1(3).

16. Akilah Amira, A., Kabel Ahmed, M. and Alharthi Huda, A., 2017. New perspectives in male infertility. GSC Biological and Pharmaceutical Sciences, 1(3).

17. Punab, M., Poolamets, O., Paju, P., Vihljajev, V., Pomm, K., Ladva, R., Korrovits, P. and Laan, M., 2017. Causes of male infertility: a 9-year prospective monocentre study on 1737 patients with reduced total sperm counts. Human reproduction, 32(1), pp.18-31.

18. Dimitriadis, F., Adonakis, G., Kaponis, A., Mamoulakis, C., Takenaka, A. and Sofikitis, N., 2017. Pre-testicular, testicular, and post-testicular causes of male infertility. Endocrinology of the testis and male reproduction. Cham: Springer, pp.1-47.

19. Bonyadi, M.R., Madaen, S.K. and Saghafi, M., 2013. Effects of varicocelectomy on anti-sperm antibody in patients with varicocele. Journal of reproduction & infertility, 14(2), p.73.

20. Restrepo, B. and Cardona-Maya, W., 2013. Antisperm antibodies and fertility association. Actas Urológicas Españolas (English Edition), 37(9), pp.571-578.

21. Kolanska, K., Alijotas-Reig, J., Cohen, J., Cheloufi, M., Selleret, L., D'argent, E., Kayem, G., Valverde, E.E., Fain, O., Bornes, M. and Darai, E., 2021. Endometriosis with infertility: a comprehensive review on the role of immune deregulation and immunomodulation therapy. American Journal of Reproductive Immunology, 85(3), p.e13384.

22. Leisegang, K. and Dutta, S., 2021. Do lifestyle practices impede male fertility?. Andrologia, 53(1), p.e13595.

23. Penzias, A., Bendikson, K., Butts, S., Coutifaris, C., Falcone, T., Gitlin, S., Gracia, C., Hansen, K., Jindal, S., Kalra, S. and Mersereau, J., 2018. Smoking and infertility: a committee opinion. Fertility and sterility, 110(4), pp.611-618.

24. Asare-Anane, H., Bannison, S.B., Ofori, E.K., Ateko, R.O., Bawah, A.T., Amanquah, S.D., Oppong, S.Y., Gandau, B.B.N. and Ziem, J.B., 2016. Tobacco smoking is associated with decreased semen quality. Reproductive Health, 13(1), pp.1-6.

25. Sharma, R., Harlev, A., Agarwal, A. and Esteves, S.C., 2016. Cigarette smoking and semen quality: a new meta-analysis examining the effect of the 2010 World Health Organization laboratory methods for the examination of human semen. European urology, 70(4), pp.635-645.

26. Jensen, T.K., Swan, S., Jørgensen, N., Toppari, J., Redmon, B., Punab, M., Drobnis, E.Z., Haugen, T.B., Zilaitiene, B., Sparks, A.E. and Irvine, D.S., 2014. Alcohol and male reproductive health: a cross-sectional study of 8344 healthy men from Europe and the USA. Human reproduction, 29(8), pp.1801-1809.

27. Leisegang, K. and Dutta, S., 2021. Do lifestyle practices impede male fertility?. Andrologia, 53(1), p.e13595.

28. Aboulmaouahib, S., Madkour, A., Kaarouch, I., Sefrioui, O., Saadani, B., Copin, H., Benkhalifa, M., Louanjli, N. and Cadi, R., 2018. Impact of alcohol and cigarette smoking consumption in male fertility potential: Looks at lipid peroxidation, enzymatic antioxidant activities and sperm DNA damage. Andrologia, 50(3), p.e12926.

29. Chang, R.C., Skiles, W.M., Chronister, S.S., Wang, H., Sutton, G.I., Bedi, Y.S., Snyder, M., Long, C.R. and Golding, M.C., 2017. DNA methylation-independent growth restriction and altered developmental programming in a mouse model of preconception male alcohol exposure. Epigenetics, 12(10), pp.841-853.

30. Gunes, S., Hekim, G.N.T., Arslan, M.A. and Asci, R., 2016. Effects of aging on the male reproductive system. Journal of assisted reproduction and genetics, 33(4), pp.441-454.

31. Rato, L., Alves, M.G., Cavaco, J.E. and Oliveira, P.F., 2014. High-energy diets: a threat for male fertility?. Obesity Reviews, 15(12), pp.996-1007.

32. Karayiannis, D., Kontogianni, M.D., Mendorou, C., Douka, L., Mastrominas, M. and Yiannakouris, N., 2017. Association between adherence to the Mediterranean diet and semen quality parameters in male partners of couples attempting fertility. Human reproduction, 32(1), pp.215-222.

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

34. Durairajanayagam, D., Agarwal, A. and Ong, C., 2015. Causes, effects and molecular mechanisms of testicular heat stress. Reproductive biomedicine online, 30(1), pp.14-27.

35. Epstein, Y. and Moran, D.S., 2006. Thermal comfort and the heat stress indices. Industrial health, 44(3), pp.388-398.

36. Hamerezaee, M., Dehghan, S.F., Golbabaei, F., Fathi, A., Barzegar, L. and Heidarnejad, N., 2018. Assessment of semen quality among workers exposed to heat stress: a cross-sectional study in a Steel Industry. Safety and health at work, 9(2), pp.232-235.

37. Mima, M., Greenwald, D. and Ohlander, S., 2018. Environmental toxins and male fertility. Current urology reports, 19(7), pp.1-8.

38. Leisegang, K. and Dutta, S., 2021. Do lifestyle practices impede male fertility?. Andrologia, 53(1), p.e13595.

39. Barratt, C.L., Björndahl, L., De Jonge, C.J., Lamb, D.J., Osorio Martini, F., McLachlan, R., Oates, R.D., van der Poel, S., St John, B., Sigman, M. and Sokol, R., 2017. The diagnosis of male infertility: an analysis of the evidence to support the development of global WHO guidance—challenges and future research opportunities. Human reproduction update, 23(6), pp.660-680.

40. Schlegel, P.N., Sigman, M., Collura, B., De Jonge, C.J., Eisenberg, M.L., Lamb, D.J., Mulhall, J.P., Niederberger, C., Sandlow, J.I., Sokol, R.Z. and Spandorfer, S.D., 2021. Diagnosis and treatment of infertility in men: AUA/ASRM guideline part II. The Journal of Urology, 205(1), pp.44-51.

41. Chimote, N., 2018. Semen Analysis. Donald School Textbook of Human Reproductive & Gynecological Endocrinology, p.292.

42. Leyser-Whalen, O., Bombach, B., Mahmoud, S. and Greil, A.L., 2022. From generalist to specialist: A qualitative study of the perceptions of infertility patients. Reproductive Biomedicine & Society Online, 14, pp.204-215.

43. Sancho, S. and Vilagran, I., 2013. The boar ejaculate: sperm function and seminal plasma analyses. In Boar Reproduction (pp. 471-516). Springer, Berlin, Heidelberg.

44. Qiu, Y., Yang, H., Li, C. and Xu, C., 2020. Progress in research on sperm DNA fragmentation. Medical science monitor: international medical journal of experimental and clinical research, 26, pp.e918746-1.

45. Boitrelle, F., Shah, R., Saleh, R., Henkel, R., Kandil, H., Chung, E., Vogiatzi, P., Zini, A., Arafa, M. and Agarwal, A., 2021. The sixth edition of the WHO manual for human semen analysis: a critical review and SWOT analysis. Life, 11(12), p.1368.

46. Henkel, R., 2021. The sixth edition of the WHO manual for human semen analysis: A critical review and SWOT analysis.

47. Agarwal, A., Majzoub, A., Baskaran, S., Selvam, M.K.P., Cho, C.L., Henkel, R., Finelli, R., Leisegang, K., Sengupta, P., Barbarosie, C. and Parekh, N., 2020. Sperm DNA fragmentation: a new guideline for clinicians. The world journal of men's health, 38(4), p.412.

48. Boitrelle, F., Shah, R., Saleh, R., Henkel, R., Kandil, H., Chung, E., Vogiatzi, P., Zini, A., Arafa, M. and Agarwal, A., 2021. The sixth edition of the WHO manual for human semen analysis: a critical review and SWOT analysis. Life, 11(12), p.1368.

49. Sabeti, P., Pourmasumi, S., Rahiminia, T., Akyash, F. and Talebi, A.R., 2016. Etiologies of sperm oxidative stress. International Journal of Reproductive Biomedicine, 14(4), p.231..

50. Thompson, A., Agarwal, A. and Du Plessis, S.S., 2013. Physiological role of reactive oxygen species in sperm function: a review. Antioxidants in male infertility: a guide for clinicians and researchers. New York, USA: Springer Science and Business Media, pp.69-89.

51. Martin-Hidalgo, D., Bragado, M.J., Batista, A.R., Oliveira, P.F. and Alves, M.G., 2019. Antioxidants and male fertility: from molecular studies to clinical evidence. Antioxidants, 8(4), p.89.

52. Cho, C.-L. and Agarwal, A. Role of sperm DNA fragmentation in male factor infertility: A systematic review. Arab J. Urol. 2018, 16, 21–34. [CrossRef].

53. Selvam, M.K.P. and Agarwal, A., 2018. A systematic review on sperm DNA fragmentation in male factor infertility: laboratory assessment. Arab Journal of Urology, 16(1), pp.65-76.

54. Kumar, N. and Singh, A.K., 2018. Reactive oxygen species in seminal plasma as a cause of male infertility. Journal of gynecology obstetrics and human reproduction, 47(10), pp.565-572.

55. Villaverde, Ana Izabel Silva Balbin, Jacob Netherton, and Mark A. Baker, 2019."From past to present: the link between reactive oxygen species in sperm and male infertility." Antioxidants 8.12, 616.

56. Ahmad, G., Almasry, M., Dhillon, A.S., Abuayyash, M.M., Kothandaraman, N. and Cakar, Z., 2017. Overview and sources of reactive oxygen species (ROS) in the reproductive system. In Oxidative stress in human reproduction (pp. 1-16). Springer, Cham.

57. Al-Mamary, M.A. and Moussa, Z., 2021. Antioxidant activity: The presence and impact of hydroxyl groups in small molecules of natural and synthetic origin. Antioxidants—Benefits, Sources, Mechanisms of Action, pp.318-377.

58. Kapoor, D., Singh, S., Kumar, V., Romero, R., Prasad, R. and Singh, J., 2019. Antioxidant enzymes regulation in plants in reference to reactive oxygen species (ROS) and reactive nitrogen species (RNS). Plant Gene, 19, p.100182.

59. Nowicka-Bauer, K. and Nixon, B., 2020. Molecular changes induced by oxidative stress that impair human sperm motility. Antioxidants, 9(2), p.134.

60. Agarwal, A., Durairajanayagam, D., Halabi, J., Peng, J. and Vazquez-Levin, M., 2014. Proteomics, oxidative stress and male infertility. Reproductive biomedicine online, 29(1), pp.32-58.

61. Garrido, N., Meseguer, M., Simon, C., Pellicer, A. and Remohi, J., 2004. Pro-oxidative and anti-oxidative imbalance in human semen and its relation with male fertility. Asian journal of andrology, 6(1), pp.59-66.

62. Kumar, N. and Singh, A.K., 2018. Reactive oxygen species in seminal plasma as a cause of male infertility. Journal of gynecology obstetrics and human reproduction, 47(10), pp.565-572.

63. Dutta, S., Sengupta, P., Slama, P. and Roychoudhury, S., 2021. Oxidative stress, testicular inflammatory pathways, and male reproduction. International Journal of Molecular Sciences, 22(18), p.10043.

64. Saleh, S.R., Manaa, A., Sheta, E., Ghareeb, D.A. and Abd-Elmonem, N.M., 2022. The Synergetic Effect of Egyptian Portulaca oleracea L.(Purslane) and Cichorium intybus L.(Chicory) Extracts against Glucocorticoid-Induced Testicular Toxicity in Rats through Attenuation of Oxidative Reactions and Autophagy. Antioxidants, 11(7), p.1272.

65. Selvam, M.K.P., Agarwal, A., Sharma, R., Samanta, L., Gupta, S., Dias, T.R. and Martins, A.D., 2021. Protein fingerprinting of seminal plasma reveals dysregulation of exosome-associated proteins in infertile men with unilateral varicocele. The World Journal of Men's Health, 39(2), p.324.

66. Ahmed, O.M. and Mohammed, M.T., 2020. Oxidative stress: The role of reactive oxygen species (ROS) and antioxidants in human diseases. Plant Arch, 20, pp.4089-4095.

67. Varesi, A., Chirumbolo, S., Campagnoli, L.I.M., Pierella, E., Piccini, G.B., Carrara, A., Ricevuti, G., Scassellati, C., Bonvicini, C. and Pascale, A., 2022. The role of antioxidants in the interplay between oxidative stress and senescence. antioxidants, 11(7), p.1224.

68. Baskaran, S., Finelli, R., Agarwal, A. and Henkel, R., 2021. Reactive oxygen species in male reproduction: a boon or a bane?. Andrologia, 53(1), p.e13577.

69. Wang, R., Li, H., Sun, J., Zhang, L., Jiao, J., Wang, Q. and Liu, S., 2021. Nanomaterials facilitating microbial extracellular electron transfer at interfaces. Advanced Materials, 33(6), p.2004051.

70. Harchegani, Asghar Beigi, et al. "Effects of zinc deficiency on impaired spermatogenesis and male infertility: the role of oxidative stress, inflammation and apoptosis." Human Fertility (2018)..

71. Thompson, A., Agarwal, A., & Du Plessis, S. S. (2013). Physiological role of reactive oxygen species in sperm function: a review. Antioxidants in male infertility: a guide for clinicians and researchers. New York, USA: Springer Science and Business Media : s.n. 69-89.

72. Harchegani, A.B., Dahan, H., Tahmasbpour, E. and Shahriary, A., 2018. Effects of zinc deficiency on impaired spermatogenesis and male infertility: the role of oxidative stress, inflammation and apoptosis. Human Fertility.

73. Fujii, J. and Tsunoda, S., 2011. Redox regulation of fertilisation and the spermatogenic process. Asian Journal of Andrology, 13(3), p.420.

74. Thompson, A., Agarwal, A. and Du Plessis, S.S., 2013. Physiological role of reactive oxygen species in sperm function: a review. Antioxidants in male infertility: a guide for clinicians and researchers. New York, USA: Springer Science and Business Media, pp.69-89.

75. Dutta, S., Majzoub, A. and Agarwal, A., 2019. Oxidative stress and sperm function: A systematic review on evaluation and management. Arab journal of urology, 17(2), pp.87-97.

76. Maitan, P., Bromfield, E.G., Stout, T.A., Gadella, B.M. and Leemans, B., 2021. A stallion spermatozoon's journey through the mare's genital tract: In vivo and in vitro aspects of sperm capacitation. Animal Reproduction Science, p.106848.

77. Agarwal, A., Virk, G., Ong, C. and Du Plessis, S.S., 2014. Effect of oxidative stress on male reproduction. The world journal of men's health, 32(1), pp.1-17.

78. *Sun, C., Lv, T., Huang, L., Liu, X., Jin, C. and Lin, X., 2020. Melatonin ameliorates aluminum toxicity through enhancing aluminum exclusion and reestablishing redox homeostasis in roots of wheat. Journal of Pineal Research, 68(4), p.e12642.*

79. Aitken, R.J., Baker, M.A. and Nixon, B., 2015. Are sperm capacitation and apoptosis the opposite ends of a continuum driven by oxidative stress?. Asian Journal of Andrology, 17(4), p.633.

80. Aitken, R.J., 2017. Reactive oxygen species as mediators of sperm capacitation and pathological damage. Molecular reproduction and development, 84(10), pp.1039-1052.

81. Atshan, M., Kakavand, K., Hosseini, S.H., Sadighi Gilani, M.A., Mohseni Meybodi, A. and Sabbaghian, M., 2020. Evaluation of sperm DNA fragmentation and chromatin structure in infertile men with immotile short-tail sperm defect. Andrologia, 52(1), p.e13445.

82. Khosrowbeygi, A. and Zarghami, N., 2007. Fatty acid composition of human spermatozoa and seminal plasma levels of oxidative stress biomarkers in subfertile males. Prostaglandins, Leukotrienes and Essential Fatty Acids, 77(2), pp.117-121.

83. Dutta, S., Majzoub, A. and Agarwal, A., 2019. Oxidative stress and sperm function: A systematic review on evaluation and management. Arab journal of urology, 17(2), pp.87-97.

84. Aitken, R.J., Gibb, Z., Baker, M.A., Drevet, J. and Gharagozloo, P., 2016. Causes and consequences of oxidative stress in spermatozoa. Reproduction, Fertility and Development, 28(2), pp.1-10.

85. Adewoyin, M., Ibrahim, M., Roszaman, R., Md Isa, M.L., Mat Alewi, N.A., Abdul Rafa, A.A. and Anuar, M.N.N., 2017. Male infertility: the effect of natural antioxidants and phytocompounds on seminal oxidative stress. Diseases, 5(1), p.9.

86. Agarwal, A., Parekh, N., Selvam, M.K.P., Henkel, R., Shah, R., Homa, S.T., Ramasamy, R., Ko, E., Tremellen, K., Esteves, S. and Majzoub, A., 2019. Male oxidative stress infertility (MOSI): proposed terminology and clinical practice guidelines for management of idiopathic male infertility. The world journal of men's health, 37(3), pp.296-312.

87. Gualtieri, R., Kalthur, G., Barbato, V., Longobardi, S., Di Rella, F., Adiga, S.K. and Talevi, R., 2021. Sperm oxidative stress during in vitro manipulation and its effects on sperm function and embryo development. Antioxidants, 10(7), p.1025.

88. Saleh, S.R., Manaa, A., Sheta, E., Ghareeb, D.A. and Abd-Elmonem, N.M., 2022. The Synergetic Effect of Egyptian Portulaca oleracea L.(Purslane) and Cichorium intybus L.(Chicory) Extracts against Glucocorticoid-Induced Testicular Toxicity in Rats through Attenuation of Oxidative Reactions and Autophagy. Antioxidants, 11(7), p.1272.

89. Bardaweel, S.K., Gul, M., Alzweiri, M., Ishaqat, A., ALSalamat, H.A. and Bashatwah, R.M., 2018. Reactive oxygen species: The dual role in physiological and pathological conditions of the human body. The Eurasian journal of medicine, 50(3), p.193.

90. Gao, S., Li, C., Chen, L. and Zhou, X., 2017. Actions and mechanisms of reactive oxygen species and antioxidative system in semen. Molecular & Cellular Toxicology, 13(2), pp.143-154.

91. Saleh, S.R., Manaa, A., Sheta, E., Ghareeb, D.A. and Abd-Elmonem, N.M., 2022. The Synergetic Effect of Egyptian Portulaca oleracea L.(Purslane) and Cichorium intybus L.(Chicory) Extracts against Glucocorticoid-Induced Testicular Toxicity in Rats through Attenuation of Oxidative Reactions and Autophagy. Antioxidants, 11(7), p.1272.

92. Baskaran, S., Finelli, R., Agarwal, A. and Henkel, R., 2021. Reactive oxygen species in male reproduction: a boon or a bane?. Andrologia, 53(1), p.e13577.

93. Sharma, D., Martineau, C.N., Le Dall, M.T., Reidy, M., Masison, D.C. and Kabani, M., 2009. Function of SSA subfamily of Hsp70 within and across species varies widely in complementing Saccharomyces cerevisiae cell growth and prion propagation. PloS one, 4(8), p.e6644.

94. Wang, X., Zhang, Y., Fei, S., Awais, M.M., Zheng, H., Feng, M. and Sun, J., 2021. Heat Shock Protein 75 (TRAP1) facilitate the proliferation of the Bombyx mori nucleopolyhedrovirus. International Journal of Biological Macromolecules, 175, pp.372-378.

95. Kim, J.H. and Kang, J.C., 2016. The immune responses and expression of metallothionein (MT) gene and heat shock protein 70 (HSP 70) in juvenile rockfish, Sebastes schlegelii, exposed to waterborne arsenic (As3+). Environmental Toxicology and Pharmacology, 47, pp.136-141.

96. Khamis, I. and Heikkila, J.J., 2013. Enhanced HSP30 and HSP70 accumulation in Xenopus cells subjected to concurrent sodium arsenite and cadmium chloride stress. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 158(3), pp.165-172.

97. Kampinga, H.H. and Craig, E.A., 2010. The HSP70 chaperone machinery: J proteins as drivers of functional specificity. Nature reviews Molecular cell biology, 11(8), pp.579-592.

98. Khan, S., Jabeen, R., Deeba, F., Waheed, U., Khanum, P. and Iqbal, N., 2021. Heat shock proteins: classification, functions and expressions in plants during environmental stresses. Journal of Bioresource Management, 8(2), p.9.

99. Pockley, A.G., Muthana, M. and Calderwood, S.K., 2008. The dual immunoregulatory roles of stress proteins. Trends in biochemical sciences, 33(2), pp.71-79.

100. Huusko, J.M., Tiensuu, H., Haapalainen, A.M., Pasanen, A., Tissarinen, P., Karjalainen, M.K., Zhang, G., Christensen, K., Ryckman, K.K., Jacobsson, B. and Murray, J.C., 2021. Integrative genetic, genomic and transcriptomic analysis of heat shock protein and nuclear hormone receptor gene associations with spontaneous preterm birth. Scientific reports, 11(1), pp.1-13.

101. Arnos, A.A., Tameemi, W.F.A. and Ali, N.M., 2018. Evaluation of heat shock protein 70 (HSP-70) & heat shock protein 90 (HSP-90) in diffuse large B-cell non-Hodgkin's lymphoma patients. Hematol Transfus Int J, 6(4), pp.125-128.

102. Yadav, A., Singh, J., Ranjan, K., Kumar, P., Khanna, S., Gupta, M., Kumar, V., Wani, S.H. and Sirohi, A., 2020. Heat shock proteins: master players for heat-stress tolerance in plants during climate change. Heat stress tolerance in plants: physiological, molecular and genetic perspectives, pp.189-211.

103. Vostakolaei, M.A., Hatami-Baroogh, L., Babaei, G., Molavi, O., Kordi, S. and Abdolalizadeh, J., 2021. Hsp70 in cancer: A double agent in the battle between survival and death. Journal of cellular physiology, 236(5), pp.3420-3444.

104. Liu, Q., Liang, C. and Zhou, L., 2020. Structural and functional analysis of the Hsp70/Hsp40 chaperone system. Protein Science, 29(2), pp.378-390.

105. Bracher, A. and Verghese, J., 2015. GrpE, Hsp110/Grp170, HspBP1/Sil1 and BAG domain proteins: nucleotide exchange factors for Hsp70 molecular chaperones. The Networking of Chaperones by Co-chaperones, pp.1-33.

106. Urashima, T., Hirabayashi, J., Sato, S. and Kobata, A., 2018. Human milk oligosaccharides as essential tools for basic and application studies on galectins. Trends in Glycoscience and Glycotechnology, 30(172), pp.SE51-SE65.

107. Mei, S., Chen, P., Lee, C.L., Zhao, W., Wang, Y., Lam, K.K., Ho, P.C., Yeung, W.S., Fang, C. and Chiu, P.C., 2019. The role of galectin-3 in spermatozoa-zona pellucida binding and its association with fertilization in vitro. Molecular human reproduction, 25(8), pp.458-470.

108. Hong, M.H., Lin, W.H., Weng, I.C., Hung, Y.H., Chen, H.L., Chen, H.Y., Chen, P., Lin, C.H., Yang, W.Y. and Liu, F.T., 2020. Intracellular galectins control cellular responses commensurate with cell surface carbohydrate composition. Glycobiology, 30(1), pp.36-48.

109. Arciniegas, E., Carrillo, L.M., Rojas, H., Ramírez, R. and Chopite, M., 2019. Galectin-1 and galectin-3 and their potential binding partners in the dermal thickening of keloid tissues. The American Journal of Dermatopathology, 41(3), pp.193-204.

110. Kim, C.H., 2022. Glycobiology in Innate Immunology. Nature Springer Publishing, Co.: Singapore.

111. Salvagno, G.L. and Pavan, C., 2016. Prognostic biomarkers in acute coronary syndrome. Annals of translational medicine, 4(13).

112. Romero, A. and Gabius, H.J., 2019. Galectin-3: is this member of a large family of multifunctional lectins (already) a therapeutic target?. Expert Opinion on Therapeutic Targets, 23(10), pp.819-828.

113. Suthahar, N., Meijers, W.C., Silljé, H.H., Ho, J.E., Liu, F.T. and de Boer, R.A., 2018. Galectin-3 activation and inhibition in heart failure and cardiovascular disease: an update. Theranostics, 8(3), p.593.

114. Ou, S.M., Tsai, M.T., Chen, H.Y., Li, F.A., Lee, K.H., Tseng, W.C., Chang, F.P., Lin, Y.P., Yang, R.B. and Tarng, D.C., 2022. Urinary Galectin-3 as a Novel Biomarker for the Prediction of Renal Fibrosis and Kidney Disease Progression. Biomedicines, 10(3), p.585.

115. Peixoto, A., Relvas-Santos, M., Azevedo, R., Santos, L.L. and Ferreira, J.A., 2019. Protein glycosylation and tumor microenvironment alterations driving cancer hallmarks. Frontiers in oncology, 9, p.380.

116. Al Attar, A., Antaramian, A. and Noureddin, M., 2021. Review of galectin-3 inhibitors in the treatment of nonalcoholic steatohepatitis. Expert Review of Clinical Pharmacology, 14(4), pp.457-464.

117. World Health Organization, 2010. Reference values and semen nomenclature. WHO Laboratory Manual for the Examination and Processing of Human Semen,, pp.223-225.

118. Zhang, M., Zhang, Q.S., Zheng, H.S., Wang, X.Y., Feng, S.Q., Tian, W.J.and Fan, H.T.(2016) _Clinical, demographic and psychological characteristics of infertile male smokers in Northeast China', Journal of International Medical Research, 44(1), pp.75-80.

119. Alshahrani, S., Agarwal, A., Assidi, M., Abuzenadah, A.M.,Durairajanayagam, D., Ayaz, A., Sharma, R. and Sabanegh, E.(2014) _Infertile men older than 40 years are at higher risk of sperm DNA damage',Reproductive Biology and Endocrinology, 12(1), pp.1-9.

120. Öztekin, Ü., Caniklioğlu, M., Sarı, S., Selmi, V., Gürel, A. and Işıkay, L., (2019) _ Evaluation of male infertility prevalence with clinical outcomes in middle Anatolian region', Cureus, 11(7).

121. Epstein, Y., & Moran, D. S. (2006). Thermal comfort and the heat stress indices. Industrial Health, 44(3), 388–398.

122. Hamerezaee, M., Dehghan, S. F., Golbabaei, F., Fathi, A., Barzegar, L., &Heidarnejad, N. (2018). Assessment of semen quality among workersexposed to heat stress: A cross-sectional study in a steel industry. Safety and Health at Work, 9(2), 232–235.

123. Wang, C., & Swerdloff, R. S. (2014). Limitations of semen analysis as a test of male fertility and anticipated needs from newer tests. Fertility and Sterility, 102(6), 1502–1507.

124. Gatimel, N., Moreau, J., Parinaud, J. and Léandri, R.D., 2017. Sperm morphology: assessment, pathophysiology, clinical relevance, and state of the art in 2017. Andrology, 5(5), pp.845-862.

125. Mustafa, H.A., Majid, H.H., Abdulqader, A.T., Mustafa, M.A. and Salih, A.A., 2019. Study On Some Physiological, Biochemical And Hormonal Parameters Of Seminal Fluid Of Infertile Men. Biochem. Cell. Arch, 19(Supplement 1), pp.1943-1947.

126. Abdulrahman, S.J., AL-Ali, I.A., Hassan, T.A.A. and Mohammed, A., 2018. INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES.

127. Öztekin, Ü., Caniklioğlu, M., Sarı, S., Selmi, V., Gürel, A. and Işıkay, L., 2019. Evaluation of male infertility prevalence with clinical outcomes in middle Anatolian region. Cureus, 11(7).

128. Punab, M., Poolamets, O., Paju, P., Vihljajev, V., Pomm, K., Ladva, R., Korrovits, P. and Laan, M., 2017. Causes of male infertility: a 9-year prospective monocentre study on 1737 patients with reduced total sperm counts. Human reproduction, 32(1), pp.18-.

129. Katib, A.(2015) _Mechanisms linking obesity to male infertility', Central European journal of urology, 68(1), p.79.

130. Aitken, R.J. and Drevet, J.R., 2020. The importance of oxidative stress in determining the functionality of mammalian spermatozoa: a two-edged sword. Antioxidants, 9(2), p.111.

131. Aitken, R.J. and Baker, M.A., 2020. The role of genetics and oxidative stress in the etiology of male infertility—a unifying hypothesis?. Frontiers in endocrinology, 11, p.581838.

132. Nowicka-Bauer, K. and Nixon, B., 2020. Molecular changes induced by oxidative stress that impair human sperm motility. Antioxidants, 9(2), p.134.

133. Yurchuk, T.O., Pavlovich, O.V., Gapon, G.O., Pugovkin, A.Y. and Petrushko, M.P., 2021. Lipid peroxidation and DNA fragmentation in fresh and cryopreserved spermatozoa of men at different spermatogenesis state. Ukr. Biochem. J, 93, pp.24-29.

134. Aworu, A.M., Ben-Chioma, A., Elekima, I., Brown, H. and Bartimaeus, E.A.S., Pattern of Some Sperm Proteins, Anti-oxidants, Testosterone, and Prostate-Specific Antigen of Infertile Males with Sperm Cell Deformities in Port Harcourt, Rivers State.

135. Ferlin, A., Speltra, E., Patassini, C., Pati, M.A., Garolla, A., Caretta, N. and Foresta, C., 2010. Heat shock protein and heat shock factor expression in sperm: relation to oligozoospermia and varicocele. The Journal of urology, 183(3), pp.1248-1252.

136. Agarwal, A., Hamada, A. and Esteves, S.C., 2012. Insight into oxidative stress in varicocele-associated male infertility: part 1. Nature Reviews Urology, 9(12), pp.678-690.

137. Mei, S., Chen, P., Lee, C.L., Zhao, W., Wang, Y., Lam, K.K., Ho, P.C., Yeung, W.S., Fang, C. and Chiu, P.C., 2019. The role of galectin-3 in spermatozoa-zona pellucida binding and its association with fertilization in vitro. Molecular human reproduction, 25(8), pp.458-470.

138. Davalieva, K., Kiprijanovska, S., Noveski, P., Plaseski, T., Kocevska, B. and Plaseska-Karanfilska, D., 2012. Human seminal plasma proteome study: a search for male infertility biomarkers. Balkan Journal of Medical Genetics: BJMG, 15(Suppl), p.35.

139. Giacomini, E., Ura, B., Giolo, E., Luppi, S., Martinelli, M., Garcia, R.C. and Ricci, G., 2015. Comparative analysis of the seminal plasma proteomes of oligoasthenozoospermic and normozoospermic men. Reproductive biomedicine online, 30(5), pp.522-531.

140. Martin-Hidalgo, D., Bragado, M.J., Batista, A.R., Oliveira, P.F. and Alves, M.G., 2019. Antioxidants and male fertility: from molecular studies to clinical evidence. Antioxidants, 8(4), p.89.

141. Cho, C.L. and Agarwal, A., 2018. Role of sperm DNA fragmentation in male factor infertility: A systematic review. Arab journal of urology, 16(1), pp.21-34.

142. Selvam, M.K.P. and Agarwal, A., 2018. A systematic review on sperm DNA fragmentation in male factor infertility: laboratory assessment. Arab Journal of Urology, 16(1), pp.65-76.

143. Walczak–Jedrzejowska, R., Wolski, J.K. and Slowikowska–Hilczer, J., 2013. The role of oxidative stress and antioxidants in male fertility. Central European journal of urology, 66(1), p.60.

144. Alahmar, A. T., Calogero, A. E., Sengupta, P., & Dutta, S. (2021). Coenzyme Q10 improves sperm parameters, oxidative stress markers and sperm DNA fragmentation in infertile patients with idiopathic oligoasthenozoospermia. The world journal of men's health.

145. Abdulrahman, S.J., AL-Ali, I.A., Hassan, T.A.A. and Mohammed, A., 2018. INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES.

146. Palani, A.F. and Asdallh, N.S., 2019. Effects of Low Seminal Plasma Antioxidant Potential on Semen Quality and Male Fertility. Passer Journal of Basic and Applied Sciences, 1(1), pp.4-7.

147. Krisfalusi, M., Miki, K., Magyar, P.L. and O'Brien, D.A., 2006. Multiple glycolytic enzymes are tightly bound to the fibrous sheath of mouse spermatozoa. Biology of reproduction, 75(2), pp.270-278.

148. Aworu, A. M., Ben-Chioma, A., Elekima, I., Brown, H., & Bartimaeus, E. A. S.2022. Pattern of Some Sperm Proteins, Anti-oxidants, Testosterone, and Prostate-Specific Antigen of Infertile Males with Sperm Cell Deformities in Port Harcourt, Rivers State.

149. Ferlin, A., Speltra, E., Patassini, C., Pati, M. A., Garolla, A., Caretta, N., & Foresta, C. (2010). Heat shock protein and heat shock factor expression in sperm: relation to oligozoospermia and varicocele. The Journal of urology, 183(3), 1248-1252.

150. Waheed, M.M., El-Bahr, S.M. and Al-Haider, A.K., 2013. Influence of seminal plasma antioxidants and osteopontin on fertility of the Arabian horse. Journal of Equine Veterinary Science, 33(9), pp.705-709.

151. Cedenho, A. P., Lima, S. B., Cenedeze, M. A., Spaine, D. M., Ortiz, V., & Oehninger, S. (2006). Oligozoospermia and heat-shock protein expression in ejaculated spermatozoa. Human Reproduction, 21(7), 1791-1794.

152. Wu, Y., Yuan, Y., Chen, L., Wang, M., Yang, Y., Wang, Y., Quan, C., Chen, D., Chen, Y., Huang, X. and Zhou, T., 2019. Quantitative proteomic analysis of human seminal plasma from normozoospermic and asthenozoospermic individuals. BioMed research international, 2019.

153. Bracke, A., Peeters, K., Punjabi, U., Hoogewijs, D. and Dewilde, S., 2018. A search for molecular mechanisms underlying male idiopathic infertility. Reproductive biomedicine online, 36(3), pp.327-339.

154. Fung, K.Y., Glode, L.M., Green, S. and Duncan, M.W., 2004. A comprehensive characterization of the peptide and protein constituents of human seminal fluid. The prostate, 61(2), pp.171-181.

155. Tsuruya, S., Tsuda, R., Ikematsu, K., Matsumoto, H., Kagawa, S. and Nakasono, I., 2006. Homology between ABH-carrier α2-seminoglycoprotein and Mac-2 binding protein. Legal Medicine, 8(5), pp.273-278.

156. Bair EL, Nagle RB, Ulmer TA, Laferté S, Bowden GT. 90K/Mac-2 binding protein is expressed in prostate cancer and induces promatrilysin expression. Prostate 2006 Feb 15 and 66(3):283-93.

157. Kovak MR, Saraswati S, Schoen DJ, Diekman AB. Investigation of galectin-3 function in the reproductive tract by identification of binding ligands in human seminal plasma. Am J Reprod Immunol 2014 and 72(4):403-412.

158. Overstreet JW, Coats C, Katz DF, Hanson FW: The importance of seminal plasma for sperm penetration of human cervical mucus. Fertil Steril 1980 and 34:569–572.

159. Layali, I., Tahmasbpour, E., Joulaei, M., Jorsaraei, S.G.A. and Farzanegi, P., 2015. Total antioxidant capacity and lipid peroxidation in semen of patient with hyperviscosity. Cell Journal (Yakhteh), 16(4), p.554.

160. Schallmoser, A., Bakjaji, F., Königsberger, S., John, J., Färber, C., Schmidt, E., Breitenbach-Koller, H., Allam, J.P., Verguts, J. and Sänger, N., 2021. Effect of mild α-chymotrypsin treatment of highly viscous semen samples on fertilization rates. Translational Andrology and Urology, 10(1), p.448.

161. Siciliano, L., Tarantino, P., Longobardi, F., Rago, V., De Stefano, C. and Carpino, A., 2001. Impaired seminal antioxidant capacity in human semen with hyperviscosity or oligoasthenozoospermia. Journal of andrology, 22(5), pp.798-803.

162. Harchegani, A.B., Rahmani, H., Tahmasbpour, E. and Shahriary, A., 2019. Hyperviscous semen causes poor sperm quality and male infertility through induction of oxidative stress. Current urology, 13(1), pp.1-6.

163. Curtis, D.A. and Araki, C.J., 2003. Whatever Happened to Exploratory Data Analysis? An Evaluation of Behavioral Science Statistics Textbooks.

164. Moraes, C.R. and Meyers, S., 2018. The sperm mitochondrion: Organelle of many functions. Animal reproduction science, 194, pp.71-80.

165. Pahune, P.P., Choudhari, A.R. and Muley, P.A., 2013. The total antioxidant power of semen and its correlation with the fertility potential of human male subjects. Journal of clinical and diagnostic research: JCDR, 7(6), pp.991-995.

166. Fazeli, F. and Salimi, S. Correlation of Seminal Plasma Total Antioxidant Capacity and Malondialdehyde Levels With Sperm Parameters in Men With Idiopathic Infertility. Avicenna J. Med. Biochem. 2016. [CrossRef].

167. Krzyściak, W., Papież, M., Bąk, E., Morava, E., Krzyściak, P., Ligęzka, A., ... & Janeczko, J. (2020). Sperm antioxidant biomarkers and their correlation with clinical condition and lifestyle with regard to male reproductive potential. Journal of clinical.

168. Aleksandra, K. and Slawomir, K. and Ewa, B. (2004),Values of malondialdehyde in human seminal plasma. Progress in Med Res., 2, 1-10.

169. Colagar, A. H., Pouramir, M., Marzony, E. T., & Jorsaraei, S. G. A. (2009). Relationship between seminal malondialdehyde levels and sperm quality in fertile and infertile men. Brazilian Archives of Biology and Technology, 52, 1387-1392.

170. Waheed, Magdi M., Ahmad MA Meligy, and Suror A. Dhalam. "The relationship between seminal plasma and serum trace elements and semen parameters of dromedary camels (Camelus dromedarius)." Reproduction in Domestic Animals 53.6 (2018): 1367-1374.

171. Agarwal, A., Hamada, A., & Esteves, S. C. (2012). Insight into oxidative stress in varicocele-associated male infertility: part 1. Nature Reviews Urology, 9(12), 678-690.

172. Nixon, B., Bromfield, E. G., Cui, J., & Iuliis, G. N. D. (2017). Heat shock protein A2 (HSPA2): regulatory roles in germ cell development and sperm function. The Role of Heat Shock Proteins in Reproductive System Development and Function, 67-93.

173. Green KI, Nwachuku EO. Seminal Analysis as a Tool to Determine the Infertility Prevalence among Men Reported to Infertility Clinic in Port Harcourt. Asian Journal of Medicine and Health. 2018 and 11(1):1-6.

174. Colagar HA, Karimi F, Jorsaraei AGS. Correlation of Sperm Parameters with Semen lipid peroxidation and total antioxidants levels in astheno- and oligoasheno- teratospermic men. Iranian Red Crescent Medical Journal. 2013: 15(9): 780 - 785.

175. Martin-Hidalgo, D.; Bragado, M.J.; Batista, A.R.; Oliveira, P.F.; Alves, M.G, 2019 Antioxidants and Male Fertility:From Molecular Studies to Clinical Evidence. Antioxidants, 8.

176. Panner Selvam, M.K. and Agarwal, A, 2018 .A systematic review on sperm DNA fragmentation in male factorinfertility: Laboratory assessment. Arab J. Urol., 16, 65–76.

177. Sisti G, Kanninen TT, Ramer I, Witkin SS, 2015 Interaction between the inducible 70-kDa heat shock protein and autophagy: effects on fertility and pregnancy. Cell Stress Chaperones 20(5):753-8.

Appendix

Name:	Phone No.		File No.:
Age (years):	Weight (Kg):	Height (m^2):	BMI (Kg/m^2):
Occupation:			
Residency (rural, urban):			Environment (Pollutions):
Infertility Period: Infertility Type (1° or 2°):			
Cause of Infertility: -			
Male Factor:	Female Factor:	Combined:	Unexplained:
Male Factor: -			
Asthenozoospermia:	Oligoasthenozoospermia:	Other:	
History of IUI or IVF: Outcome:			
Medical History: -	Chronic Disease:	Drugs: / Mumps:/ Others:	
Surgical History: -			
Varicocele :-	Side (Lt., Rt., Bilateral):		Grade:
Hydrocele:	Hernia:	Orchiopexy:	Others:
Smoking: -			
Alcohol			
SFA: (separate paper)			

Figure 2.1: Detailed questionnaire taken from the patients

المـلخــص

من الواضح أن سلامة الحيوانات المنوية أمر بالغ الأهمية بالنسبة لنقل المواد الوراثية بشكل صحيح إلى الأجيال القادمة وسلامة الحيوانات المنوية المسؤولة عن الإخصاب. في حين أن أنواع الأكسجين التفاعلية هي جزيئات إشارات مهمة في العمليات الفسيولوجية، فقد تلعب أيضًا دورًا في العمليات المرضية التي تؤثر على الجهاز التناسلي والتكاثر إذا كانت موجودة بكميات زائدة.

كان الغرض الرئيسي من هذه الدراسة هو تحديد المؤشرات الحيوية التي تساعد في تشخيص ومراقبة القدرة الإنجابية للذكور. أجرينا تقييم مضادات الأكسدة في البلازما المنوية وبروتين الصدمة الحرارية 70 وبروتين ربط ال Galectin-3والحالة السريرية ونمط حياة الأشخاص الذين يعانون من مشاكل في الإنجاب.

تم إجراء دراسة مراقبة الحالة على 119 عينة من السائل المنوي، و31 رجلاً يعانون من استسقاء النطاف، و28 رجلاً يعانون من قلة النطاف، في حين أن 60 آخرين يتمتعون بصحة جيدة. تم جمع عينات السائل المنوي من المرضى العراقيين الذين أتو إلى عيادات العقم لتشخيص العقم وتقنيات الإنجاب المساعدة في كربلاء للفترة من تشرين الثاني 2021 إلى أيار 2022.

تم وضع إجابات جميع المشاركين على الاستبيان على ورقة بيانات وإعطائهم رقم هوية تسلسلي. تم منع الأخطاء باستخدام إدخالات متعددة. تم استخدام الحزمة الإحصائية للعلوم الاجتماعية، الإصدار 28.0، (IBM، SPSS، شيكاغو، إلينوي، الولايات المتحدة الأمريكية). لإنشاء تحليل البيانات لهذا المشروع تم تشغيل الإحصائيات الوصفية على بيانات المشاركين من كل مجموعة. تم استخدام متوسط SDلتوضيح قيم المتغيرات المستمرة. تم استخدام اختبار شابيرو لفحص توزيع البيانات. تم استخدام نسب الأرجحية (ORs) ونطاق فاصل الثقة بنسبة 95٪، والتي تم حسابها باستخدام أي انحدار لوجستي مشروط، لتقدير العلاقة بين المكونات التي تم فحصها. تم استخدام الاختبارات الإحصائية التحليلية لتأكيد وجود اختلافات معنوية في المتغيرات الفئوية بين المعلمات. تم اعتبار نتائج كل اختبار فرضية بقيمة p ثنائية الجانب تبلغ 0.05 أو أقل ذات دلالة إحصائية

أظهر متوسط مستوى مضادات الأكسدة الكلية للسائل المنوي أعلى مستوى (3.53 ± 0.64 وحدة / مل) في المجموعة الضابطة، وأدنى مستوى ارتبط مع قلة النطاف (1.88 ± 0.15 نانوجرام / لتر). كما أظهرت نتائج دراستنا أن متوسط مستوى بروتين الصدمة الحرارية 70 الأدنى لوحظ في حالة المجموعة الضابطة (40.17 ± 1.94 نانوجرام / لتر) وأعلى مستوى ارتبط مع قلة النطاف (7.39 ± 50.02 نانوجرام / لتر). أما بالنسبة لمتوسط معدل بروتين ربط Galectin-3فلم يكن ذا دلالة إحصائية بين المجموعة الضابطة و Asthenozoospermeaو Oligoasthenozoospermia، ارتبط بروتين الصدمة الحرارية 70 مع حالة الأكسدة الكلية للسائل المنوي ارتباط عكسي معنوي.

توجد علاقة ارتباط بين بروتين الصدمة الحرارية 70 ومضادات الأكسدة الكلية للسائل المنوي وهذا يدل على أهمية قياس كل منهما في حالات العقم وعدم الرضا عن أحدهما على الآخر.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء
كلية الطب
فرع الكيمياء والكيمياء الحياتية

تقييم بروتين Galectin-3 الملزم وبروتين الصدمة الحرارية 70 وعلاقتهما بالعلامات الحيوية المضادة للأكسدة في عينة من الرجال العراقيين

رسالة ماجستير

إلى مجلس كلية الطب/ فرع الكيمياء والكيمياء الحياتية/ جامعة كربلاء كجزء من متطلبات نيل درجة الماجستير في الكيمياء السريرية

من قبل

احمد عبد الجليل سعيد

بكالوريوس كلية العلوم فرع الكيمياء جامعة كربلاء 2010 - 2011

إشراف

أ. م. د. شيماء زهراو ندا
كلية الطب / جامعة كربلاء

أ.د. وسن غازي
كلية الطب / جامعة كربلاء

2022

1444 هجـ