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THE EFFECT OF IL-8 AND IGF-1 LEVELS ON DNA FRAGMENTATION IN SEMEN AND THE STANDARD SPERM PARAMETERS

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THE EFFECT OF IL-8 AND IGF-1 LEVELS ON DNA FRAGMENTATION IN SEMEN AND THE STANDARD SPERM PARAMETERS

By Mohammed Noaman Hamid HAMID

July 2023

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

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ABSTRACT

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July 2023

Since many studies have demonstrated that there is an influence on several elements of DNA fragmentation in sperm, we will discuss the effects of interleukin 8 and IGF-1 in this study. This study was conducted at Kamal Al-Samarrai Hospital in Baghdad, where 120 random samples were selected from patients to reveal the type of infertility and the reasons that were medically approved in this field. The fundamental principle of the test was carried out using a commonly available kit and the quantitative sandwich enzyme immunoassay method. The kit we are employing is based on the Enzyme-Linked Immunosorbent Assay (ELISA), a technology based on a biotin double antibody sandwich. The wells had previously been pre-coated with an IL-8 monoclonal antibody. After adding IL-8 to the wells, the plates were incubated. Next, biotin-tagged IL-8 antibodies were added so they could combine with streptavidin-HPR to form an immunological complex. The plates were then washed to remove any unbound enzymes. This was done to make sure that there were no unattached enzymes. As a result, the objective of this research was to investigate the viability of employing the insulin-like growth factor I (IGF-1) complex in order to improve the quality of sperm that had previously been incubated.

2023, 83 pages

Keywords: Fragmentation, IL-8, IGF-1, Sperm

ÖZET

IL-8 VE IGF-1 DÜZEYLERİNİN SEMENDEKİ DNA FRAGMENTASYONU VE STANDART SPERM PARAMETRELERİ ÜZERİNDEKİ ETKİSİ

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Birçok çalışma spermdeki DNA parçalanmasının çeşitli unsurları üzerinde bir etki olduğunu gösterdiği için, bu çalışmada interlökin 8 ve IGF-1'in etkilerini tartışacağız. Bu çalışma, Bağdat'taki Kamal Al-Samarrai Hastanesi'nde, infertilite türünü ortaya çıkarmak ve bu alanda tıbbi olarak onaylanmış nedenleri tespit etmek için hastalardan rastgele 120 örnek seçilerek gerçekleştirilmiştir. Testin temel prensibi, yaygın olarak bulunan bir kit ve kantitatif sandviç enzim immunoassay yöntemi kullanılarak gerçekleştirilmiştir. Kullanmakta olduğumuz kit, bir biyotin çift antikor sandviçine dayalı bir teknoloji olan Enzim Bağlantılı İmmünosorbent Testine (ELISA) dayanmaktadır. Kuyucuklar önceden bir IL-8 monoklonal antikoru ile önceden kaplanmıştır. Kuyucuklara IL-8 eklendikten sonra plakalar inkübe edilmiştir. Daha sonra, bir immünolojik kompleks oluşturmak üzere streptavidin-HPR ile birleşebilmeleri için biyotin etiketli IL-8 antikorları eklenmiş, plakalar daha sonra herhangi bir bağlanmamış enzimi çıkarmak için yıkanmıştır. Bu işlem, bağlanmamış enzim olmadığından emin olmak için yapılmıştır. Sonuç olarak, bu araştırmanın amacı, daha önce inkübe edilmiş sperm kalitesini artırmak için insülin benzeri büyüme faktörü I (IGF-1) kompleksi kullanmanın uygulanabilirliğini araştırmak olmuştur.

2023, 83 sayfa

Anahtar Kelimeler: Fragmentasyon, IL-8, IGF-1, Sperm

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Mohammed Noaman Hamid HAMID

Çankırı-2023

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LIST OF SYMBOLS

°C	Degree celsius
min	Minute
%	Percentage
±	Plus, minus

LIST OF ABBREVIATIONS

Bcl-2	B-cell lymphoma 2
DFI	DNA fragmentation index
EIF4E	Eukaryotic translation initiation factor 4E
FACS	Fluorescence-activated cell sorter
FSH	Follicle-stimulating hormone
GPCRs	G protein-coupled receptors
GSH	Glutathione (glutamic acid, cysteine and glycine)
GRO	Growth-related oncogenes
HPLC	High-performance liquid chromatography
IGF-1	Insulin-like growth factor
IL-8	Interleukin 8
ICSI	Intracytoplasmic sperm injection
IVF	In vitro fertilisation
LSD	Least significant difference
LFA-1	Lymphocyte function-associated antigen 1
MDA	Malondialdehyde
OAT	Oligoasthenoteratozoospermia
OS	Oxidative stress
PF4	Platelet factor four
PCO	Polycystic ovary
ROS	Reactive oxygen species
SFA	Seminal fluid analysis
SDF	Sperm DNA fragmentation
SOD	Superoxide dismutase
TESA	Testicular sperm aspiration
TESE	Testicular sperm extraction
WBC	White blood cells
WHO	World health organization

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1. INTRODUCTION

1.1 Interleukin 8

Macrophages, as well as other types of cells like epithelial cells, smooth muscle cells in the airways, and endothelial cells, produce IL-8, a type of chemokine. It is also known as chemokine (C-X-C motif) ligand 8 (CXCL8). Other names for IL-8 include CXCL8 and chemokine (C-X-C motif) ligand 8 (Murphy 2019). Endothelial cells store their IL-8 reserves in structures called Weibel-Palade bodies, which are vesicles used for storage (El-Mansi and Nightingale 2021). The CXCL8 gene in humans is responsible for the production of the protein known as IL-8. IL-8 begins its life as a precursor peptide made up of 99 amino acids. This peptide is cleaved in order to produce a variety of active isoforms of IL-8, each of which has a specific function (Pan *et al.* 2020). The most common form that is secreted by macrophages when they are grown in culture is a peptide that contains 72 amino acids (García-Burgos *et al.* 2020).

The forms of IL-8 receptors on the surface membrane that are known as G protein-coupled serpentine receptors CXCR1 and CXCR2, respectively, are the ones that receive the most attention (Avazi *et al.* 2019). The expression of the two receptors as well as their affinity for IL-8 are distinct from one another (CXCR1 has a higher affinity than CXCR2). IL-8 is an important immune response mediator that is created as a byproduct of a series of metabolic events and is then secreted into the body. This process takes place during the innate immune system response (Zha *et al.* 2020).

IL-8, which is also known as the neutrophil chemotactic factor, serves two purposes. These functions are as follows (Czepiel *et al.* 2018). It does this by stimulating a process called chemotaxis, which causes the target cells, most of which are neutrophils but also other granulocytes, to move closer to the site of the infection. Once they arrive at their destination, IL-8 is in charge of stimulating phagocytosis. In addition, it is common knowledge that interleukin-8 acts as a powerful stimulator of angiogenesis. Ca^{2+} levels inside the cell rise, and the process of exocytosis (such as histamine release) begins

(Gonzalez-Aparicio and Alfaro 2020). Among other physiological reactions, the respiratory burst is one of the physiological effects that IL-8 has on target cells. Both migration and phagocytosis need these responses to work properly, and both of these processes need IL-8 (Hafez *et al.* 2021).

CR^{2+} naive T cells, which are sometimes referred to as "recent thymic emigrants," are the cells responsible for the production of the signature chemokine known as IL-8. Any cell involved in the innate immune response that possesses toll-like receptors has the potential to release this chemokine (Olmos-Ortiz *et al.* 2019). In the majority of instances, macrophages are the first cells to recognize the presence of an antigen. As a consequence of this, macrophages are also the first cells to produce IL-8 in order to recruit other cells. It has been proven that IL-8, in both its monomer and homodimer forms, is an effective inducer of the chemokine receptors CXCR1 and CXCR2. This was done by studying the interaction between the two forms of IL-8. Even though methylating the Leu25 residue can stop the homodimer from working, this change can actually make the homodimer more powerful. There is a widespread belief that IL-8 has a role in the development of bronchiolitis, a condition of the respiratory tract that is typically brought on by a viral infection (Bohmwald *et al.* 2019).

IL-8 is a member of the CXC chemokine family, which also includes other chemokines. This gene, along with the genes that code for the other eleven members of the CXC chemokine family, is located in a cluster on chromosome 4q. This cluster also contains the CXC chemokine gene cluster.

1.2 CXCL-8 Mediated Chemotaxis of the Neutrophil

Chemotaxis refers to the movement of neutrophils toward the site of an injury or infection. CXCL8 is the primary cytokine that is involved in this process. In order for neutrophil chemotaxis to be successful, several factors need to be present. These factors include an increased expression of high-affinity adhesion molecules, which are responsible for securing the neutrophil to the endothelium close to the affected site (and preventing it from being washed away into the circulatory system). Additionally, the neutrophil needs to be able to digest its way through the basement membrane and the extracellular matrix (ECM) to reach the affected site. Inducing the necessary cell signaling to bring about these changes is dependent on CXCL8, which plays a crucial role in this process (Bohmwald *et al.* 2019).

To begin, the release of histamine at the site of infection causes vasodilation of the capillaries near the injured area (Abazari *et al.* 2022). This slows blood flow in the region and encourages leukocytes, such as neutrophils, to migrate closer to the endothelium and away from the center of the lumen, which is where blood flow is at its quickest. When this takes place, there is a formation of tepid interactions between the selections that are expressed on neutrophils and endothelial cells (whose expression is also increased through the action of CXCL8 and other cytokines). Seins of the type L are located on neutrophils, whereas seins of the types P and E are located on endothelial cells. As a direct consequence of this, chemotaxis moves into the "rolling" phase (Zaferani *et al.* 2021).

When a neutrophil moves along the endothelium, it comes into contact with a CXCL8 molecule that is expressed on the surface (Buffone *et al.* 2019). This interaction stimulates the cell signaling pathway by way of a G-coupled protein receptor. When CXCL8 binds to CXCR1/2 on neutrophils, it stimulates the expression of an integrin called LFA-1. This integrin helps ICAM-1 receptors on the endothelium form high-affinity bonds. LFA-1 expression and affinity are significantly increased so that the amount of binding can be maximized (Persson *et al.* 2019). Because of this, the neutrophil's rate of movement is slowed even further, and eventually it comes to a complete stop. Another significant function that CXCL8 stimulation of cell signaling triggers is the start of the oxidative burst. This process allows proteolytic enzymes and reactive oxygen species (ROS) to build up. Both are needed for the breakdown of the extracellular matrix (ECM) and the basement membrane (Grosche *et al.* 2018). Secretory granules are responsible for the release of these as well as additional integrins. The release of reactive oxygen species (ROS) and enzymes that are damaging to the host is controlled to reduce the amount of damage done to the host; however, ROS and damaging enzymes still make it to the site of infection to carry out their effector functions (Maldonado *et al.* 2020).

1.3 The Effect of Interleukin 8 on DNA

Striking a balance between cell creation and cell death is crucial to keeping the body's cell count within ranges that are deemed physiologically normal (Kent *et al.* 2020). An increase in the number of hematopoietic effector cells is one of the distinguishing characteristics of inflammatory disorders. The accumulation of cells may have been brought on by either an increase in the rate of cell production or a reduction in the rate of cell death (Tang *et al.* 2021). Apoptosis, which is characterized by a series of distinct morphological changes in the dying cell, is the most common type of physiologic cell death. These changes take place in the dying cell. In inflammation, it is generally accepted that there is an increase in the production of new cells; however, the significance of a decreased rate of apoptosis is not yet fully understood (Śledziński *et al.* 2018).

It is unknown how survival and death factors affect apoptosis in vivo, despite the fact that these factors can modulate apoptosis in neutrophils in vitro. In addition, the intracellular mechanisms that these factors utilize in order to regulate apoptosis in neutrophils are still a mystery (Zhang *et al.* 2021). Although it is evident that neutrophils express members of the Bcl-2 and caspase families, the functional role that these families may play as potential apoptosis regulators in these inflammatory effector cells has not been investigated in vivo or ex vivo (Dostert *et al.* 2019).

The Bcl-2 family contains members that can function either as anti-apoptotic or pro-apoptotic proteins. In apoptotic pathways, the protein known as Bax plays the role of a death agonist (Dostert *et al.* 2019). Bax is a member of the Bcl-2 family. It has been demonstrated that the formation of heterodimers between Bcl-2 and Bax inhibits the pro-apoptotic activity of Bax. It has been shown that neutrophils express Bax but not Bcl-2; therefore, it is likely that neutrophils contain functional Bax homodimers, which are at least partially responsible for the short lifespan of neutrophils. Bcl-2 is known to be expressed in neutrophils. The liberation of cytochrome c from mitochondria, possibly through the formation of a channel, and the subsequent activation of the caspase cascade may be the mechanisms through which Bax's pro-apoptotic activity is mediated (Wolf *et al.* 2022).

In this study, we present evidence that several neutrophilic inflammatory diseases are characterized by delayed neutrophil apoptosis. There was a correlation between delayed neutrophils, increased expression of neutrophil survival factors, and significantly lower levels of Bax within the cells (Gray *et al.* 2018). Through the use of specific Bax antisense oligodeoxynucleotides, it was directly demonstrated that Bax plays an important role in the regulation of neutrophil apoptosis. As a consequence of this, cytokine-mediated reduction of intracellular Bax levels appears to be a key mechanism in many inflammatory disorders, which ultimately results in neutrophil expansion (Ge *et al.* 2021).

1.4 Effect of IGF-1 on Sperm DNA

Although the connection between growth hormone, and more specifically insulin-like growth factor 1 (IGF-1), and testicular development and spermatogenesis has been thoroughly investigated, there is a dearth of information regarding either the hepatic or the paracrine forms of IGF-1 (Olojede *et al.* 2022). IGF-1 is essential for the process of spermatogenesis. The use of frozen and thawed samples of buffalo, yak, canine, and ovine sperm, for instance, resulted in increased levels of the growth factor IGF-1, which is associated with spermatozoa motility, mitochondrial membrane potential, and oocyte cleavage rate (Santos *et al.* 2022).

Oligoasthenoteratozoospermia is a complicated condition that is marked by low sperm count (oligozoospermia), poor sperm movement (asthenozoospermia), and abnormal sperm shape (Monteiro *et al.* 2018). Oligoasthenoteratozoospermia (OAT) is a condition that affects male fertility (teratozoospermia). Oligoasthenoteratozoospermia (OAT) is a condition that affects male fertility (teratozoospermia). IGF-I has been shown to stimulate the maturation of spermatozoa, increase sperm motility, and significantly correlate with total sperm count (Mora Rodríguez *et al.* 2019). These findings suggest that IGF-1 treatment may improve sperm quality. For the objective of this study, we examined the impact of IGF-1 on sperm parameters in a patient with primary infertility and severe OAT (Kups *et al.* 2022).

Recent research conducted by Jenkins *et al.* (2018) focused on DNA methylation, chromatin remodeling, histone modifications, and sperm RNAs when reviewing the epigenetics of male fertility. They found out, to their surprise, that there is a lack of information known about DNA methylation and a lack of information known about sperm RNAs. Very little is known about sperm RNAs, despite the fact that a number of studies have investigated how the methylation of sperm DNA affects male fertility and the number of sperm produced.

It has been discovered that human spermatozoa contain thousands of different RNAs, both coding and non-coding. These cells contain RNAs that most likely derive from unmethylated or hypomethylated genes (Vrba and Futscher 2018). These genes are accessible to the transcriptional apparatus and serve as indicators of the activity of sperm transcription. H19 and IGF2 are the differentially methylated genes that have received the most research attention, and it has been discovered that infertile patients have different patterns of gene methylation than control subjects do. H19 is the genetic code for a non-coding RNA that plays a role in the inhibition of IGF-1R expression, and IGF-2 is the genetic code for the homonymous growth factor insulin-like growth factor 2 (IGF-2) (Cannarella *et al.* 2022). Both of them are located on chromosome 11p15.5, which is significant because it is the location of the first pattern of imprinted genes ever discovered (Noordermeer and Feil 2020).

H19 is a gene that is passed down through the maternal line and is expressed by the maternal allele, whereas IGF-2 is a gene that is passed down through the paternal line (meaning that it is expressed by the paternal allele). Enhancers that are downstream of H19 are shared by H19 and IGF-2 gene expression. Upstream of the H19 gene is a region known as the H19 differentially methylated region (H19 DMR), which controls their transcription. In the maternal allele, the H19 DMR does not have any methylation. This prevents access to the IGF2 enhancer, which in turn enables the expression of H19 while simultaneously inhibiting IGF-2. On the other hand, the H19 in the paternal allele has been methylated. The expression of IGF-2 is increased as a result, while the expression of H19 is decreased (Yamaguchi *et al.* 2019).

The H19 DMR is largely unmethylated in human fetal spermatogonia, but in mature spermatogonia, it is heavily methylated. This difference is seen only in humans. According to the findings of experimental research, the rate of H19 DMR methylation affects the human sperm count. This is shown by the fact that sperm from people with oligozoospermia methylate at a slower rate than sperm from people with normal sperm counts. However, the mechanism that is responsible for this effect is not currently known (Barbieri and Kouzarides 2020).

There has been no investigation into whether the H19 and IGF2 genes are expressed at the sperm level or whether sperm count is correlated with their level of transcription. These queries have not both been looked into (Le *et al.* 2019).

1.5 DNA Fragmentation in Sperm

The quality of sperm is often used as an indirect indicator of male infertility. According to the World Health Organization (WHO), the ejaculate volume, the sperm concentration, the motility, and the morphology of the sperm are the most important parameters that are evaluated in infertility centers as part of routine semen analysis (Feferkorn *et al.* 2022). The genetic make-up of a newborn is the result of the information contained in both the oocyte and the sperm, and this information needs to be unaltered for the subsequent stages of embryonic and fetal development to result in a healthy offspring (Innocenzi *et al.* 2019). The reproductive process can be hampered by the presence of any type of damage in the DNA of either the male or female gametes. It is the most common cause of paternal DNA anomaly transmission to progeny and is found in a high percentage of spermatozoa from men who are subfertile or infertile (Boguenet *et al.* 2021). Sperm DNA fragmentation can be found in men who are subfertile or infertile. A number of hypotheses have been proposed regarding the molecular mechanism that is responsible for the fragmentation of sperm DNA (Cecchele *et al.* 2022). Of these, apoptosis, abnormal chromatin packaging, and reactive oxygen species are considered to be the most important. Numerous studies have demonstrated that spermatozoa with DNA fragmentation are capable of fertilizing an oocyte; however, these spermatozoa are linked to abnormal embryo quality, a halt in the development of the blastocyst, and lower pregnancy rates, regardless of whether the fertilization took place naturally or through the assisted reproductive technologies of intrauterine insemination (IUI), In vitro fertilization (IVF), or Intracytoplasmic sperm injection (ICSI) (Kopp *et al.* 2023). It has been demonstrated in several studies that oocytes and embryos maintain the ability to repair DNA damage that may be present in the genome of the father. However, it is not known whether all types of DNA damage can be repaired (Schumacher *et al.* 2021). For instance, it would appear that breaks in double-stranded DNA are less repairable than breaks in single-stranded DNA, and as a consequence, they have a greater influence on the quality of embryos and/or the development of embryos. In addition, an oocyte's maturity level, the age of its mother, and the environment all have an impact on its ability to repair DNA damage (Aitken 2022). This review provides a concise summary of the factors that contribute to sperm DNA fragmentation, including their relationship to seminal parameters and paternal age, as well as the impact these factors have on assisted reproduction procedures (Dahan *et al.* 2021).

1.6 Human Sperm Chromatin Structure

Germ cells are absolutely necessary to the survival of the human race as they enable the transmission of genetic information from one generation to the next. The production of highly specialized sperm cells is the result of spermatogenesis, which is a process that is ongoing and meticulously regulated (Cheng *et al.* 2022). This process involves extremely noticeable cellular, genetic, and chromatin changes that lead to the production of sperm cells (Figure 1.1) (Kejnovsky and Jedlicka 2022). Spermatogonial stem cells divide, differentiate into primary spermatocytes, and then undergo genetic recombination to create round haploid spermatids (Ibtisham and Honaramooz 2020). The process of spermiogenesis, which involves significant cellular, epigenetic, and chromatin remodeling, is then carried out on the rounded spermatids that have been produced (Sharma *et al.* 2019). After the nucleosomes and histones have been disassembled, the highly positively charged protamines form compact toroidal complexes. These complexes are responsible for the organization of 85–95% of the human sperm DNA (Gaikwad *et al.* 2019). In human spermatozoa, we find two distinct forms of the amino acid protamine (P1 and P2). The chromatin of human sperm is less stable than that of other organisms because it has fewer thiol groups available for disulfide bonding (Dos Santos Hamilton and Assumpção 2020). In conclusion, as the cysteines travel through the epididymis, they gradually undergo oxidation, which results in the formation of inter- and intraprotamine disulfide bonds (Le Blévec *et al.* 2020). These bonds, in conjunction with zinc bridges, serve to completely compact and stabilize the chromatin. Mammalian DNA is the most condensed form of eukaryotic DNA because of the total of all of these interactions, which enables it to adjust to the incredibly restricted space of the sperm nucleus (Greenberg and Bourc'his 2019).

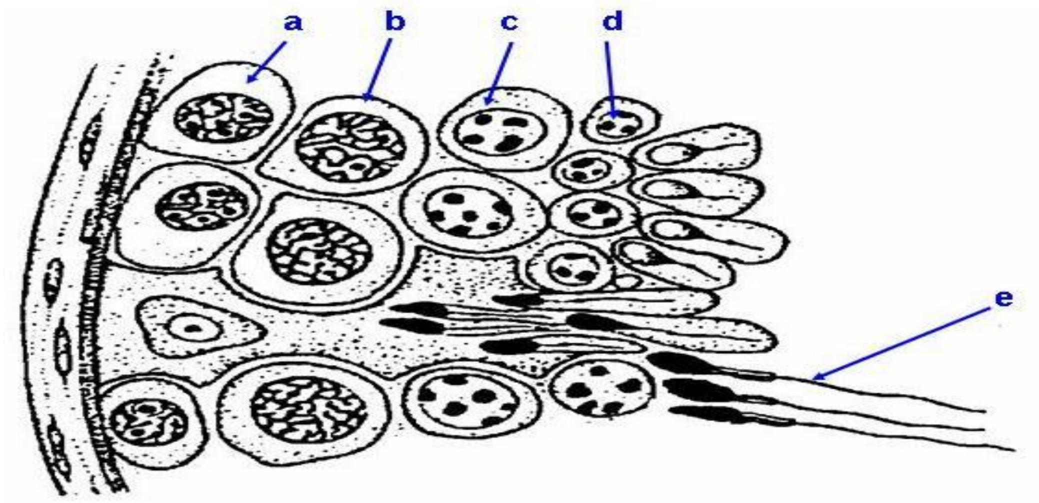

Figure 1.1 Espermatogenesis. The following are listed in order: (a) spermatogonia (2n); (b) primary spermatocytes (2n); (c) secondary spermatocytes (n); (d) spermatids (n); and (e) spermatozoa

The organization of chromatin is essential during both the process of fertilization and the early stages of embryonic development (Kresoja-Rakic and Santoro 2019). Crystalline, insoluble, tightly packed, and well-organized, sperm chromatin is made up of 27 kilobytes of DNA loop domains. Sperm chromatin is a well-organized structure (Abdelhedi *et al.* 2019). These loops are connected to the nuclear matrix at their bases, which can be observed using fluorescent in situ hybridization (FISH). During the process of sperm decondensation, the DNA maintains its attachment to the base of the tail, which suggests the presence of a structure in human sperm that is analogous to the nuclear annulus. This DNA organization not only permits the transfer of the extremely securely packaged genetic information to the egg but also guarantees that the DNA is delivered in a physical and chemical form that the developing embryo can access (Krimsky 2019).

1.7 Causes of DNA Fragmentation

Apoptosis, defects in chromatin remodeling during spermiogenesis, and oxygen radical-induced DNA damage can all cause sperm DNA fragmentation (Dutta *et al.* 2020).

1.8 Apoptosis

It is possible to keep track of the germ cell population that Sertoli cells will maintain during the process of spermiogenesis thanks to the apoptosis process. Additionally, this process enables the regulation of sperm cell overproduction and the elimination of abnormal cells (Pedrana *et al.* 2021). Apoptosis of sperm is mediated by proteins of the type Fas, and the concentration of these proteins is higher than fifty percent in males with abnormal seminal parameters. Cells that have been labeled with Fas proteins are phagocytosed and destroyed by Sertoli cells, which are associated with these cells (Horibe *et al.* 2019). However, some defective germ cells will go through the process of sperm remodeling while spermiogenesis is taking place. These cells will later show up in the ejaculate looking normal but will have genetic defects. Apoptosis is distinguished from other cell death processes by the rupturing of the cell membrane, the rearrangement of the cytoskeleton, the condensation of the nucleus, and the fragmentation of intranucleosomal DNA into multiple 185-bp pieces (Spangler 2022).

1.9 Damage During Chromatin Packing in the Spermiogenesis

The DNA and sperm nuclear proteins that make up sperm chromatin are arranged in a convoluted fashion, and there are varying degrees of compaction present in order to reduce nuclear volume and head size (Ariyasinghe 2019). Then, DNA fragmentation could be the result of unresolved strand breaks that were created during the normal process of spermiogenesis to relieve the torsional stresses involved in packing a very large amount of DNA into a very small sperm head. This was done to prevent the DNA from becoming overly twisted (Press *et al.* 2020). Phosphorylation of H2Ax, activation of nuclear poly (ADP-ribose) polymerase and topoisomerase, and other processes are responsible for the correction of these physiological strand breaks (Curtin and Szabo 2020).

1.10 Oxygen Radical-Induced DNA Damage by Reactive Oxygen Species

Byproducts of oxygen metabolism are oxidizing agents called reactive oxygen species (ROS), which are also known as free radicals (Madkour 2019). Because they contain at least one unpaired electron, they are capable both of producing highly reactive molecules (such as hydroxyl ion [OH], superoxide ion [O^{2-}], nitric oxide [NO], peroxyl [RO_2], lipid peroxyl [LOO], and thyl [RS]) and non-radical molecules (singlet oxygen [O_2], hydrogen peroxide [H_2O_2], hypochloric acid [HOCl], lipid peroxide [LOOH], and ozone [O_3]) (Capanoglu *et al.* 2020).

It has been demonstrated that the chromatin found in the nucleus of sperm is susceptible to oxidative damage, which can lead to the modification of bases and the fragmentation of DNA. Electromagnetic radiation is the cause of ROS production, which decreases the motility and vitality of human spermatozoa and damages DNA (De Iuliis *et al.* 2009, Gautam *et al.* 2022) claim that a number of toxins are released, including those in the form of metals (cadmium, chromium, lead, manganese, and mercury) and those released from industrial products or structural materials (benzene, methylene chloride, hexane, toluene, trichloroethane, styrene, and phthalates). Additionally, drinking and smoking enhance the formation of ROS and result in DNA strand breakage, which decreases sperm motility and triggers death (Henkel and Leisegang 2020).

In addition, the activation of sperm caspases and endonucleases by ROS results in the fragmentation of sperm DNA. Cui *et al.* (2017) found that exposing the mouse testis in vivo to temperatures between 40 and 42 degrees Celsius causes caspase and endonuclease to become active, which leads to a significant increase in the amount of DNA fragmentation in the epididymis (Takeshima *et al.* 2021). It is possible to limit the amount of damage that sperm might sustain during their journey through the epididymis if they are removed from the testicles before they enter the epididymis. Extraction of testicular sperm can improve clinical outcomes for patients who have high levels of DNA fragmentation in their sperm and multiple failures of in vitro fertilization (IVF) (TESE or TESA) (Esteves *et al.* 2020).

The chromatin of human sperm gets cross-linked in situations of high oxidative stress, which increases the frequency of DNA strand breaks (Aitken *et al.* 2020). When there is only a small amount of damage to the DNA of the spermatozoa, the spermatozoa can perform self-repair and may potentially regain the ability to fertilize the oocyte and continue development. The oocyte is indeed capable of repairing damaged sperm DNA; however, if the machinery within the oocyte is insufficient to repair DNA damage, the embryo may not be able to develop or implant successfully in the uterus (Griffiths *et al.* 2020).

2. LITERATURE REVIEW

2.1 IL-8

In addition to other cell types like epithelial cells, smooth muscle cells in the lungs, and endothelial cells, macrophages also produce interleukin 8 as a type of chemokine. After this, the precursor peptide must be disassembled into several active isoforms of IL-8. In culture, macrophages primarily secrete amino acid peptides (Cui *et al.* 2017).

The G protein-coupled serpentine receptors CXCR1 and CXCR2 are the ones that garner the most attention from researchers. These receptors are located on the surface membrane and are among the many different types of surface membrane receptors that are capable of binding IL-8. Both CXCR1 and CXCR2 have varying degrees of affinity for IL-8, and they express this cytokine in a variety of distinct ways. CXCR1 is the precursor to CXCR2. The production of the protein known as IL-8 requires a number of distinct metabolic events to take place. Within the context of the response of the innate immune system, it plays a significant role as a modulator of the immunological response (Zhou *et al.* 2020).

2.1.1 Structure of IL-8

Chemokines are signaling proteins that cells release in response to a variety of stimuli, including infection and damage (Chen *et al.* 2018). They make a concentration gradient for chemotaxis, which guides leukocytes with chemokine receptors to the damaged cells and tells them to kill the infectious agent through phagocytosis and other ways. This is done by guiding the leukocytes to the damaged cells (Malak *et al.* 2020). This allows the leukocytes to find the damaged cells more easily. The presence of chemokines has been associated with a variety of diseases, including cancer and several immune disorders. Two examples that exemplify the complexity of the system are the ability of individual chemokines to interact with multiple receptors and the ability of individual receptors to interact with multiple chemokines. Both of these examples are shown below. In humans, there are around fifty distinct chemokines, each of which can interact with one of twenty-three distinct G protein-coupled receptors. Chemokines play an important role in the immune system through GPCRs (Stappler *et al.* 2017).

Chemokines are globular proteins that are typically less than one hundred residues in length. Cells secrete chemokines. There is very little sequence homology between members of different classes of polypeptides, but the polypeptides do have some secondary and tertiary structural similarities in common with one another (Munawar *et al.* 2018). They are split up into four distinct groups according to their characteristics. In particular, they have one or two disulfide links, which serve to stabilize the protein fold and can be classified according to where in the sequence they occur. In other words, disulfide linkages are a type of protein fold stabilization. The structure of the protein is made up of three strands connected by small loops, a C-terminal helix, a long N-loop that is complemented by a turn of the 310 helix, and flexible N-terminal residues starting with the first cysteine residue. Interleukin-8 is a chemokine that belongs to the CXC class of chemokines. Other chemokines in this class also have the essential glutamate-leonine-arginine (ELR) sequence motif for biological activity. Because of how similar their structures are, what we learn about how a single chemokine is made, how it moves, and how it interacts with other molecules is likely to apply to a wide range of chemokine receptor combinations and functions. This is due to the fact that chemokines and their receptors have a lot in common due to the similarities in their structural make-up (David and Kubes 2019).

The very first chemokine ever found was given the designation interleukin-8 (IL-8 for short; IL-8, CXCL-8). This form, which has 72 residues and is a homodyne at high concentrations despite being a monomer at low concentrations, is the one in which it interacts with the receptor for which it was developed. Low concentrations of the monomer form do not allow it to engage with the receptor. X-ray crystallography and solution nuclear magnetic resonance were the methods that were initially utilized in order to figure out the structure of the wild-type IL-8 dimer. Since that time, after being subjected to a diverse set of circumstances and undergoing a significant number of mutations, its structure has been determined (Errichelli *et al.* 2017).

With only slightly more extensive changes to the amino acid sequence, it is possible to make IL-8 samples that can exist in solution as stable monomers or dimers. When a disulfide bond forms across the dimerization interface, IL-8 dimers are made that can't break apart. For this article, the monomeric form of IL-8 was made by replacing the natural amino acid Leu25 at the dimerization interface with the artificial amino acid N-methyl leonine. Changes were made to Leu25, Val27, and Glu29 to break up the hydrogen bonding network that keeps the demerit form stable, and the last six residues of the protein were taken out to break up the interface between the two molecules (Kazlauskas 2018). The production of the monomeric form of IL-8 proceeded in this manner. Both the demerit wild-type IL-8 and the monomeric IL-8 with N-methyl leonine have structures with small differences in where the first strand is and how the residues are arranged in helices. These differences may be seen in the demerit wild type of IL-8. These variations can be seen when comparing the two types of IL-8 (Ellwanger *et al.* 2020).

CXCR1 and CXCR2, two chemokine receptors that are highly similar to one another in terms of their sequences, were among the first to be discovered. Chemokine receptors make up six of the forty GPCR structures that have been discovered so far. These structures include CXCR1, CXCR4, CCR2, CCR9, and CCR5, in addition to the viral receptor US28. The structure of IL-8 bound to a chemokine receptor has not yet been determined; however, CXCR4 and US28 have been co-crystallized with viral chemokines to gain more insight into the structure of the complex (Sonawani *et al.* 2022). IL-8 is shown to have interactions with two major binding sites on CXCR1 that may be located in close proximity to one another, according to the experimental data that is currently available. The extracellular N-terminal portion of the receptor, where binding site-I is found, contains a total of 38 different residues in its structure. It has been demonstrated that a synthetic peptide composed of these two extracellular loops joined together by a linker is capable of binding IL-8 with a KD of 0.5 M. Arg119, Arg203 in extracellular loop 2, and Asp265 in extracellular loop 3 are some of the other residues that can be found in Binding Site-II (Berkamp 2018).

Under physiological conditions, there is evidence to suggest that wild-type IL-8 (1-72) interacts with CXCR1 as a monomer. These conditions are: Neutrophil chemo taxis and

Ca^{2+} mobilization assays have shown that monomeric IL-8 (1-66) possesses the same level of efficacy (Boyles *et al.* 2020).

The sequence diversity of the residues in Binding Site-I of chemokine receptors is high. This diversity is what accounts for the specificity with which the chemokine binds to the receptor. Switching the N-terminal regions of CXCR1 and CXCR2 between the two receptors changes their ability to bind specific molecules. This is because the N-terminal regions of CXCR1 and CXCR2 differ the most from one another (Moussouras *et al.* 2017). The observation that the adaptable N-terminal region appears to be a defining characteristic of chemokine receptors served as the impetus for the study of these residues in soluble peptides apart from the rest of the receptor. This research was carried out in order to better understand how chemokine receptors work. When measured in the low micromolar range, IL-8's affinity for the many N-terminal peptides derived from CXCR1 is at its highest. Nuclear magnetic resonance (NMR) investigations of IL-8 bound to a 21-residue peptide whose sequence corresponds to residues 9–29 of CXCR1 yielded the first insights at atomic resolution into the interaction between the two molecules. When bound to IL-8, the peptide takes on a more discernible three-dimensional structure than it does on its own. It was found that the residues Pro21, Pro22, Asp24, Glu25, Asp26, and Pro29, which are grouped together in the middle of the peptide, are involved in the binding of IL-8 (Padilla *et al.* 2017).

More recently, Berkamp (2018) showed that interactions with IL-8 change the chemical shifts of other CXCR1 residues like Asn16, Thr18, and Gly19 in a big way. This was done by demonstrating that these residues are affected. Also, it has been shown that IL8 can bind to different lengths of CXCR1 N-terminal peptides with affinities that are increased by micelles. This shows that lipids may affect how effectively IL-8 interacts with CXCR1, which indicates that lipids may affect how well IL-8 interacts with CXCR1 (Berkamp 2018). There are signs that the Tarp residue in the 34-mer peptide made from the N-terminus of CXCR1 interacts with DOPC lipid bilayers. This interaction takes place in the N-terminal region of the protein. Another one of our demonstrations has shown that the N-terminus of CXCR1 interacts with the surface of the membrane when IL-8 is not present, but these interactions are disrupted when IL-8 is present. This was shown to be the case by the fact that the N-terminus of CXCR1 engages in interactions with the surface of the membrane (Kukielka *et al.* 2021).

Researchers have isolated and characterized the IL-8 residues that are crucial to the function of CXCR1. Even though it has been shown that the unique ELR residues (Glu4, Leu5, and Arg6) are needed for CXCR1 to be activated, IL-8 does not attach to N-terminal peptides when they are present. This is the case despite the fact that CXCR1 activation requires these residues. Even if these remnants are present, this conclusion can still be drawn. The main binding sites on the IL-8 molecule have been found to be the N-loop (residues 12–18), the 40-s loop, and the third strand (residues 4–51). These spots are made up of charged residues and hydrophobic residues in equal measure (Ngo *et al.* 2020).

It appears that the N-loop residues Tyr13, Lys15, and Phe21 are responsible for imparting specificity to certain receptors. In addition, it has been demonstrated that the interaction of IL-8 with the peptide that corresponds to CXCR1's N-terminus does not result in a significant change in the conformation of the IL-8 molecule. Furthermore, there is evidence that IL-8 dimers split apart when they bind the N-terminal residues of CXCR1, which suggests that there are only a few minor differences between the binding of obligatory IL-8 monomers and IL-8 dimers (Novak *et al.* 2021).

2.1.2 Function of IL-8

Neutrophil chemotactic factor, also known as IL-8, primarily operates in two different ways (Gonzalez-Aparicio and Alfaro 2020). Inducing the target cells, primarily neutrophils but also other granulocytes, into chemotaxis causes these cells to migrate toward the site of the infection. Once the invaders have arrived at their destination, IL-8 also stimulates phagocytosis. In addition, it has been demonstrated that IL-8 is a potent agent that promotes the formation of new blood vessels. IL-8 causes a number of physiological changes in target cells. Some of these changes are an increase in the amount of Ca2+ inside the cell, the release of histamine from the cell through a process called exocytosis, and the respiratory burst. IL-8 is the culprit behind these processes, which are required for migration and phagocytosis to take place (Luo *et al.* 2018).

It has been demonstrated that IL-8 is a signatory chemokine of CR^{2+} naive T cells. Additionally, any cells involved in the innate immune response that have toll-like receptors can secrete IL-8. Because macrophages are the first cells to detect an antigen, they are also the first cells to release IL-8, which causes other cells to become attracted to the area. It has been shown that both the monomer and homodyne forms of IL-8 have a strong effect on the expression of the chemokine receptors CXCR1 and CXCR2. Although the homodyne is more efficient, the methylation of Leu25 has the potential to inhibit homodyne activity (Padilla *et al.* 2017).

It is believed that IL-8 plays a role in the development of bronchiolitis, which is a respiratory disease that is frequently caused by viral infections. IL-8 is a chemokine that belongs to the family of CXC chemokines. This gene and the genes for the other ten members of the CXC chemokine family are grouped on the chromosome 4q region of the genome (Fu *et al.* 2017).

2.2 IGF-1

The role of IGF-1 in reproduction as well as the growth of various cancers and benign proliferations has become known as a result of recent discoveries on the endocrine, paracrine, and anticrime involvement of IGF-1 in the proliferation of many tissues. These discoveries have led to the understanding that IGF-1 plays an important role in the proliferation of many tissues (Hernández-Silva *et al.* 2020). IGF-1 will be discussed in the context of gynecology throughout the course of this article. Women before menopause who have high levels of IGF-1 but low levels of an IGF binding protein (IGFBP) like IGFBG-3 have an increased risk of developing breast cancer. There is a correlation between elevated levels of IGF-1 and an increased risk of cervical, ovarian, and endometrial cancer in postmenopausal as well as premenopausal women. Both in vitro and in vivo studies have shown that the presence of IGF-1 promotes the growth of myomas, which are the most common type of benign uterine tumor found in women (AlAshqar *et al.* 2019). According to the findings of various studies, IGF-1 is hypothesized to play a part in the differentiation process of a variety of reproductive tissues, including the endometrium and the tissues of the ovaries. Insulin resistance and an increase in plasma IGF-1 are two symptoms that frequently appear in patients who suffer from polycystic ovary syndrome (PCO). Endometriosis and polycystic ovary syndrome both cause a decrease in IGF levels locally in the endometrium, which may help explain infertility. Plasma IGF-1 levels are found to be higher in more severe cases of endometriosis. IGF has recently been shown to facilitate the implantation of human embryos into the endometrium during in vitro fertilization procedures. It is a conundrum that different immune systems need to cooperate for implantation and continued pregnancy to take place. It would appear that IGF is the agent that catalyzes the joining of the two epithelia (Qin and Xu 2022). A disturbance can result in pregnancy complications such as spontaneous miscarriage, preeclampsia, or embryo defects. Other pregnancy complications include premature labor and delivery. IGF is therefore a useful indicator that a pregnancy is progressing normally. If we have a better mechanistic understanding of how IGF-1 acts on the cellular level, the practitioner may find it interesting to use IGF-1's diagnostic potential as a marker for a variety of diseases. Not only will this provide scientists with more sophisticated mechanistic explanations, but it will also not yet be determined how the levels of IGF-1 in local tissues relate to the levels of IGF found throughout the body under certain conditions (Yan and Charles 2018).

2.2.1 Biochemistry of IGF-1

IGF molecules are small polypeptides that contain 70 amino acids for IGF-1 and 67 amino acids for IGF-2. There is 62% amino acid sequence homology between the two types of IGF molecules. Their structure is very similar to that of insulin in that it is made up of a single polypeptide chain connected by three sulfur-sulfur bridges. The gene for IGF-2 is located on chromosome 11, and the gene for IGF-1 is located on chromosome 12. The primary site of its production is in the cells of the liver, and it is regulated by both GH and the factors of the diet. IGFs are transported through the bloodstream attached to specific proteins, including the IGFBPs (Allard and Duan 2018).

2.2.2 Structure of IGF-1

IGFs, also known as insulin-like growth factors, are essential to the development of a healthy fetus and infant after birth. The type 1 IGF receptor, also known as IGF-IR, initiates the physiological response in vivo to the ligand. This receptor binds to IGF-I with high affinity (LeRoith *et al.* 2021). Although with a lower affinity, IGF-IR is also able to bind to IGF-II, which is a factor that contributes to the mutagenic effects that the polypeptide has on the development of the fetus. An earlier genetic study that linked the insulin receptor to the growth-promoting effects of IGF-II has been supported by more recent research that has shown that the insulin receptor (IR), which is alternately spliced and lacks exon 11, binds IGF-II with high affinity (Liefers-Visser *et al.* 2017).

Insulin, IGF-I, and IGF-II are all examples of ligands that share a common three-dimensional (3D) architecture and are capable of binding to both IR and IGF-IR competitively. There is no known ligand for her, and knockouts of IRR have no discernible effect on the organism's phenotype. When both alleles of Igf-1r are deleted in the germ line, severe growth retardation occurs during the second half of gestation. This occurs because of the lack of IGF-1R. Fibroblast cell lines derived from IGF-1R knockout mice are resistant to oncogenic transformation by a wide variety of viral and cellular oncogenes when cultured in an environment rich in serum (Ngo *et al.* 2021). These cell lines also display impaired progression through the cell cycle. On the other hand, ligand-dependent overexpression of IGF-IRs facilitated the transformation of cell lines into neoplastic forms. Therefore, the IGF axis is essential for both the development of healthy cells as well as the transformation of cells into cancer. As a direct consequence of this, IGF-IR has emerged as a potential therapeutic target in the treatment of human cancer (Lyu *et al.* 2018).

2.2.2.1 IGF-I receptor: discovery and sequence

In 1974, different proteins from pure rat liver plasma membranes and detergent-solubilized fractions were used to label 25-I-labeled insulin and 25-I-labeled NSILAs, which is a soluble fraction of insulin-like activity that can't be turned off. This finding provided the first piece of evidence suggesting the presence of an IGF receptor that was distinct from IR (Makkar *et al.* 2022). The IGF-IR was utilized as a homodyne by means of SDS gel electrophoresis, which demonstrates that it is composed of two a-chains and two b-chains joined together by disulfide receptors. The mature 22 receptors are produced from a precursor of IGF-IR that has a molecular weight of 180 kilodaltons and has been glycosylated, dimerized, and put through the processing of proteolysis. The next important discovery was the demonstration that IGF-IR is a tyrosine kinase that becomes activated and self-phosphorylated after it binds to IGF-I (Velloso *et al.* 2017). This was a very important discovery.

In the year 1986, the human IGF-IR canal was successfully cloned and its sequence was determined. It contains 4,989 nucleotides and the genetic code for 1.367 amino acids in their precursor forms (Figure 2.1). Both the 30-residue signal peptide (residues 30 to -1) and the Arg-Lys/Arg-Arg furin protease cleavage (residues 708–711) are present in the pre-preceptor monomer, which, upon cleavage, produces one a-chain and one -chain. The signal peptide begins at residue 30 and ends at residue 1. The extracellular portion of the IGF-IR consists of the a-chain (residues 1–707), which has eleven potential N-linked glycosylation sites, and the b-chain, which has 195 residues. Both of these chains have eleven potential N-linked glycosylation sites. The tyrosine kinase is housed within a cytoplasmic domain consisting of 408 residues and a single membrane segment belonging to Tran (residues 906–929) (Residues 930–1337). The canal for the third member of the IR family has also been cloned and sequenced. Additionally, its organization is very similar to that of the human IR canal (Hernández-Corbacho *et al.* 2017).

Figure 2.1 IGF-IR canal

Both human IGF-IR and human IR have their own unique sequences of amino acids. For the L1/Cys-rich/L2 domains and the tyrosine kinase domain, the secondary structural assignments are shown above the sequences as cylinders for -helices and arrows for -strands. Below the sequences is a representation of the demarcation borders that separate the many different domains and modules (Adams *et al.* 2000)

The length of the human IGF-IR gene is greater than one hundred thousand base pairs, and it has ten exons in the plus chain and eleven exons in the minus chain. In a different human IGF-IR mRNA transcript, the deletion of three base pairs results in the substitution of Argo for Thr898Gly899 (Figure 2.1) (Escribano *et al.* 2017). This occurs eight residues upstream from the beginning of the human IGF-IR transmembrane region. The CAG-isoform displays decreased internalization and improved signaling characteristics in comparison to the CAG+ isoform.

The high-IR, which is very glycosylated and possesses 16 potential N-linked glycosylation sites like the hire, is also highly glycosylated. The third member of this receptor subfamily is called the, and it possesses 11 N-linked sites as opposed to the human's total of 18 N-linked sites (Cuccui *et al.* 2017). The vast majority of studies have considered hiring. Analytical ultracentrifugation revealed that the hire protein expressed in CHO-K1 cells contained between 58 and 64 different types of carbohydrates (Romauch 2020). There are oligosaccharides of both the complex and high mannose types; the latter contains residues of fuses in addition to N-acetyl glucosamine, GA lactose, and silica acid. Oligosaccharides can also be of the high-mannose type. The O-linked glycosylation process has only been demonstrated for the hire subunit. According to studies that investigated the effects of removing N-linked glycosylation sites, there are a lot of redundant processes involved in the glycosylation of hire (Fuertes-Martín *et al.* 2020). Each site can be changed independently, with one notable exception, without affecting the ligand-receptor binding, the processing of the receptor, or the expression on the cell surface. 30-36 It would appear that the major receptor domains, particularly those that are close to the N-terminus (L1, Cyst-rich, L2), need at least one intact glycosylation site in order to fold and process correctly. When combinations of glycosylation sites are examined, this requirement becomes clear (Chalkley and Baker 2017).

2.2.3 Function of IGF-1

IGF-1 is the primary mediator of the effects of growth hormone (GH) (Kraemer *et al.* 2017). The anterior pituitary gland is responsible for producing growth hormone, which is then secreted into the bloodstream, where it stimulates the production of IGF-1 by the liver. IGF-1 then promotes systemic body growth and has effects that promote growth on almost all of the body's cells, particularly those found in the skeletal muscle, cartilage, bone, liver, kidney, nerve, skin, and hematopoietic systems (Cheng *et al.* 2021). IGF-1 also has effects that promote growth in almost all of the body's nerve cells. IGF-1 is capable of controlling the synthesis of DNA in living things, in addition to having effects that are analogous to those of insulin.

IGF-1 is capable of attaching to the insulin receptor as well as at least two other cell surface receptors that are tyrosine kinases, one of which is the IGF-1 receptor. In addition, IGF-1 can bind to at least two other cell surface receptors that are tyrosine kinases (IGF-1R) (Zhang *et al.* 2020). It binds to IGF-1R, a particular receptor that is present on the surface of numerous different cell types in numerous organs, mediating the primary function. This receptor may be found in many different sorts of organisms. The mechanism of intracellular signaling is kicked off whenever the IGF-1R molecule is bound (Crudden *et al.* 2018). IGF-1 is one of the natural activators of the AKT signaling pathway that is considered to be among the most efficient. It does this by inhibiting the process of programmed cell death, encouraging the growth and proliferation of cells, and stimulating the formation of new cells. IGF-1 receptors have a significantly higher propensity to bind IGF-1 than insulin receptors do, which lends support to the idea that the IGF-1 receptor is the "physiologic" receptor (Owen 2017). IGF-1 is just 0.1 times as effective as insulin when it comes to its ability to activate the insulin receptor. Insulin is the more powerful of the two hormones. There is a potential that some of this signaling is carried out by heterodimers made up of IGF-1R and insulin receptors (the reason for the confusion is that binding studies show that IGF-1 binds the insulin receptor 100-fold less well than insulin, which doesn’t correlate with the actual potency of IGF-1 in vivo at inducing phosphorylation of the insulin receptor and hypoglycemia) (Yoshida and Delafontaine 2020).

IGF-1 binds to and activates its own receptor, the IGF-1R. It then sends signals through a number of intracellular transduction cascades when RTKs are made on the cell surface (Aghajani *et al.* 2019). After RTKs have been expressed, this occurs. When RTKs are made on the surface of the cell, IGF-1 binds to its own receptor, the IGF-1R, and turns it on. It then sends signals through a number of intracellular transduction cascades. This happens after RTKs have been expressed (Hwang *et al.* 2020). Phosphatidylinositol-3 kinase (PI3K) and its downstream partner, the motor, are two of the most important regulators of one of the most important pathways. A significant mechanistic pathway is also involved in mediating the cascading impact that is caused by this pathway. This effect is caused by this pathway (the mammalian target of kanamycin). Kanamycin is able to suppress the activity of the mTORC1 complex as a result of its interaction with the enzyme FKBPP12 (Xiong *et al.* 2017). MTORC1 is suppressed while MTORC2 is untouched, and the cell responds by boosting the activity of AKT, which directs signals through the protein that is unaffected by the inhibition of MTORC1. The phosphorylation of eukaryotic translation initiation factor 4E by motor proteins interferes with the capacity of EIF4E-binding protein 1 (EIF4EBP1) to inhibit eukaryotic translation initiation factor 4E and decrease metabolism (EIF4E). The skin, internal organs, and secondary lymph nodes are the most common locations for the development of tumors. The growth of cancerous tumors (Kaposi sarcoma) is strongly linked to mutations in the PI3K-AKT-mTOR signaling pathway. IGF-1R enables activation of these signaling pathways, which in turn regulate cellular viability and the metabolic absorption of biogenic chemicals (Liu *et al.* 2020). Ganitumab could lead to the development of a therapeutic strategy that aims to reduce tumor collection. Ganitumab is a potentially monoclonal antibody that targets the IGF-1R in an antagonistic manner. Ganitumab inhibits the activation of the PI3K-mTOR signaling pathway by binding to the IGF-1 receptor and preventing IGF-1 from binding to the receptor. It is possible that the growth of tumor cells can be stopped and apoptosis can be induced in tumor cells if a pathway that promotes survival is blocked (Hsu *et al.* 2019).

IGFBP1, IGFBP2, IGFBP3, IGFBP4, IGFBP5, IGFBP6, and IGFBP7 are the seven IGF-1 binding proteins (IGFBPs) that have been shown to bind to and interact with insulin-like growth factor 1. There are also IGFBPs that inhibit growth. For example, the affinity with which IGF-1 binds to IGFBP-2 and IGFBP-5 is significantly higher than the affinity with which it binds to its receptor. As a result, there is a reduction in IGF-1 activity because serum levels of these two IGFBPs are increasing (Allard and Duan 2018).

2.2.4 IGF-1 and male fertility

Infertility is defined as the inability to conceive a child after one year of unprotected sexual activity (Akhondi *et al.* 2019). There are some medical conditions that can also cause infertility. It is estimated that male fertility factors are to blame for 50% of all cases of infertility in human couples, which accounts for 15% of all human couples. Some evidence suggests that the quality of sperm has declined, despite the fact that overall human fertility does not appear to have decreased in recent years. Up to this point, decreased sperm count and decreased sperm motility have been linked to a wide variety of factors. Some of these factors include anatomical and physical impediments, hormonal imbalances, immunologic deficiencies, genitourinary infections, environmental exposure to dangerous chemicals that are harmful to spermatogenesis, abnormal morphology, and anatomical and physical obstructions (Park 2017).

Pituitary gonadotropins and testosterone are required for the intricate developmental process of spermatogenesis. Through anticrime, paracrine, and juxtacrine mechanisms, this process controls spermatogenic cells and the way the testicles work in a roundabout way. One of the molecules that is believed to play a role in the development of germ cells is called IGF-1, which has a molecular weight of 7.6 kilodaltons and contains a total of 70 amino acids (Lee and Kim 2018). IGF-1 is responsible for mediating the cell-proliferating effects of growth hormone (GH). Steroidogenesis, metabolic processes, cell proliferation, and differentiation are all influenced by IGF-1. Plasma and the fluid that comes from tissues both contain it. IGF-1 influences several facets of reproduction, including the beginning, development, and control of spermatogenesis, which is one of those facets (Neirijnck *et al.* 2019IGFs are primarily manufactured in the liver, and their release occurs in response to the pulsatile GH secretions produced by the anterior pituitary. IGF-1 was found in the testis, which is part of the male reproductive system. Leyden cells and Sterol cells are responsible for their secretion in the testis. IGF-1 receptors have been discovered on sterol cells, Leyden cells, secondary spermatocytes, spermatids, and spermatozoa. Sterol cells are responsible for producing spermatozoa (Ramal-Sanchez *et al.* 2019). There is evidence to suggest that IGF-1 is involved in both the formation of steroids and spermatozoa. IGF-1 is also found in the peripheral circulation, where it usually lives with IGF-binding protein-3 and the acid-labile subunit in a heavy tertiary complex. IGF-1 can also be found in the central circulation (Fujimoto *et al.* 2017).

IGF-1 levels in the serum and sperm, on the other hand, have only been the focus of a limited number of research efforts. In one study, the levels of IGF-1 in infertile men were found to be variable, while another study looked at these levels in healthy, fertile men who had undergone vasectomies and found that these men were still fertile. The purpose of the present study was to determine the relationship between serum IGF-1 concentration, seminal plasma IGF-1 concentration, and abnormal sperm parameter values in order to acquire a deeper comprehension of the role that IGF-l plays in the process of fertility (Perussello 2022).

2.3 DNA

2.3.1 DNA definition

DNA, or deoxyribonucleic acid, is a specific kind of molecule that belongs to the category of nucleic acids (Cuéllar-Cruz *et al.* 2019). It is very long and extremely small. DNA is a molecule that carries the genetic code, which is unique to each person and contains the instructions for making the proteins that are necessary for human cellular function. DNA is responsible for carrying the genetic code. DNA is a molecule. The mother is responsible for contributing one half of the son's DNA, while the father is responsible for contributing the other half. DNA instructions are passed down from parents to offspring (Moore and Shenk 2017).

The genetic material, commonly known as DNA, is found in both the mitochondria of the cell and the chromosome-like structures known as chromosomes. The genetic material can be found in the nucleus of the cell. It stores the genetic information necessary for the reproduction of cells as well as the production of their organelles and components (Wolff *et al.* 2020).

2.3.2 The shape and components of DNA

Every nucleotide, which is a molecule that makes up DNA, has a nitrogen base, a phosphate group, and a sugar group in it. These three groups make up DNA. There are four distinct categories of nitrogenous bases, and some examples of each are adenine, thymine, guanine, and cytosine (Sajid *et al.* 2019).

When the nitrogenous bases in DNA are linked in pairs, they make strips of deoxyribose sugar and phosphate molecules on their sides. These strips are called AT and GC. The arrangement of the nitrogenous bases, which act as the rungs, determines the structure of this spiral ladder. The genetic code is the result of this arrangement (Satyavolu *et al.* 2019).

DNA is encased in chromatin, which is composed of tiny proteins called histones that help organize DNA into nucleosomes, which in turn create chromatin fibers that are coiled and concentrated to form chromosomes. The double helix structure of DNA contributes to the compactness of the biological molecule. The DNA is condensed into chromatin structures that can be inserted inside the nucleus (Xu *et al.* 2018).

On each chromosome, there is only room for one molecule of DNA. The human genome contains a total of 46 chromosomes, which are arranged in 23 pairs. The 21^{st} chromosome, which only contains about 3.000 genes, is the smallest of all the human chromosomes, while the first chromosome, which contains 8.000 genes, is the largest (Belser *et al.* 2021).

2.3.3 Protein production process

There are two primary stages in the manufacture of proteins, and these are as follows:

In the course of the transcription process, the nitrogenous base thymine (T) is exchanged for pal uracil. This results in the production of messenger RNA, which is a single-stranded DNA transcript (U) (Wu *et al.* 2020).

Tran is responsible for the conversion of mRNA into amino acids. As an illustration, the letter combination (GUG) denotes the amino acid valise. Codons are read from messenger RNA (mRNA), and each codon stands for a particular amino acid or structural component of a protein. Codons consist of three letters (Abbasi *et al.* 2020).

2.3.4 DNA discovery

The discovery of DNA was made by a German biologist named Friedrich Mescher in the year 1869 AD (LaFleur 2019). However, it wasn't until 1953, when James Watson, Francis Crick, Maurice Wilkins, and Rosalind Franklin discovered the double helix structure of DNA, that scientists started to comprehend the significance of this molecule. Around the same time, they started to entertain the idea that DNA might store information of a biological nature (Kim and Poslad 2019).

2.3.5 DNA duplication

DNA replicates itself by first separating into two distinct strands, each of which acts as a template for the subsequent replication of a new strand (Brown 2020). After this, the new strands are translated by utilizing the same hydrogen bonds between the bases in the double structure to produce two new molecules of double-stranded DNA. Each of these new molecules contains one copy of the original DNA. This method of replication, which is also referred to as semiconservative replication, is necessary for the maintenance of genetic characteristics on both the original strand and the new strand (Cohen *et al.* 2018).

2.3.6 DNA test

DNA testing can be utilized in the process of diagnosing genetic disorders as well as determining whether or not an individual is a genetic mutation carrier (Camunas-Soler *et al.* 2018). An individual's DNA not only contains information about their ancestry, but it also has the potential to occasionally reveal whether or not they are predisposed to developing certain diseases. He is the one who gets to choose whether or not to pass it on to his children, as well as evaluate his own risk of developing a genetic disease (Moore 2017).

2.3.7 The importance of DNA

A significant number of unsolved criminal cases have been resolved thanks to DNA because it can be used to identify the person who committed a crime by comparing a sample of their DNA to evidence found at the crime scene and, similarly, because evidence found at one crime can be linked to evidence found at other crimes (Campbell *et al.* 2017).

If there is a high risk of chromosomal or genetic disorders in the fetus before birth, it may be necessary to take a DNA sample from the expectant mother while she is pregnant. This is done if there is a high risk of these conditions being present (Sanz *et al.* 2020).

DNA testing is appropriate for people who have a family history of genetic disorders, are at high risk of developing a specific genetic disorder, or are currently being treated for another genetic disorder (Boycott *et al.* 2017).

The production of vaccines involves the use of plasmids that contain particular DNA sequences; the antigens contained in these plasmids are then translated into the specific antigens that cause an immune response in the body (Jahanafrooz *et al.* 2020).

2.4 Sperm DNA

Sperm, or spermatozoon (Spermatozoid), to give it its full scientific name, is the reproductive cell found in males. Sperm is also known as the reproductive cell (plural: spermatozoa). Spermatozoa, also known as motile sperm that have a flagellum and a tail, are produced by animals (Lehti and Sironen 2017).

Spermatogenesis is the process by which sperm cells are created. Sperm cells are created through spermatogenesis. It is located in the seminiferous tubules of the testicles in amniotes like humans (reptiles and mammals). This process involves the development of several subsequent sperm cell precursors after beginning with the spermatogonia (Ben

Maamar *et al.* 2021). They go through a process known as spermatocyte differentiation. After that, spermatocytes go through the process of meiosis, which reduces the number of chromosomes in their cells. The next step is the production of spermatids. Spermatogenesis is the final stage of the process known as spermatogenesis. During development, spermatids transform into spermatozoa that are complete (Rahman *et al.* 2018).

The production of these testicular cells initiates itself during the puberty stage. The genetic material (DNA) that it carries in its head is what will be transferred to the egg when it fertilizes it. It is mobile and possesses a tail at the same time (Ruiz and Granja 2017). In humans, between 150 and 200 million sperm are released during the act of copulation. However, in order for the egg to become fertilized, there must be only one sperm cell present. When it enters an egg cell (oocyte), a sperm loses its tail on the outside of the cell, travels through the egg membrane, and then transfers its genetic material to the egg (Stadelman *et al.* 2017). Sperm are kept in a viscous liquid. This fluid is known by a few different names, including semen (resay), ejaculate, and more commonly just semen. Spermatocytogenesis is the process by which stem cells divide in order to replenish themselves and create a population of cells that will eventually develop into mature sperm. This population of cells will eventually become mature sperm (Sharma *et al.* 2018).

The genetic material can be found in the "baker's shovel"-shaped head of the spermatozoa, which is the location of the spermatozoa. The acrosome is a structure that surrounds the head of the spermatozoon and contains the enzymes that are necessary for the spermatozoon to penetrate the wall of the egg cell in order to fertilize the egg (Simons and Olson 2018). When the sperm cell reaches the egg, the enzymes that are contained within the acrosome membrane are activated and released, making it possible for the sperm to enter the egg. It is provided with n of the male DNA molecules, which then combine with n of the female DNA molecules to produce 2n of the DNA molecules (this cell is called a zygote) (Favilla *et al.* 2021). The cells that comprise the zygote will then begin to divide and expand. The X and Y chromosomes that are carried by spermatozoa are primarily responsible for the formation of sexuality. The female reproductive cell half of male reproductive cells contains an X chromosome, while the male reproductive cell half of male reproductive cells contains a Y chromosome. In the event that an X-chromosome-carrying sperm fertilizes a female reproductive cell, the resulting chromosomes will be XX, and the individual will have female genitalia. There are, however, a few notable exceptions to this rule (Madan 2020). For instance, there is a possibility that there are people in the world who suffer from hereditary diseases such as XXY and X0 (Zore *et al.* 2018).

2.4.1 Sperm parameters

Antoine van Leeuwenhoek was able to see spermatozoa in a sample of semen in the year 1677 by making use of a single-lens microscope (Jegou 2018). This was significantly more powerful than the 20–30x magnification of the compound microscopes that were used in the late 14th century. With the aid of the microscope, he was able to discover "animalcules," which are defined as "tiny motile components in semen that assist in fertilization." However, in 1875, Oscar Herter demonstrated using sea urchins that the sperm head actually fused with the female germ cells to form the nucleus of a new being. This was the most significant advance in scientific knowledge at the time. John Macleod, a scientist who worked in New York, United States, made two significant discoveries between the years 1920 and 1950 (MacLeod and Urquiola 2021). One of these discoveries is that oxygen metabolism is a factor in the cause of altered sperm quality, and the other discovery is that semen analysis can be used as an index of human fertility through sperm concentration, motility, and morphology. The procedures for sperm analysis have been standardized by the World Health Organization (WHO) thanks to the creation of a guidance manual for sperm analysis, which receives an update once every ten years (Esteves 2022). These sperm parameters are what used today to determine whether or not a male is sterile or subfertile.

The process known as spermatogenesis requires approximately 70 days to reach its conclusion. The process begins in the epididymis with spermatogonia and continues through primary, secondary, and spermatid spermatocytes before culminating in the formation of mature spermatozoa. Large terminal deletions of the Y-chromosome long arm (Yq) are found in men who have azoospermia (Aitken 2022). This finding suggests that azoospermia factors necessary for spermatogenesis are located within the achromatic region of Yq. Two hormones, testosterone from the testes and follicle-stimulating hormone (FSH) from the pituitary gland, control the production of spermatozoa. The follicle-stimulating hormone (FSH) makes the first meiotic division happen in primary spermatocytes (Santi *et al.* 2020). The luteinizing hormone tells the Leyden cells of the testes to make and release testosterone, which helps the secondary spermatocytes change into mature spermatozoa. This process takes place under the control of luteinizing hormone (Derkach *et al.* 2022). The production of spermatozoa, their maturation, and their lifespan are all negatively affected at an early stage in the pathogenesis of many diseases. The parameters of a man's sperm can, according to some reports, reveal information about his health (Skinner *et al.* 2017).

According to the definition that is given in its broadest sense, health is "a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity." This definition describes health as "a state of complete physical, mental, and social well-being." The presence of a disease, the development of a depressive illness, and cognitive decline with age are all linked to prolonged exposure to psychological stress. Cancer, cardiovascular diseases, diabetes mellitus, infections, and hypertension are the leading causes of death around the world, other than accidents, homicide, and suicide, which are the leading causes of death (Cunningham *et al.* 2018).

2.5 Sperm DNA Fragmentation

DNA-damaged spermatozoa can successfully fertilize an egg (Mazur *et al.* 2021). The most likely effects that the introduction of nucleotide or DNA damage into the maternal genome during fertilization by the paternal genome, which has not been repaired by the oocyte, will have on normal embryonic and fetal development are still unknown (Wu *et al.* 2017). A sizeable percentage of women do not go on to have children because they are unable to conceive, despite the fact that there does not appear to be a male or female factor that contributes to infertility. It is likely that the genomic male factor of infertility is present in many of these couples. This factor can cause infertility due to sperm DNA damage, meiotic changes, or sperm aneuploidy, among other things (Dissanayake *et al.* 2019). Even though very little is known about the human oocyte and embryo's ability to repair DNA damage, a number of studies on gene expression have suggested that both the oocyte and the embryo possess defense mechanisms that may allow them to deal with some paternal DNA anomalies. This is despite the fact that very little is known about the human oocyte's and embryo's capacity to repair DNA damage. To begin, the cytoplasmic and genomic quality of the oocyte, both of which are significantly impacted by increasing age, will determine how well the oocyte is able to initiate repair (Setti *et al.* 2021). This is the first step. Second, the decrease in pregnancy rate (PR) observed in older women may be made worse by the growing evidence linking paternal age to the quality of sperm DNA present in a spermatozoon. This evidence links paternal age to the quality of sperm DNA present in a spermatozoon (Chapuis *et al.* 2017).

It has been hypothesized that the presence of unrepaired DNA damage above a critical threshold in embryos created in vivo and in vitro is what causes the block in embryo development seen after embryo implantation in embryos with a normal karyotype (Yang *et al.* 2021). This block can be seen in embryos that have a normal karyotype. Recent studies have shown that this type of damage, which is referred to as a late paternal effect, can manifest at any time during or after the implantation process. It is believed that some preimplantation embryos pass away between the stages of postembryonic genome activation and the blastocyst stage. Additionally, there are hints that link low blastocyst viability to high DNA damage levels in sperm samples (Zheng *et al.* 2018).

It is important to note that the DNA damage that was discovered in the embryo may not always be connected to the DNA damage that was present in the spermatozoon that fertilized the oocyte. The oocyte is a major contributor to DNA irregularities in the organism (Ma *et al.* 2017). If studies on aneuploidy are used as an illustration, then problems with the oocyte will continue to be the most important factor contributing to the condition. It is possible that in the future, analytical techniques will concentrate on combining chromosomal probes and DNA fragmentation testing in embryo biopsies (Coonen *et al.* 2020). This could enable a more thorough analysis, which would include not only the diagnosis of monogenic diseases and aneuploidy but also the degree to which

DNA is damaged in the embryo. This would make it possible to select embryos that have the highest genomic quality. Examining the fragmentation of DNA in trophoblastic cells that have been taken from a blastocyst biopsy is another method that can be used to determine whether the oocyte or the embryo has repaired the DNA damage caused by the sperm (Newman *et al.* 2022).

2.5.1 Mechanisms of sperm DNA fragmentation

How does sperm DNA damage take place?

There are primarily six different mechanisms that can result in DNA damage in spermatozoa (Bisht and Dada 2017). This damage can occur in either the nuclear or mitochondrial DNA. These can occur either during the production of sperm cells or while the sperm cells are being transported, and include (Figure 2.2) the following: apoptosis during spermatogenesis; DNA strand breaks caused by sperm chromatin remodeling during spermiogenesis; post-testicular DNA fragmentation caused primarily by oxygen radicals, such as the hydroxyl radical and nitric oxide, during sperm transport through the seminiferous tubules and the epididymis; DNA fragmentation caused by endogenous caspases and endonucleases; and DNA damage caused by radiation (Takeshima *et al.* 2021).

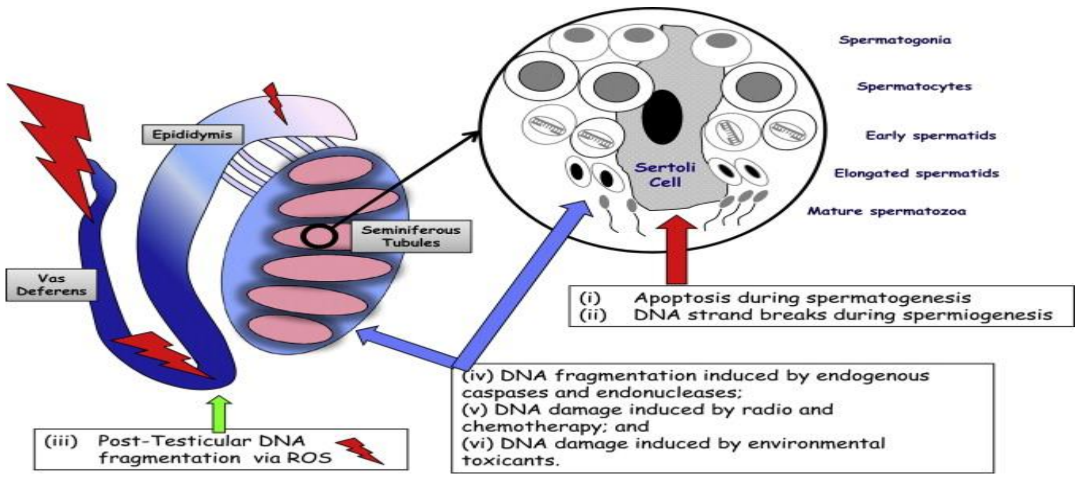

Figure 2.2 The main mechanism that can cause DNA damage in spermatozoa during the production and transport of sperm cells

The following are the major mechanisms that can cause DNA damage in spermatozoa during either the production or the transport of sperm cells:

(i) Apoptosis during the process of spermatogenesis.

(ii) DNA strand breaks are produced during the remodeling of sperm chromatin during the process of spermatogenesis.

(iii) During sperm transport through the seminiferous tubule, mostly oxygen radicals cause DNA to break up after it leaves the testes.

The post-testicular damage that occurs while sperm are being transported through the epididymis is one of these six mechanisms that has the potential to make a significant contribution to the DNA fragmentation of sperm (Park *et al.* 2018). This is supported by previous research, which found that the level of DNA fragmentation in the caudal epididymis and ejaculated sperm was higher than that found in testicular spermatozoa. Recent findings lend credence to this hypothesis. However, it is still unknown whether errors that occurred during the process of spermatogenesis made the sperm more susceptible to damage that occurred after the testes (Parrish *et al.* 2017).

2.6 The Effect of IL-8 on DNA Fragmentation

Infertility affects approximately 15% of couples around the world who are of reproductive age, with the male partner being responsible for 30–40% of these cases (Deshpande and Gupta 2019). The first step in figuring out if a man is infertile is to look at his sperm. This involves measuring the concentration, movement, vitality, shape, and number of round cells and white blood cells in the sperm using cutoff values set by the World Health Organization (WHO). There is a condition known as leukocytospermia in which there are more than 1.106 white blood cells (WBC) per milliliter of semen (Wu *et al.* 2020). This condition affects 10% to 20% of infertile men. Leukocytospermia is a well-known indicator of an infection in the male urogenital system, which can have a negative impact on the quality of the sperm. Interleukin 6 and IL8 are both types of cytokines that are produced by leucocytes. It is believed that these cytokines are important factors and sensitive markers for the prediction of acute seminal inflammation. It has been demonstrated that seminal cytokines have a detrimental effect on the process of spermatogenesis, which in turn results in low-quality sperm (Fallah *et al.* 2018).

The inflammatory responses that occur within the male genital tract are inextricably linked to the oxidative stress that occurs there (OS). A factor that influences fertility is reactive oxygen species, also known as ROS (Darbandi *et al.* 2018). These are leukocyte-produced molecules that prevent male reproduction. In fact, oxidative stress is brought on not only by an excessive amount of ROS but also by the lipid peroxidation that takes place at the plasma membrane (Yusupov *et al.* 2017). When ROS enter sperm, they harm the genetic material there. More specifically, they accelerate the destruction of mitochondrial DNA (mtDNA), speed the fragmentation of sperm DNA, and prevent cells from producing ATP.

Malondialdehyde (MDA), which is one of the byproducts of lipid peroxidation, is found in much higher concentrations in people with leukocytospermia (Wang *et al.* 2019). Spermatozoa are protected from the damaging effects of oxidative stress by antioxidants that act as free radical scavengers. Some examples of these antioxidants include catalase (CAT), glutathione (GSH), and superoxide dismutase (SOD) (Zulaikhah 2017). Researchers are already debating the findings of the various studies in regards to their concentrations in seminal plasma. According to the findings of a number of studies, leukocytes have the tendency to weaken the antioxidant defense system of sperm. In contrast, patients with leukocytospermia have been shown to have significantly elevated levels of antioxidant activity in other studies (Derakhshan *et al.* 2018).

2.7 IL-8 and Sperm DNA Fragmentation

It is believed that the DNA fragmentation index (DFI), which measures the number of DNA breaks that occur in sperm cells, can more accurately predict male fertility than conventional semen parameters (Khatun *et al.* 2018). Furthermore, the high levels of sperm DNA fragmentation (SDF) adversely affected key aspects of assisted reproduction, including fertilization, blastula formation, pregnancy rates, and live birth rates. Because seminal leucocytes are thought to be the primary producers of reactive oxygen species

(ROS), which cause subsequent inflammatory processes and stromal cell death (SDF) (Hatef *et al.* 2017), a significant amount of research has been done to investigate the effect that they have on the integrity of DNA. Leucocyte spermia has been shown to have a detrimental impact on the integrity of the sperm chromatin by a number of authors, in addition to a significant increase in SDF in leucocyte sperm samples (Zandieh *et al.* 2018). This finding was made possible by the fact that leucocyte spermia causes a significant increase in SDF in leucocyte sperm samples. Men who had higher levels of seminal leucocytes also had greater sperm chromatin damage. Leucocytospermia, on the other hand, had very little of an impact on DNA impairment, as reported by Moskovtsev *et al.* (2007). They came to the conclusion that leucocytes may not have as significant an impact on the pathology of SDF as had been previously believed. This lack of correlation between SDF and the volume of seminal leucocytes has been independently verified by three separate groups of researchers (Petersen *et al.* 2018). In addition to the production of reactive oxygen species (ROS), seminal leucocytes are capable of producing a wide variety of pro-inflammatory cytokines (Zeinali *et al.* 2017). Two of these cytokines, IL-6 and IL-8, are thought to be possible indicators of male infertility. Several studies have been conducted to investigate the effect that these cytokines have on the structure of DNA chromatin, but the results are inconclusive. In an in vitro study, the combination of IL-6 and IL-8 led to an increase in DNA strand breaks (Aghazarian *et al.* 2019). Other researchers contested the correlation between elevated levels of IL-6 and impaired sperm chromatin that was discovered in vivo. In addition, a comprehensive search of the relevant literature revealed that no in vivo studies had been conducted to investigate the link between IL-8 and DNA fragmentation. The process of spermatogenesis and the maturation of the epididymis are responsible for the development of sperm characteristics, especially concentration, motility, and shape. The relationship between typical sperm parameters and DNA integrity is a hotly debated topic (Elbashir *et al.* 2018). This is due to the fact that the development of sperm characteristics is dependent on the maturation of the epididymis. DNA fragmentation is viewed as an independent measure of male fertility, independent of conventional sperm parameters, despite the fact that some authors support the idea that chromatin impairment is attributed to compromised conventional sperm parameters. DNA fragmentation is viewed as an independent measure of male fertility, independent of conventional sperm parameters (Santi *et al.* 2020).

2.8 IL-8 and Male Infertility

Inflammation is facilitated by the chemokine CXC-chemokine interleukin-8 (IL-8), also known as CXCL8 (Alfaro *et al.* 2017). The large family of chemokines is composed of small cytokines that share four conserved cysteine residues and are linked together by disulfide bonds. The first two cysteines, which are either adjacent in CC chemokines or separated by one amino acid in CXC chemokines, serve as a defining characteristic between the two subfamilies of chemokines (Saxena and Singh 2021). In CC chemokines, the first two cysteines are adjacent, while in CXC chemokines, the first two cysteines are either adjacent or separated. On chromosome 4, humans have a cluster of CXC chemokine genes, and on chromosome 17, humans have a cluster of CC chemokine genes. The transcription of the IL-8 gene results in the production of a protein that contains 99 amino acids (Nguyen *et al.* 2017). This protein is then processed, resulting in the production of a protein that contains 77 amino acids in non-immune cells and 72 amino acids in monocytes and macrophages, both of which are capable of signaling. Although other hormone-responsive elements and NF-IL-6 consensus sites have been characterized on the IL-8 gene promoter, the primary transcriptional regulators of IL-8 expression are activating proteins and/or nuclear Factor-B-mediated transcriptional activity (Kujundžić *et al.* 2019). This is the case despite the fact that the IL-8 gene promoter has been studied. Accordingly, it has been demonstrated that a wide variety of stimuli, including inflammatory signals (such as tumor necrosis factor and IL-1), chemical and environmental stressors (such as exposure to chemotherapeutic agents and hypoxia), and steroid hormones (e.g., androgens, estrogens, and dexamethasone), can all affect the amount of IL-8 that is expressed in a cell (Harshman *et al.* 2020).

The first CXC chemokines for which a three-dimensional structure has been determined are platelet factor four (PF4) and IL-8. In their monomeric structures, they each have an NH2-terminal loop, three antiparallel strands connected by loops, and a COOH-terminal helix. IL-8 is capable of forming globular dimers in a solution (Williamson 2018). These dimers are composed of two antiparallel helices that cross a six-stranded antiparallel sheet (composed of the three strands of each subunit) (Ciudad *et al.* 2020). It is generally assumed that the dimer is the biologically relevant form because most chemokines exist in solution as dimers, and this practice will continue until it is demonstrated that IL-8 can function as a monomer. In spite of the fact that biological activities can be observed at concentrations as low as nanomolar, the dissociation constants are almost always found in the micromolar range (Garnier *et al.* 2017). Data from size exclusion high-performance liquid chromatography (HPLC), analytical ultracentrifugation, chemical cross-linking, and titration microcalorimetry provide support for the conclusion that IL-8 occurs primarily as a monomer at physiological concentrations. This conclusion was drawn based on the findings of the previous three methods (Schünemann *et al.* 2019).

Chemokines exert their effects through membrane receptors that contain seven trans The binding of two G protein-coupled receptors on the surface of cells, known as CXCR1 and CXCR2, is the mechanism by which IL-8 exerts its biological effects (Sharma *et al.* 2018). It is possible that gene duplication led to the development of these receptors because of the striking structural similarity between them. Signals are transmitted across the membrane as a result of ligand-induced conformational changes (Lister *et al.* 2017). These changes expose epitopes on the intracellular loops and carboxyl-terminal tail of the receptor, which encourage coupling to functional heterotrimeric G proteins. In the past, researchers have found that the presence of pertussis toxin dampens the chemotactic response induced by CXC chemokine (Petri and Sanz 2018). This finding suggests that G is the primary G protein that is coupled to this family of receptors. On the other hand, the fact that some responses induced by IL-8 are not sensitive to pertussis toxin suggests that these receptors may couple to and activate additional G proteins that are not well understood at this time. It is possible that the promiscuous G protein coupling is caused by the differential cell-specific expression of the G proteins, which disrupts the affinity-based equilibrium that is established between the receptors and the intracellular pool of G proteins (Peterson and Luttrell 2017). On the basis of the expanding knowledge of the role that G subunits play in the process of signaling to primary effectors and the discovery that G protein-coupled receptors can also signal through additional non-G protein-dependent pathways, it is also possible to comprehend the various signaling pathways that are activated by CXCR1 and CXCR2. Both of these factors make it possible to comprehend the various signaling pathways that are activated by CXCR1 and CXCR2 (Meng *et al.* 2020).

Neutrophils include both the CXCR1 and CXCR2 receptors. The amino acid sequences of the two sets of genes are 77% identical and are situated near one another on chromosome 2q35. These two receptors show that the pharmacology of ligand binding differs dramatically between CXCR1 and CXCR2 (Yang *et al.* 2022). CXCR1 receptors do not become operational until after IL-8 and granulocyte chemotactic protein-2 have first bound to them. On the other hand, CXCR2 can be activated by a variety of CXC chemokines, such as the neutrophil-activating peptide, granulocyte chemotactic protein-2, growth-related oncogenes (GRO), and GRO (Alsharidah *et al.* 2017).

Even though monocytes, basophils, and eosinophils possess IL-8 receptors as well, their reaction to IL-8 is significantly less intense than that of neutrophils. The Northern blotting method did not find any T lymphocytes without either of the IL-8 receptors. This suggests that their levels of expression may be low (Wang *et al.* 2019). It was determined through fluorescence-activated cell sorting (FACS) analysis and the use of monoclonal antibodies that CXCR1 and CXCR2 are present on all neutrophils and monocytes but only on a small percentage of lymphocytes. This information was obtained from the study. They are not found in $CD4^+$ T cells or B cells, but they are found in NK cells and $CD8^+$ T cells, albeit in very low concentrations (Nicolai *et al.* 2020).

Depending on the function of the target cell, the pleiotropic effects of pro-inflammatory cytokines can act in one of three different ways: synergistically, additively, or antagonistically. IL-8 encourages the development of inflammatory reactions by collaborating with IL-1 to promote neutrophil activation, phagocytosis, and the removal of bacteria at the site of inflammation (Zeng *et al.* 2019). There are many different types of cells that are capable of secreting IL-8. Some of these cell types include activated T lymphocytes, monocytes, neutrophils, eosinophils, fibroblasts, synovial cells, adipocytes, endothelial, epithelial, and keratinocyte cells (Kitala *et al.* 2019). In addition to its primary function, which is to activate neutrophils by drawing them to sites of inflammation, it also has a potent pro-antigenic effect on basophils and T cells (Kitala *et al.* 2019).

2.9 IGF-1 and Sperm DNA Fragmentation

It is possible to improve fertilization through the manipulation of sperm both in vivo and in vitro, as well as through the manipulation of spermatozoa for clinical IVF (Beirão *et al.* 2019). One method for controlling the behavior of sperm is called the centrifugation of spermatozoa. During the process of spermatozoa capacitation, which is both biochemical and physiological, a number of complex reactions take place. During the capacitation process, events such as the activation of enzymes, the motility of spermatozoa, and the modification and characterization of membranes take place (Gautam *et al.* 2019). One of the disadvantages of centrifuging the sperm is that it leads to an increase in the production of ROS by the spermatozoa. The increase in ROS production that occurs after centrifugation is thought to be the result of a complex process that can be caused by chemical reactions that take place either within or outside of the organelles of the cell. In addition to their role in the fusion of spermatozoa and oocytes, reactive oxygen species are also critically important in the induction of hyperactivity and capacitation, as well as the acrosome reaction (Mahajan *et al.* 2022). However, when there is an abundance of ROS, the antioxidant defense mechanisms in spermatozoa and seminal plasma are unable to combat their effects. As a result of this excess, DNA chain reactions will be stopped, glycolytic enzymes will become inactive, fatty acids, particularly polyunsaturated fatty acids, which are crucial components of the phospholipid layer of the sperm membrane, will be damaged, sperm motility will be reduced, and sperm will eventually die (Nowicka-Bauer and Nixon 2020).

The building blocks of sperm are called spermatozoa, and they are found suspended in a fluid medium called seminal plasma. A fluid called seminal plasma, which is complex fluid, mediates the chemical function of the ejaculate (Morgan and Watkins 2020). Insulin-like growth factor (IGF-I) is one of the components that can be found in seminal plasma. It has been hypothesized that this growth factor plays a direct or indirect role in the processes of steroid genesis and spermatogenesis in the testes and that the dysregulation of this process is one of the contributing factors to male infertility. A protein known as the IGF-I complex can be found in seminal plasma. IGF-I stands for insulin-like growth factor I (Van Doorn 2020).

A complex consisting of three different protein molecules is produced whenever insulin-like growth Factor-I binds to an insulin-like growth factor-binding protein. A single molecule of insulin-like growth Factor-I (subunit) with a molecular weight of 7.6 kDa, a single molecule of insulin-like growth factor binding protein (subunit) with a molecular weight of 53 kDa, and a single molecule of an acid-labile subunit (subunit) with a molecular weight of 53 kDa are all components of this complex (Yan *et al.* 2017). The acid-labile subunit that associates with IGF-I contributes to an increase in molecular weight by imparting a complicated function to the bond that exists between IGF and IGF-3. Seminal plasma, which is produced by Leyden and Sterol cells in the testes, has been found to contain IGF-I. These cells are responsible for its production. Reports indicate that the growth factor IGF-I plays a significant role in the motility of spermatozoa as well as the maturation and development of germ cells (Castro-Arnau *et al.* 2022).

3. MATERIALS AND METHODS

3.1 Study Subjects

This prospective study was carried out between November 2021 and February 2022. In addition to the Toxicity Consultation Center and the Specialist Surgeries Hospital, the Kamal al-Samarea Hospital can be found within Baghdad's Medical City. It is generally agreed that these two hospitals are among the most important medical facilities in all of Baghdad. After taking out all of the patients whose seminal fluid parameters were already within the normal range and all of the office workers, this study included a total of 120 males with abnormal seminal fluid analyses, military workers, and atypical body forms. Patients with atypical body types were more likely to have abnormal seminal fluid analyses than patients with typical body types. They were split up into two groups, one for the patients and one for the controls; a spot in one of the groups was allotted to each individual patient. The age range of the male patients in the patient group was from 19 to 51 years old, and there were a total of 80 male patients. These patients were separated into two subgroups: fifty patients with newly diagnosed primary infertility and thirty patients with secondary infertility. Both groups of patients had been attempting to conceive for a period of two years. Before collecting the samples for the study, seminal fluid analysis (also known as SFA) was performed on each individual patient because the patients who took part in the research project all had abnormalities in the characteristics of their seminal fluid. Forty healthy males with a family size of at least two children and no known history of any kind of systemic issue were recruited for the purpose of acting as a control group for the study. Every patient was given a questionnaire case sheet, which was then filled out with the patient's age, occupation, infertility type, infertility time, environment, surgical history, smoking history, and a report on the patient's previous medical history. The questionnaire case sheets were then given back to the patients. In addition, a report on the examination of the seminal fluid was provided to every patient.

3.1.1 Sample collection

Following the collection of 5 milliliters of venous blood from each patient, the blood was placed in a gel tube, allowed to coagulate, and then centrifuged at a rate of 3000 revolutions per minute for a period of 5 minutes in order to obtain blood sera. After that, the blood sera were poured into tiny tubes, labeled, and put in a freezer at a temperature of -20 degrees Celsius until it was time to measure the amounts of IL-8 and IGF-1.

3.2 Materials

3.2.1 Instruments

The instruments used in the study are shown in Table 3.1.

Table 3.1 List of instruments

Instrument	Company	Country
ELISA instrument	BIOTEK	USA
Centrifuge	Kokusan	Japan
Incubator	Yamato	Japan
Shaker	TKA	Italy
Deep freezer (-20℃)	Electrolux	England
Refrigerator	Dynex	Turkey
Mglomy	China	China
Microscope	Novel	China
Disposable syringes	AFCO	Jordan
Eppendrof tubes	Local	Iraq
Glass slides	Local	USA
Tips (different size)		
Urine specimen cup		

3.2.2 Reagents

The reagents used in the study are shown in Table 3.2.

Table 3.2 List of kits and reagents

Kit and reagent	Company	Country
IL-8 kit	Biomerieux	France
IGF-1	China	China
Aniline Blue	Sigma-Aldrich Chemie	USA

3.3 Methods

3.3.1 Assessment of IL-8

The frozen serum was thawed at room temperature to measure the IL-8 by using an ELISA instrument.

Principle of ELISA;

The fundamental principle of the test was carried out using a commonly available kit and the quantitative sandwich enzyme immunoassay method.

The kit we employ is based on the Enzyme-Linked Immunosorbent Assay (ELISA), a technology based on a biotin double antibody sandwich.

The wells had previously been pre-coated with an IL-8 monoclonal antibody. After adding IL-8 to the wells, the plates were incubated. Next, biotin-tagged IL-8 antibodies were added so they could combine with streptavidin-HPR to form an immunological complex. The plates were then washed to remove any unbound enzymes. This was done to make sure that there were no unattached enzymes.

After that, substrates A and B were introduced, and the complex then underwent an acidic reaction, which caused it to change color from purple to yellow.

3.3.2 Assessment of IGF-1

IGF-1 measurements were made using the meglomy device.

3.3.3 Detection of DFI (DNA fragmentation index)

It took 10 microns of sperm fluid that had been liquified and dispersed over the slide, followed by 3-5 minutes of drying time, in order to fix the sperm that were already present on the slide. After that, the slide was cooked in a 4% formalin solution for 25 minutes in a special cooker.

After the allotted 25 minutes had elapsed, the slide was removed from the formalin and set aside to dry. After the slide was allowed to dry completely, it was immersed in pigment (aniline blue) for five minutes.

After five minutes, the slide was lightly burned to remove excess pigment and set aside to dry before moving on to the next reading.

3.4 Statistical Analysis

The data were initially pre-coded before being entered into a computer running version 18 of the Statistical Package for Social Science software. This enabled statistical analysis to be performed on the data (using SPSS). In order to construct a summary of the data, we made use of the mean as well as the standard deviation. For the purpose of doing group comparisons, the independent sample t-test was utilized, and it was applied to the quantitative variables in question. For the purpose of determining the degree of relationship between the various variables, Pearson correlation coefficients were computed (Source: SPSS Inc., Chicago, Illinois, United States).

The comparison of significance (p-value) in any test was considered as follows:

- P value<0.05 was considered statistically significant (S)
- P value<0.01 was considered highly significant (HS)
- P value>0.05 was considered non-significant (NS)

4. RESULTS AND DISCUSSION

It has been shown that variations in the levels of IGF-I found in seminal plasma can have an effect on the stimuli for the growth and maturation of germ cells as well as the motility of spermatozoa. As a result, the objective of this research was to investigate the viability of employing the insulin-like growth factor I (IGF-1) complex in order to improve the quality of sperm that had previously been incubated (Malivindi *et al.* 2018) (Table 4.1).

Table 4.1 Analysis of the parameters with comparisons between the various groups

Group	**Mean ± SE**		
	DFI	**IL-8**	**IGF-1**
Control	15.80 ±0.85 b	50.45±3.06	49.55 ±3.58 a
Teratospermia	52.85 ±2.58 a	44.87 ±3.47	7.12 ±0.52 b
Astenosprmia	56.32 ±2.68 a	52.50 ±2.86	8.67 ±0.67 b
LSD value	6.180 **	8.809 NS	5.951 **
P-value	0.0001	0.211	0.0001
Significant differences were found between the means having different letters in the same column (P≤0.01) Averages that carry different letters differ significantly, and averages that carry similar letters do not differ significantly. The highest average takes the letter a, and so on downward. If there is an average, it takes two letters, such as ab. This does not differ from the average that carries a or from the average that carries b.			

4.1 The Distribution of IL-8 Among Study Group

The median levels of IL-8 in astenospermia were 52.50±2.86, whereas they were 44.87±3.47 in teratospermia and 50.45±3.06 in comparison to the control group, with a non-significant relationship (p-value of 0.211), as shown in Figure 4.1.

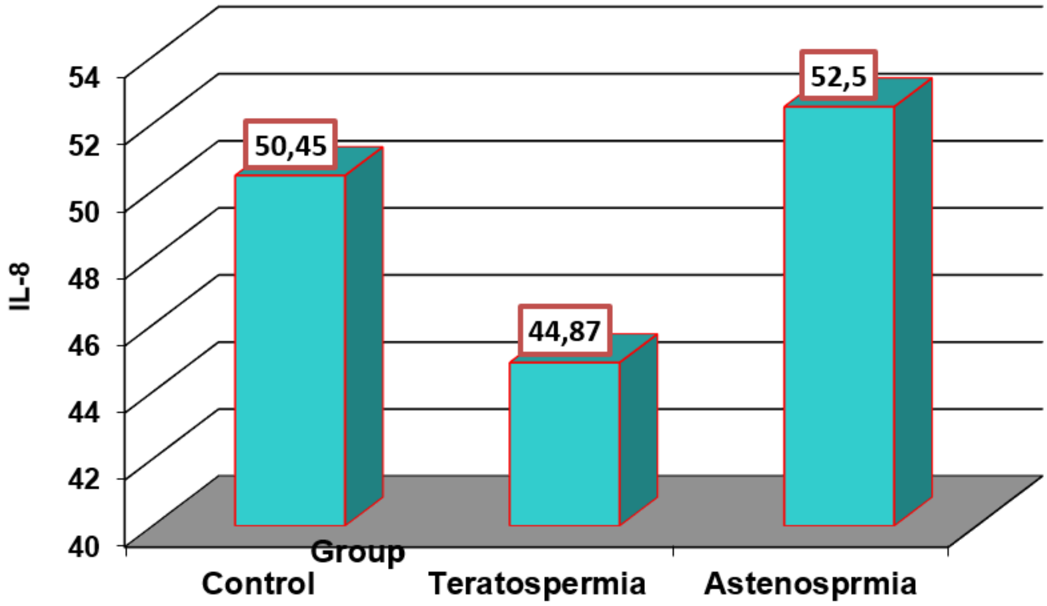

Figure 4.1 Comparison between difference groups in IL-8

4.2 The Distribution of DFI Among Study Group

The mean DFI in asthenospermia was 56.32±2.68, while the mean DFI in teratospermia was 52.85±2.58 and 15.80±0.85 compared to the control group, a non-significant relationship with a p-value of 0.0001, as shown in Figure 4.2.

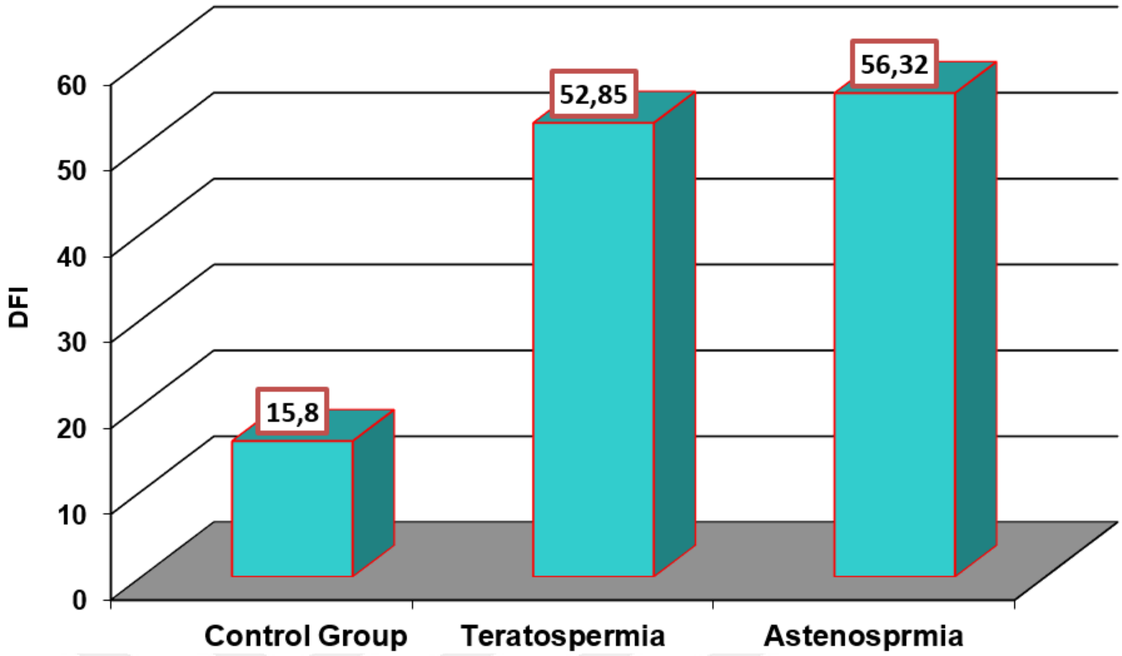

Figure 4.2 Comparison between difference groups in DFI

4.3 The Distribution of IGF-1 Among Study Group

The mean IGF-1 was 8.67±0.67 in asthenospermia, 7.12±0.52 in teratospermia, and 49.55±3.58 compared to the control group, a non-significant association with a p-value of 0.0001, as shown in Figure 4.3

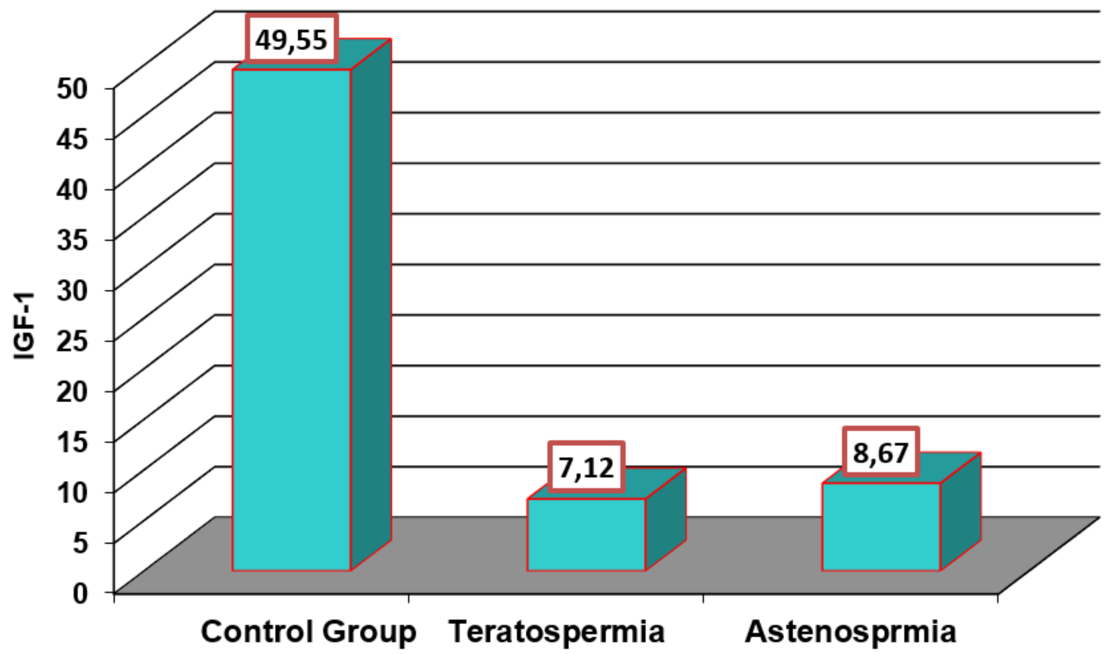

Figure 4.3 Comparison between difference groups in IGF-1

4.4 Discussion

Sperm DNA fragmentation, which can also result in abnormal embryo quality and lower conception rates, may have an impact on male fertility. Reactive oxygen species, aberrant chromatin packing, and apoptosis are some of the factors that contribute to fragmentation. The maturity of the oocyte and the mother's age are two variables that affect the ability of oocytes to repair damage. Our results suggest that DNA fragmentation affects.

IL-8 is a type of protein that plays a role in inflammation and the immune response. Elevated levels of IL-8 in sperm have been linked to teratospermia and other types of male infertility, according to research. The mechanism by which IL-8 influences sperm shape may involve oxidative stress and inflammation, both of which can alter sperm function and structure (Luo *et al.* 2018).

IGF is a hormone-like substance that plays an important role in cell growth and development regulation. It has a similar structure to insulin and acts on cells in the same way, promoting cell proliferation and inhibiting apoptosis (cell death). White blood cells

(WBC), primarily macrophages, produce inflammatory cytokines in response to foreign antigens and pathogens (infection challenge) and also in chronic inflammation (immunological activation). Acute and chronic infections may contribute to male infertility. However, the clinical significance of a silent infection in asymptomatic patients is unclear. Furthermore, the interpretation of the markers commonly used for diagnosis is debatable, such as the role of seminal leukocytes and clinically significant thresholds.

Even though there wasn't a statistically significant difference, IL-8 levels were much higher in samples from oligozoospermic men whose sperm were less mobile and had a lower total number of mobile sperm. Standard morphology, viability, or seminal plasma fructose concentration had no relationship.

Higher IL-8 levels were linked to decreased sperm motility and a lower total motile sperm count, but not to other factors like standard morphology, viability, or seminal plasma fructose concentration.

There is a strong link between the number of leukocytes and the amount of IL-8 in sperm and in the seminal fluid. High levels of IL-8 were found in 52.5% of samples with high leukocyte ratios, and the median levels of IL-8 in samples with leukocytospermia were four times higher. Low IL-8 levels did not result in There was also a link between IL-8 concentrations and the total number of leukocytes in the ejaculate. Also, leukocyte screening results showed a significant correlation with IL-8 concentrations in seminal fluid, with positive paper strip testing in 30.3% of samples with high IL-8.

IL-8 levels in seminal fluid did not correlate with testicular volume, consistency, or previous genital trauma, surgery, mumps, etc. IL-8 concentrations were higher in patients who had previously had genital tract infections, but the differences were not statistically significant. The duration of infertility, as well as stress related to work or infertility, had no significant association with IL-8 concentrations. Patients reporting occasional sexual dysfunction had a slightly higher frequency of high IL-8 concentrations, but this was not statistically significant. Finally, there was no statistically significant relationship between IL-8 concentration and male age.

The amount of IL-8 in the seminal plasma was linked to the number of leukocytes in the seminal fluid and to some clinically important measures of sperm quality, but not to the results of sperm cultures. All testing was performed under standardized conditions, and the same individuals performed functional assays. The investigators were not aware of the IL results throughout the study, which allowed for the exclusion of inter-observer variations.

Cytokines rarely act in isolation, and the concentrations of IL-8 in the seminal fluid were much higher compared to serum concentrations. The concentrations of IL-8 were significantly related and were within a wide inter-individual range. However, the results of this study and other studies are limited by the one-point determination of these interleukins.

These results were consistent with the findings of a previous review. Testing was done in a controlled environment. After at least five days of sexual abstinence, all ejaculates were collected in the hospital early in the morning (between 7:00 and 9:00), and they were all immediately analyzed after liquefaction. Antibiotics were not administered to any of the guys while the trial was ongoing. The same persons (R.B. and M.H.) carried out functional studies including sperm movement testing, SCMPT, immunocytochemical round cell differentiation, and radial immunodiffusion. Since neither researcher was aware of the IL outcomes during the entire investigation, any inter-observer differences could be ruled out (Humar *et al.* 1999).

Cytokines rarely act in isolation in cytokine networks, and the concentrations of IL-8 in the seminal fluid were much higher than in serum. The concentrations of IL-8 were significantly interrelated and varied greatly between individuals. The results of this and other studies, though, are limited by the fact that these interleukins were only measured at one point. Future research will need to show intra-individual variation over a longer period of time as well as possible factors that could affect the results. Populations of cytokine-soluble receptors must also be considered.

Cytokines may also be involved in the regulation of sperm motility and capacitation, as well as sperm-female genital tract interaction. For example, it has been demonstrated that IL-8 increases sperm motility and has a chemotactic effect on spermatozoa. These findings imply that cytokines may play a role in male fertility in addition to their role in the immune system.

The results of some studies have shown that Strong polypeptides called cytokines are generated by inflammatory cells as components of the host response. Numerous cell types, including neutrophils, T cells, and monocytes and macrophages, can release IL-8 in response to a range of signals, which are usually triggered by infection or injury. According to multiple studies (Rees 1992, Rutanen 1993, Fiocchi *et al.* 1994, Galley and Webster 1996, Grimm *et al.* 1996, Henderson *et al.* 1996, Sharkey 1998), they are important mediators of a variety of physiological and pathological processes. Allograft rejection also involves cytokines. Major depression has been linked to an increased production of several pro-inflammatory cytokines during immunological responses (Maes *et al.* 1997). The production of cytokines is also significantly influenced by genetic variables (Westendorp *et al.* 1997).

Overall, the complex and multifactorial nature of male infertility makes determining the precise role of cytokines in the pathogenesis of this condition difficult.

Furthermore, the development of new techniques for detecting and measuring sperm cytokines may provide new insights into the mechanisms underlying male infertility, leading the way to the development of novel diagnostic and therapeutic strategies.

Chronic exposure to IL-8 can make these receptors less sensitive, which can change how leukocytes move in response to chemicals and could make you more likely to get sick. IL-8 also attracts lymphocyte populations to the site of emission, which can influence the immune response and potentially contribute to tumor development and antitumor response suppression.

Insulin-like growth factor (IGF) has been shown to increase testosterone production, increase the number of LH receptors in Leydig cells, promote thymidine inclusion in Sertoli cell DNA, control glucose and lactate metabolism in Sertoli cells, and stimulate the production of plasminogen activator in Sertoli cells. Moreover, IGF is required for the survival of the Sertoli population as well as the development, formation, and migration of germ cells (Qin and Xu 2022).

The insulin growth factor family, which includes insulin and insulin-like growth factors I and II, is critical for controlling growth, metabolism, and reproduction. The insulin/IGF system regulates cell growth, proliferation, differentiation, and survival in nearly every organ and system in the body, including testis development and function. Local gonadal factors like the insulin/IGF family influence reproductive performance, and IGF signaling is essential for both body growth and development and reproductive function.

Low IGF-1 levels have been linked to poor sperm morphology and motility. Growth hormone and IGF-1 have been shown in lab animals and humans to influence Sertoli cell proliferation and testicular volume, both of which are associated with sperm output. More research is needed, however, to determine the exact mechanism of action of IGF-1 on spermatogenesis.

While IGF-1 has been shown to impair human sperm motility in vitro, growth hormone treatment has been shown to increase seminal plasma IGF-1 and sperm motility in males suffering from infertility. IGF-1 production stimulates spermatozoa maturation in a paracrine or autocrine manner, increasing sperm motility and IGF-1 levels that correlate linearly and significantly with total sperm count.

The correlation between serum or seminal IGF-1 and semen quality has only been examined in a small number of studies. The lowest mean and median values of seminal

IGF-1 were seen in the studies' male subjects who were infertile because of oligospermia. These levels were considerably different from those in the immuno-infertile group, the fertile group, and the other subgroups of male-factor infertiles. These results imply that oligospermia and decreased seminal IGF-1 levels are related (Colombo and Naz 1999, Qin and Xu 2022). In this investigation, we did find a significant association between semen parameters and the serum level of IGF-1; however, we did not notice a significant relationship between semen parameters and seminal IGF-1.

Male smokers have been found to have lower amounts of IGF-1 in their seminal fluid and morphologically normal sperm. Particularly, infertile smokers, infertile nonsmokers, fertile smokers, and control participants had the lowest seminal IGF-1 values (Hassan *et al.* 2009). These results suggest that smoking and infertility are linked to decreased IGF-1.

Additionally, it has been demonstrated that seminal IGF-1 significantly positively correlates with the quantity of rapidly moving and motile spermatozoa (Lee *et al.* 1995). In a paracrine or autocrine mechanism, IGF-1 synthesis promotes spermatozoa maturation, improving sperm motility. So, in male Germ cells, IGF-1 might serve as a differentiation marker. Additionally, Colombo and Naz (2020) demonstrated a substantial linear association between the seminal IGF-1 level and the overall sperm count. Despite being indirectly examined in the current study; group D's sperm count is indirectly considered. We saw lower seminal IGF-1 levels in this group, which is consistent with earlier studies, but the difference was not statistically significant. The people in the normal sperm parameter group were also male patients who were infertile, which poses a drawback to this study's analysis of IGF-1 levels.

We discovered that, when compared to the normal group, the groups with aberrant sperm parameters had considerably lower serum IGF-1 levels. However, the groups examined here did not significantly differ in terms of seminal plasma IGF-1 levels. To pinpoint the precise mechanisms by which GH and IGF-1 affect fertility, more research is necessary.

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

Sperm DNA fragmentation, which can lead to suboptimal embryo quality and lower conception rates, may have an effect on male fertility. Aploches, abnormal chromatin packing, reactive oxygen species, and others are some of the elements that cause fragmentation. Our findings imply that DNA fragmentation had an impact.

Although there was no statistically significant difference, IL-8 concentrations in oligozoospermic men were considerably greater in samples with lower motility and a lower total motile sperm count.

Additionally, cytokines may regulate sperm-female genital tract contact as well as sperm motility and capacitation. IL-8, for instance, has been shown to improve sperm motility and have a chemotactic effect on spermatozoa.

In a paracrine/autocrine mechanism, IGF-1 synthesis promotes spermatozoa maturation, and IGF-1 levels that significantly and linearly correspond to total sperm count.

5.2 Recommendation

Men experiencing infertility should seek out specialized medical professionals and follow their instructions, recommendations, and treatments in order to overcome the condition.

Alternatively, exposure to pollutants and treatments that have detrimental effects on sperm or weaken the male reproductive system can potentially result in the absence of sperm production.

Men who have a background of pollutant exposure or have been assessed to have a changeable lifestyle risk factor should be given the opportunity for Sperm DNA

Fragmentation (SDF) testing. This test can help predict fertility potential, emphasize the importance of lifestyle modifications (such as quitting smoking and receiving antioxidant therapy), and monitor the patient's response to treatment.

REFERENCES

Abazari, M., Ghaffari, A., Rashidzadeh, H., Badeleh, S. M. and Maleki, Y. 2022. A systematic review on classification, identification, and healing process of burn wound healing. The International Journal of Lower Extremity Wounds, 21(1): 18-30.

Abbasi, A. A., Mazinani, M. and Hosseini, R. 2020. Chaotic evolutionary-based image encryption using RNA codons and amino acid truth table. Optics & Laser Technology, 132: 106465.

Abdelhedi, F., Chalas, C., Petit, J. M., Abid, N., Mokadem, E., Hizem, S. and Dupont, J. M. 2019. Altered three-dimensional organization of sperm genome in DPY19L2-deficient globozoospermic patients. Journal of Assisted Reproduction and Genetics, 36: 69-77.

Adams, T. E., Epa, V. C., Garrett, T. P. J. and Ward, C. W. 2000. Structure and function of the type 1 insulin-like growth factor receptor. Cellular and Molecular Life Sciences CMLS, 57: 1050-1093.

Aghajani, M., Mansoori, B., Mohammadi, A., Asadzadeh, Z. and Baradaran, B. 2019. New emerging roles of CD133 in cancer stem cell: Signaling pathway and miRNA regulation. Journal of Cellular Physiology, 234(12): 21642-21661.

Aghazarian, A., Huf, W., Pflüger, H. and Klatte, T. 2019. The association of seminal leucocytes, interleukin-6 and interleukin-8 with sperm DNA fragmentation: A prospective study. Andrologia, 51(11): e13428.

Aitken, R. J. 2022. Role of sperm DNA damage in creating de novo mutations in human offspring: the ‘post-meiotic oocyte collusion’hypothesis. Reproductive Biomedicine Online, 22(1):1-9.

Aitken, R. J., De Iuliis, G. N. and Nixon, B. 2020. The sins of our forefathers: paternal impacts on de novo mutation rate and development. Annual Review of Genetics, 54: 1-24.

Akhondi, M. M., Ranjbar, F., Shirzad, M., Ardakani, Z. B., Kamali, K. and Mohammad, K. 2019. Practical difficulties in estimating the prevalence of primary infertility in Iran. International Journal of Fertility & Sterility, 13(2): 113.

AlAshqar, A., Patzkowsky, K., Afrin, S., Wild, R., Taylor, H. S. and Borahay, M. A. 2019. Cardiometabolic risk factors and benign gynecologic disorders. Obstetrical & Gynecological Survey, 74(11): 661.

Alfaro, C., Sanmamed, M. F., Rodríguez-Ruiz, M. E., Teijeira, Á., Oñate, C., González, Á. and Melero, I. 2017. Interleukin-8 in cancer pathogenesis, treatment and follow-up. Cancer Treatment Reviews, 60: 24-31.

Allard, J. B. and Duan, C. 2018. IGF-binding proteins: why do they exist and why are there so many?. Frontiers in Endocrinology, 9: 117.

Alsharidah, A. S., Alzogaibi, M. A., Bayoumy, N. M. and Alghonaim, M. 2017. Neutrophil chemokines levels in different stages of nephrotic syndrome. Saudi Journal of Kidney Diseases and Transplantation, 28(6): 1256-1263.

Ariyasinghe, N. 2019. Biophysical characterization of cancer cell phenotypes. PhD. Thesis, Arizona State University, 93 page, Arizona.

Avazi, D. O., Awasum, A. C., Hassan, A. Z., Ayo, J. O., Aluwong, T., Simon, A. Y. and Kudi, A. C. 2019. Evaluation of levels of interleukin-6, interleukin-8 and some haematologic parameters of dogs with cutaneous wounds. Cytokine, 113: 128-138.

Barbieri, I. and Kouzarides, T. 2020. Role of RNA modifications in cancer. Nature Reviews Cancer, 20(6): 303-322.

Beirão, J., Boulais, M., Gallego, V., O'Brien, J. K., Peixoto, S., Robeck, T. R. and Cabrita, E. 2019. Sperm handling in aquatic animals for artificial reproduction. Theriogenology, 133: 161-178.

Belser, C., Baurens, F. C., Noel, B., Martin, G., Cruaud, C., Istace, B. and Aury, J. M. 2021. Telomere-to-telomere gapless chromosomes of banana using nanopore sequencing. Communications Biology, 4(1): 1047.

Ben Maamar, M., Nilsson, E. E. and Skinner, M. K. 2021. Epigenetic transgenerational inheritance, gametogenesis and germline development. Biology of Reproduction, 105(3): 570-592.

Berkamp, S. 2018. Interaction studies of the Chemokine Interleukin-8 with the G protein-coupled receptor CXCR1 by nuclear magnetic resonance spectroscopy. PhD. Thesis, University of California, 141 page, San Diego.

Birkenmeier, G., Glander, H. J., Kratzsch, J. and Weisbrich, C. 1996. Insulin-like growth factor-I and alpha 2–macroglobulin in seminal plasma. Human Reprod, 11(11): 2454-60.

Bisht, S. and Dada, R. J. F. B. 2017. Oxidative stress: Major executioner in disease pathology, role in sperm DNA damage and preventive strategies. Front Biosci, 9(3): 420-47.

Boguenet, M., Bouet, P. E., Spiers, A., Reynier, P. and May-Panloup, P. 2021. Mitochondria: their role in spermatozoa and in male infertility. Human Reproduction Update, 27(4): 697-719.

Bohmwald, K., Gálvez, N. M., Canedo-Marroquín, G., Pizarro-Ortega, M. S., Andrade-Parra, C., Gómez-Santander, F. and Kalergis, A. M. 2019. Contribution of cytokines to tissue damage during human respiratory syncytial virus infection. Frontiers in Immunology, 10: 452.

Boycott, K. M., Rath, A., Chong, J. X., Hartley, T., Alkuraya, F. S., Baynam, G. and Lochmüller, H. 2017. International cooperation to enable the diagnosis of all rare genetic diseases. The American Journal of Human Genetics, 100(5): 695-705.

Boyles, J. S., Beidler, C. B., Strifler, B. A., Girard, D. S., Druzina, Z., Durbin, J. D. and Witcher, D. R. 2020. Discovery and characterization of a neutralizing pan-ELR+ CXC chemokine monoclonal antibody. Taylor & Francis, 12(1): 183-188.

Brown, T. A. 2020. Gene cloning and DNA analysis: an introduction. John Wiley & Sons, 432 page, New York.

Buffone, A., Anderson, N. R. and Hammer, D. A. 2019. Human neutrophils will crawl upstream on ICAM-1 if Mac-1 is blocked. Biophysical Journal, 117(8): 1393-1404.

Campbell, R., Feeney, H., Fehler-Cabral, G., Shaw, J. and Horsford, S. 2017. The national problem of untested sexual assault kits (SAKs): Scope, causes, and future directions for research, policy, and practice. Trauma, Violence and Abuse, 18(4): 363-376.

Camunas-Soler, J., Lee, H., Hudgins, L., Hintz, S. R., Blumenfeld, Y. J., El-Sayed, Y. Y. and Quake, S. R. 2018. Noninvasive prenatal diagnosis of single-gene disorders by use of droplet digital PCR. Clinical Chemistry, 64(2): 336-345.

Cannarella, R., Mancuso, F., Arato, I., Lilli, C., Bellucci, C., Curto, R. and Calogero, A. E. 2022. Sperm-carried IGF2 Downregulates the Expression of Mitogens Produced by Sertoli Cells: A Paracrine Mechanism for Regulating Spermatogenesis?. Frontiers in Endocrinology, 2997.

Capanoglu, E., Kamiloglu, S., Demirci Cekic, S., Sozgen Baskan, K., Avan, A. N., Uzunboy, S. and Apak, R. 2020. Antioxidant activity and capacity measurement. Plant Antioxidants and Health, 1-66.

Castro-Arnau, J., Chauvigné, F., Gómez-Garrido, J., Esteve-Codina, A., Dabad, M., Alioto, T. and Cerdà, J. 2022. Developmental RNA-Seq transcriptomics of haploid germ cells and spermatozoa uncovers novel pathways associated with teleost spermiogenesis. Scientific Reports, 12(1): 1-18.

Cecchele, A., Cermisoni, G. C., Giacomini, E., Pinna, M. and Vigano, P. 2022. Cellular and molecular nature of fragmentation of human embryos. International Journal of Molecular Sciences, 23(3): 1349.

Chalkley, R. J. and Baker, P. R. 2017. Use of a glycosylation site database to improve glycopeptide identification from complex mixtures. Analytical and Bioanalytical Chemistry, 409: 571-577.

Chapuis, A., Gala, A., Ferrières-Hoa, A., Mullet, T., Bringer-Deutsch, S., Vintejoux, E. and Hamamah, S. 2017. Sperm quality and paternal age: effect on blastocyst formation and pregnancy rates. Basic and Clinical Andrology, 27(1): 1-9.

Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J. and Zhao, L. 2018. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget, 9(6): 7204.

Cheng, H., Huang, H., Guo, Z., Chang, Y. and Li, Z. 2021. Role of prostaglandin E2 in tissue repair and regeneration. Theranostics, 11(18): 8836.

Cheng, H., Shang, D. and Zhou, R. 2022. Germline stem cells in human. Signal Transduction and Targeted Therapy, 7(1): 345.

Ciudad, S., Puig, E., Botzanowski, T., Meigooni, M., Arango, A. S., Do, J. and Carulla, N. 2020. Aβ (1-42) tetramer and octamer structures reveal edge conductivity pores as a mechanism for membrane damage. Nature Communications, 11(1): 3014.

Cohen, S., Puget, N., Lin, Y. L., Clouaire, T., Aguirrebengoa, M., Rocher, V. and Legube, G. 2018. Senataxin resolves RNA: DNA hybrids forming at DNA double-strand breaks to prevent translocations. Nature Communications, 9(1): 533.

Colombo, J. B. and Naz, R. K. 1999. Modulation of insulin-like growth factor-1 in the seminal plasma of infertile men. Journal of Andrology, 20(1): 118-125.

Coonen, E., Rubio, C., Christopikou, D., Dimitriadou, E., Gontar, J., De Rycke, M. and ESHRE PGT-SR/PGT-A Working Group. 2020. ESHRE PGT Consortium good practice recommendations for the detection of structural and numerical chromosomal aberrations. Human Reproduction Open, 2020(3): hoaa017.

Crudden, C., Shibano, T., Song, D., Suleymanova, N., Girnita, A. and Girnita, L. 2018. Blurring boundaries: receptor tyrosine kinases as functional G protein-coupled receptors. International Review of Cell and Molecular Biology, 339: 1-40.

Cuccui, J., Terra, V. S., Bossé, J. T., Naegeli, A., Abouelhadid, S., Li, Y. and BRaDP1T Consortium. 2017. The N-linking glycosylation system from Actinobacillus pleuropneumoniae is required for adhesion and has potential use in glycoengineering. Open Biology, 7(1): 160212.

Cuéllar-Cruz, M., Islas, S. R., González, G. and Moreno, A. 2019. Influence of nucleic acids on the synthesis of crystalline Ca (II), Ba (II), and Sr (II) silica–carbonate biomorphs: implications for the chemical origin of life on primitive Earth. Crystal Growth & Design, 19(8): 4667-4682.

Cui, N., Hu, M. and Khalil, R. A. 2017. Biochemical and biological attributes of matrix metalloproteinases. Progress in Molecular Biology and Translational Science, 147: 1-73.

Cunningham, R. M., Walton, M. A. and Carter, P. M. 2018. The major causes of death in children and adolescents in the United States. New England Journal of Medicine, 379(25): 2468-2475.

Curtin, N. J. and Szabo, C. 2020. Poly (ADP-ribose) polymerase inhibition: past, present and future. Nature Reviews Drug Discovery, 19(10): 711-736.

Czepiel, J., Biesiada, G., Dróżdż, M., Gdula-Argasińska, J., Żurańska, J., Marchewka, J. and Garlicki, A. 2018. The presence of IL-8+ 781 T/C polymorphism is associated with the parameters of severe Clostridium difficile infection. Microbial Pathogenesis, 114: 281-285.

Dahan, M. H., Mills, G., Khoudja, R., Gagnon, A., Tan, G. and Tan, S. L. 2021. Three hour abstinence as a treatment for high sperm DNA fragmentation: a prospective cohort study. Journal of Assisted Reproduction and Genetics, 38: 227-233.

Darbandi, M., Darbandi, S., Agarwal, A., Sengupta, P., Durairajanayagam, D., Henkel, R. and Sadeghi, M. R. 2018. Reactive oxygen species and male reproductive hormones. Reproductive Biology and Endocrinology, 16(1): 1-14.

David, B. A. and Kubes, P. 2019. Exploring the complex role of chemokines and chemoattractants in vivo on leukocyte dynamics. Immunological Reviews, 289(1): 9-30.

De Iuliis, G. N., Newey, R. J., King, B. V. and Aitken, R. J. 2009. Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa in vitro. PloS One, 4(7): e6446.

Derakhshan, Z., Ferrante, M., Tadi, M., Ansari, F., Heydari, A., Hosseini, M. S. and Sadrabad, E. K. 2018. Antioxidant activity and total phenolic content of ethanolic extract of pomegranate peels, juice and seeds. Food and Chemical Toxicology, 114: 108-111.

Derkach, K. V., Bakhtyukov, A. A., Morina, I. Y., Romanova, I. V., Bayunova, L. V. and Shpakov, A. O. 2022. Comparative study of the restoring effect of metformin, gonadotropin, and allosteric agonist of luteinizing hormone receptor on spermatogenesis in male rats with Streptozotocin-induced Type 2 Diabetes Mellitus. Bulletin of Experimental Biology and Medicine, 172(4): 435-440.

Deshpande, P. S. and Gupta, A. S. 2019. Causes and prevalence of factors causing infertility in a public health facility. Journal of Human Reproductive Sciences, 12(4): 287.

Dissanayake, D. M. I. H., Keerthirathna, W. L. R. and Peiris, L. D. C. 2019. Male infertility problem a contemporary review on present status and future perspective. SAGE, 3: 1-7.

Dos Santos Hamilton, T. R. and Assumpção, M. E. O. D. Á. 2020. Sperm DNA fragmentation: causes and identification. Zygote, 28(1): 1-8.

Dostert, C., Grusdat, M., Letellier, E. and Brenner, D. 2019. The TNF family of ligands and receptors: communication modules in the immune system and beyond. Physiological Reviews, 99(1): 115-160.

Dutta, S., Henkel, R., Sengupta, P. and Agarwal, A. 2020. Physiological role of ROS in sperm function. Male Infertility: Contemporary Clinical Approaches, Andrology, ART and Antioxidants, 337-345.

Elbashir, S., Magdi, Y., Rashed, A., Ibrahim, M. A., Edris, Y. and Abdelaziz, A. M. 2018. Relationship between sperm progressive motility and DNA integrity in fertile and infertile men. Middle East Fertility Society Journal, 23(3): 195-198.

Ellwanger, J. H., Valverde-Villegas, J. M., De Lima Kaminski, V., De Medeiros, R. M., De Matos Almeida, S. E., Santos, B. R. and Chies, J. A. B. 2020. Increased IL-8 levels in HIV-infected individuals who initiated ART with CD4+ T cell counts< 350 cells/mm3–A potential hallmark of chronic inflammation. Microbes and Infection, 22(9): 474-480.

El-Mansi, S. and Nightingale, T. D. 2021. Emerging mechanisms to modulate VWF release from endothelial cells. The International Journal of Biochemistry & Cell Biology, 131: 105900.

Errichelli, L., Dini Modigliani, S., Laneve, P., Colantoni, A., Legnini, I., Capauto, D. and Bozzoni, I. 2017. FUS affects circular RNA expression in murine embryonic stem cell-derived motor neurons. Nature Communications, 8(1): 14741.

Escribano, O., Beneit, N., Rubio-Longás, C., López-Pastor, A. R. and Gómez-Hernández, A. 2017. The role of insulin receptor isoforms in diabetes and its metabolic and vascular complications. Journal of Diabetes Research, 2017.

Esteves, S. C. 2022. Evolution of the World Health Organization semen analysis manual: where are we?. Nature Reviews Urology, 19(7): 439-446.

Esteves, S. C., Santi, D. and Simoni, M. 2020. An update on clinical and surgical interventions to reduce sperm DNA fragmentation in infertile men. Andrology, 8(1): 53-81.

Fallah, A., Mohammad-Hasani, A. and Colagar, A. H. 2018. Zinc is an essential element for male fertility: a review of Zn roles in men’s health, germination, sperm quality, and fertilization. Journal of Reproduction & Infertility, 19(2): 69.

Favilla, B. P., Haddad, L. A. and Melaragno, M. I. 2021. Human chromosomes. In Human Genome Structure, Function and Clinical Considerations. Cham: Springer, 25-58.

Feferkorn, I., Azani, L., Kadour-Peero, E., Hizkiyahu, R., Shrem, G., Salmon-Divon, M. and Dahan, M. H. 2022. Geographic variation in semen parameters from data used for the World Health Organization semen analysis reference ranges. Fertility and Sterility, 118(3): 475-482.

Fiocchi, C., Binion, D. G. and Katz, J. A. 1994. Cytokine production in the human gastrointestinal tract during inflammation. Current Opinion in Gastroenterology, 10(6): 639-644.

Fu, Q., Yang, Y., Li, C., Zeng, Q., Zhou, T., Li, N. and Liu, Z. 2017. The CC and CXC chemokine receptors in channel catfish (Ictalurus punctatus) and their involvement in disease and hypoxia responses. Developmental & Comparative Immunology, 77: 241-251.

Fuertes-Martín, R., Correig, X., Vallvé, J. C. and Amigó, N. 2020. Human serum/plasma glycoprotein analysis by 1H-NMR, an emerging method of inflammatory assessment. Journal of Clinical Medicine, 9(2): 354.

Fujimoto, M., Hwa, V. and Dauber, A. 2017. Novel modulators of the growth hormone-insulin-like growth factor axis: pregnancy-associated plasma protein-A2 and stanniocalcin-2. Journal of Clinical Research in Pediatric Endocrinology, 9(2): 1.

Gaikwad, A. S., Anderson, A. L., Merriner, D. J., O'Connor, A. E., Houston, B. J., Aitken, R. J. and Nixon, B. 2019. GLIPR1L1 is an IZUMO-binding protein required for optimal fertilization in the mouse. BMC Biology, 17: 1-18.

Galley, H. F. and Webster, N. R. 1996. The immuno-inflammatory cascade. British Journal of Anaesthesia, 77(1): 11-16.

García-Burgos, M., Moreno-Fernández, J., Alférez, M. J., Díaz-Castro, J. and López-Aliaga, I. 2020. New perspectives in fermented dairy products and their health relevance. Journal of Functional Foods, 72: 104059.

Garnier, C., Devred, F., Byrne, D., Puppo, R., Roman, A. Y., Malesinski, S. and Tsvetkov, P. O. 2017. Zinc binding to RNA recognition motif of TDP-43 induces the formation of amyloid-like aggregates. Scientific Reports, 7(1): 6812.

Gautam, R., Priyadarshini, E., Nirala, J. and Rajamani, P. 2022. Impact of nonionizing electromagnetic radiation on male infertility: an assessment of the mechanism and consequences. International Journal of Radiation Biology, 98(6): 1063-1073.

Gautam, R., Singh, K. V., Nirala, J., Murmu, N. N., Meena, R. and Rajamani, P. 2019. Oxidative stress-mediated alterations on sperm parameters in male Wistar rats exposed to 3G mobile phone radiation. Andrologia, 51(3): e13201.

Ge, Y., Huang, M. and Yao, Y. M. 2021. The effect and regulatory mechanism of High Mobility Group Box-1 protein on immune cells in inflammatory diseases. Cells, 10(5): 1044.

Gonzalez-Aparicio, M. and Alfaro, C. 2020. Retracted Artıcle: Significance of the IL-8 pathway for immunotherapy. Human Vaccines & Immunotherapeutics, 16(10): 2312-2317.

Gray, R. D., Hardisty, G., Regan, K. H., Smith, M., Robb, C. T., Duffin, R. and Davidson, D. J. 2018. Delayed neutrophil apoptosis enhances NET formation in cystic fibrosis. Thorax, 73(2): 134-144.

Greenberg, M. V. and Bourc'his, D. 2019. The diverse roles of DNA methylation in mammalian development and disease. Nature reviews Molecular Cell Biology, 20(10): 590-607.

Griffiths, M. J., Winship, A. L. and Hutt, K. J. 2020. Do cancer therapies damage the uterus and compromise fertility?. Human Reproduction Update, 26(2): 161-173.

Grimm, M. C., Elsbury, S. K., Pavli, P. and Doe, W. F. 1996. Interleukin 8: cells of origin in inflammatory bowel disease. Gut, 38(1): 90-98.

Grosche, J., Meissner, J. and Eble, J. A. 2018. More than a syllable in fib-ROS-is: The role of ROS on the fibrotic extracellular matrix and on cellular contacts. Molecular Aspects of Medicine, 63: 30-46.

Hafez, S. F. M., Amer, O. T., Mostafa, O. A. E. and Samy, W. M. 2021. Polymorphism RS2227306 In The Interleukin 8 Gene and Its Relation to Bronchial Asthma. The Egyptian Journal of Hospital Medicine, 86(1): 50-53.

Harshman, L. C., Wang, V. X., Hamid, A. A., Santone, G., Drake, C. G., Carducci, M. A. and Sweeney, C. J. 2020. Impact of baseline serum IL-8 on metastatic hormone-sensitive prostate cancer outcomes in the Phase 3 CHAARTED trial (E3805). The Prostate, 80(16): 1429-1437.

Hassan, A., Abo-Azma, M., Fayed, S. M. and Mostafa, T. 2009. Seminal plasma cotinine and insulin-like growth factor-I in idiopathic oligoasthenoteratozoospermic smokers. BJU International, 103(1): 108-111.

Hatef, B., Taromchi, A., Nejatbakhsh, R., Farrokhi, A. and Shokri, S. 2017. Supplementation of freezing media with stromal cell-derived factor-1α preserves human sperm from cryodamage. Cryobiology, 79: 37-42.

Henderson, B., Poole, S. and Wilson, M. 1996. Microbial/host interactions in health and disease: who controls the cytokine network?. Immunopharmacology, 35(1): 1-21.

Henkel, R. and Leisegang, K. 2020. Origins of sperm DNA damage. Male Infertility: Contemporary Clinical Approaches, Andrology, ART and Antioxidants, 361-375.

Hernández-Corbacho, M. J., Salama, M. F., Canals, D., Senkal, C. E. and Obeid, L. M. 2017. Sphingolipids in mitochondria. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1862(1): 56-68.

Hernández-Silva, C. D., Villegas-Pineda, J. C. and Pereira-Suárez, A. L. 2020. Expression and role of the G protein-coupled estrogen receptor (GPR30/GPER) in the development and immune response in female reproductive cancers. Frontiers in Endocrinology, 11: 544.

Horibe, A., Eid, N., Ito, Y., Otsuki, Y. and Kondo, Y. 2019. Ethanol-induced autophagy in sertoli cells is specifically marked at androgen-dependent stages of the spermatogenic cycle: potential mechanisms and implications. International Journal of Molecular Sciences, 20(1): 184.

Hsu, S. K., Chiu, C. C., Dahms, H. U., Chou, C. K., Cheng, C. M., Chang, W. T. and Lin, I. L. 2019. Unfolded protein response (UPR) in survival, dormancy, immunosuppression, metastasis, and treatments of cancer cells. International Journal of Molecular Sciences, 20(10): 2518.

Humar, A., St. Louis, P., Mazzulli, T., McGeer, A., Lipton, J., Messner, H. and MacDonald, K. S. 1999. Elevated serum cytokines are associated with cytomegalovirus infection and disease in bone marrow transplant recipients. The Journal of Infectious Diseases, 179(2): 484-488.

Hwang, J. R., Byeon, Y., Kim, D. and Park, S. G. 2020. Recent insights of T cell receptor-mediated signaling pathways for T cell activation and development. Experimental & Molecular Medicine, 52(5): 750-761.

Ibtisham, F. and Honaramooz, A. 2020. Spermatogonial stem cells for in vitro spermatogenesis and in vivo restoration of fertility. Cells, 9(3): 745.

Innocenzi, E., De Domenico, E., Ciccarone, F., Zampieri, M., Rossi, G., Cicconi, R. and Grimaldi, P. 2019. Paternal activation of CB2 cannabinoid receptor impairs placental and embryonic growth via an epigenetic mechanism. Scientific Reports, 9(1): 17034.

Jahanafrooz, Z., Baradaran, B., Mosafer, J., Hashemzaei, M., Rezaei, T., Mokhtarzadeh, A. and Hamblin, M. R. 2020. Comparison of DNA and mRNA vaccines against cancer. Drug Discovery Today, 25(3): 552-560.

Jegou, B. 2018. Historic considerations in male reproduction. Encyclopedia of Reproduction, 3.

Jenkins, T. G., Aston, K. I., Cairns, B., Smith, A. and Carrell, D. T. 2018. Paternal germ line aging: DNA methylation age prediction from human sperm. BMC Genomics, 19(1): 1-10.

Kazlauskas, R. 2018. Engineering more stable proteins. Chemical Society Reviews, 47(24): 9026-9045.

Kejnovsky, E. and Jedlicka, P. 2022. Nucleic acids movement and its relation to genome dynamics of repetitive DNA: Is cellular and intercellular movement of DNA and RNA molecules related to the evolutionary dynamic genome components?. BioEssays, 44(4): 2100242.

Kent, S. A., Spires-Jones, T. L. and Durrant, C. S. 2020. The physiological roles of tau and Aβ: implications for Alzheimer's disease pathology and therapeutics. Acta Neuropathologica, 140(4): 417-447.

Khatun, A., Rahman, M. S. and Pang, M. G. 2018. Clinical assessment of the male fertility. Obstetrics & Gynecology Science, 61(2): 179-191.

Kim, R. and Poslad, S. 2019. Growable, invisible, connected toys: twitching towards ubiquitous bacterial computing. In Proceedings of the Halfway to the Future Symposium 2019, 1-9.

Kitala, D., Klama-Baryła, A., Łabuś, W., Ples, M., Misiuga, M., Kraut, M. and Kucharzewski, M. 2019. Amniotic cells share clusters of differentiation of fibroblasts and keratinocytes, influencing their ability to proliferate and aid in wound healing while impairing their angiogenesis capability. European Journal of Pharmacology, 854: 167-178.

Kopp, T. I., Pinborg, A., Glazer, C. H. and Magyari, M. 2023. Assisted reproductive technology treatment and risk of multiple sclerosis–a Danish cohort study. Fertility and Sterility, 119(2): 291-299.

Kraemer, W. J., Ratamess, N. A. and Nindl, B. C. 2017. Recovery responses of testosterone, growth hormone, and IGF-1 after resistance exercise. Journal of Applied Physiology, 122(3): 549-558.

Kresoja-Rakic, J. and Santoro, R. 2019. Nucleolus and rRNA gene chromatin in early embryo development. Trends in Genetics, 35(11): 868-879.

Krimsky, S. 2019. GMOs decoded: A skeptic's view of genetically modified foods. MIT Press, 219 page, Cambridge.

Kujundžić, R. N., Stepanić, V., Milković, L., Gašparović, A. Č., Tomljanović, M. and Trošelj, K. G. 2019. Curcumin and its potential for systemic targeting of inflamm-aging and metabolic reprogramming in cancer. International Journal of Molecular Sciences, 20(5): 1180.

Kukielka, G. L., Smith, C. W., LaRosa, G. J., Manning, A. M., Mendoza, L. H., Daly, T. J. and Entman, M. L. 2021. Interleukin-8 gene induction in the myocardium after ischemia and reperfusion in vivo. The Journal of Clinical Investigation, 95(1): 89-103.

Kups, M., Gill, K., Rosiak-Gill, A., Harasny, P., Machalowski, T., Grabowska, M. and Piasecka, M. 2022. Evaluation of selected semen parameters and biomarkers of male infertility–preliminary study. F1000 Research, 11(591): 591.

LaFleur, M. 2019. Defining mechanisms of tumor immunity. PhD. Thesis, Harvard University, 186 page, Massachusetts.

Le Blévec, E., Muroňová, J., Ray, P. F. and Arnoult, C. 2020. Paternal epigenetics: mammalian sperm provide much more than DNA at fertilization. Molecular and Cellular Endocrinology, 518: 110964.

Le, M. T., Nguyen, T. A. T., Nguyen, H. T. T., Nguyen, T. T. T., Nguyen, V. T., Le, D. D. and Cao, N. T. 2019. Does sperm DNA fragmentation correlate with semen parameters?. Reproductive Medicine and Biology, 18(4): 390-396.

Lee, K. O., Ng, S. C., Lee, P. S., Bongso, A. T., Taylor, E. A., Lin, T. K. and Ratnam, S. S. 1995. Effect of growth hormone therapy in men with severe idiopathic oligozoospermia. European Journal of Endocrinology, 132(2): 159-162.

Lee, W. S. and Kim, J. 2018. Insulin-like growth factor-1 signaling in cardiac aging. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 1864(5): 1931-1938.

Lehti, M. S. and Sironen, A. 2017. Formation and function of sperm tail structures in association with sperm motility defects. Biology of Reproduction, 97(4): 522-536.

LeRoith, D., Holly, J. M. and Forbes, B. E. 2021. Insulin-like growth factors: Ligands, binding proteins, and receptors. Molecular Metabolism, 52: 101245.

Liefers-Visser, J. A. L., Meijering, R. A. M., Reyners, A. K. L., van der Zee, A. G. J. and De Jong, S. 2017. IGF system targeted therapy: Therapeutic opportunities for ovarian cancer. Cancer Treatment Reviews, 60: 90-99.

Lister, F. G., Le Bailly, B. A., Webb, S. J. and Clayden, J. 2017. Ligand-modulated conformational switching in a fully synthetic membrane-bound receptor. Nature Chemistry, 9(5): 420-425.

Liu, Y., Jin, M., Wang, Y., Zhu, J., Tan, R., Zhao, J. and Xing, J. 2020. MCU-induced mitochondrial calcium uptake promotes mitochondrial biogenesis and colorectal cancer growth. Signal Transduction and Targeted Therapy, 5(1): 59.

Luo, H., Wang, C., Liu, M., Yin, B., Peng, A., Huang, D. and Ye, L. 2018. Inhibition of SOX9 promotes inflammatory and immune responses of dental pulp. Journal of Endodontics, 44(5): 792-799.

Lyu, H., Han, A., Polsdofer, E., Liu, S. and Liu, B. 2018. Understanding the biology of HER3 receptor as a therapeutic target in human cancer. Acta Pharmaceutica Sinica B, 8(4): 503-510.

Ma, H., O'Neil, R. C., Gutierrez, N. M., Hariharan, M., Zhang, Z. Z., He, Y. and Mitalipov, S. 2017. Functional human oocytes generated by transfer of polar body genomes. Cell Stem Cell, 20(1): 112-119.

MacLeod, W. B. and Urquiola, M. 2021. Why does the United States have the best research universities? Incentives, resources, and virtuous circles. Journal of Economic Perspectives, 35(1): 185-206.

Madan, K. 2020. Natural human chimeras: A review. European Journal of Medical Genetics, 63(9): 103971.

Madkour, L. H. 2019. Function of reactive oxygen species (ROS) inside the living organisms and sources of oxidants. Journal of Pharmaceutical and Biomedical Analysis Abbreviation, 2: 180023.

Maes, M., Bosmans, E., De Jongh, R., Kenis, G., Vandoolaeghe, E. and Neels, H. 1997. Increased serum IL-6 and IL-1 receptor antagonist concentrations in major depression and treatment resistant depression. Cytokine, 9(11): 853-858.

Mahajan, A., Sharma, P., Mishra, A. K., Gupta, S., Yadav, S., Anand, M. and Swain, D. K. 2022. Interplay mechanisms between progesterone and endocannabinoid receptors in regulating bull sperm capacitation and acrosome reaction. Journal of Cellular Physiology, 237(7): 2888-2912.

Makkar, R., Behl, T., Sehgal, A., Singh, S., Sharma, N., Makeen, H. A. and Meraya, A. M. 2022. Targeting Insulin-Like Growth Factor-I in Management of Neurological Disorders. Neurotoxicity Research, 40(3): 874-883.

Malak, H. A., Abulreesh, H. H., Organji, S. R., Elbanna, K., Shaaban, M. R., Ahmad, I. and Almalki, M. H. 2020. Immune system evasion mechanisms in Staphylococcus aureus: current understanding. Journal of Pure and Applied Microbiology, 14(4): 2219-2234.

Maldonado, E., Rojas, D. A., Morales, S., Miralles, V. and Solari, A. 2020. Dual and opposite roles of reactive oxygen species (ROS) in Chagas disease: beneficial on the pathogen and harmful on the host. Oxidative Medicine and Cellular Longevity, 2020: 1-17.

Malivindi, R., Rago, V., De Rose, D., Gervasi, M. C., Cione, E., Russo, G. and Aquila, S. 2018. Influence of all-trans retinoic acid on sperm metabolism and oxidative stress: Its involvement in the physiopathology of varicocele-associated male infertility. Journal of Cellular Physiology, 233(12): 9526-9537.

Mazur, A. A., Chelomin, V. P., Zhuravel, E. V., Kukla, S. P., Slobodskova, V. V. and Dovzhenko, N. V. 2021. Genotoxicity of polystyrene (PS) microspheres in short-term exposure to gametes of the sand dollar Scaphechinus mirabilis (Agassiz, 1864)(Echinodermata, Echinoidea). Journal of Marine Science and Engineering, 9(10): 1088.

Meng, L., Zhao, Y., Bu, W., Li, X., Liu, X., Zhou, D. and Sun, H. 2020. Bone mesenchymal stem cells are recruited via CXCL8-CXCR2 and promote EMT through TGF-β signal pathways in oral squamous carcinoma. Cell Proliferation, 53(8): e12859.

Monteiro, C., Marques, P. I., Cavadas, B., Damião, I., Almeida, V., Barros, N. and Seixas, S. 2018. Characterization of microbiota in male infertility cases uncovers differences in seminal hyperviscosity and oligoasthenoteratozoospermia possibly correlated with increased prevalence of infectious bacteria. American Journal of Reproductive Immunology, 79(6): e12838.

Moore, D. S. and Shenk, D. 2017. The heritability fallacy. Wiley Interdisciplinary Reviews: Cognitive Science, 8(2): e1400.

Moore, R. C. 2017. Childhood's domain: Play and place in child development. Routledge, 340 page, Oxfordshire.

Mora Rodríguez, J. A., Porchia, L. M., Camargo, F. and López-Bayghen, E. 2019. The use of insulin-like growth factor 1 improved the parameters of the seminogram in a patient with severe oligoasthenoteratozoospermia. SAGE Open Medical Case Reports, 7: 2050313X19834154.

Morgan, H. L. and Watkins, A. J. 2020. The influence of seminal plasma on offspring development and health. In Seminars in Cell & Developmental Biology. Academic Press, 97: 131-137.

Moskovtsev, S. I., Willis, J., White, J. and Mullen, J. B. M. 2007. Leukocytospermia: relationship to sperm deoxyribonucleic acid integrity in patients evaluated for male factor infertility. Fertility and Sterility, 88(3): 737-740.

Moussouras, N. A., Getschman, A. E., Lackner, E. R., Veldkamp, C. T., Dwinell, M. B. and Volkman, B. F. 2017. Differences in sulfotyrosine binding amongst CXCR1 and CXCR2 chemokine ligands. International Journal of Molecular Sciences, 18(9): 1894.

Munawar, A., Ali, S. A., Akrem, A. and Betzel, C. 2018. Snake venom peptides: Tools of biodiscovery. Toxins, 10(11): 474.

Murphy, P. M. 2019. Chemokines and chemokine receptors. In Clinical immunology. Elsevier, 151-170.

Neirijnck, Y., Papaioannou, M. D. and Nef, S. 2019. The insulin/IGF system in mammalian sexual development and reproduction. International Journal of Molecular Sciences, 20(18): 4440.

Newman, H., Catt, S., Vining, B., Vollenhoven, B. and Horta, F. 2022. DNA repair and response to sperm DNA damage in oocytes and embryos, and the potential consequences in ART: a systematic review. Molecular Human Reproduction, 28(1): gaab071.

Ngo, M. H. T., Jeng, H. Y., Kuo, Y. C., Nanda, J. D., Brahmadhi, A., Ling, T. Y. and Huang, Y. H. 2021. The role of IGF/IGF-1R signaling in hepatocellular carcinomas: stemness-related properties and drug resistance. International Journal of Molecular Sciences, 22(4): 1931.

Ngo, T., Stephens, B. S., Gustavsson, M., Holden, L. G., Abagyan, R., Handel, T. M. and Kufareva, I. 2020. Crosslinking-guided geometry of a complete CXC receptor-chemokine complex and the basis of chemokine subfamily selectivity. PLoS Biology, 18(4): e3000656.

Nguyen, T. T. T., Nguyen, H. T., Wang, P. C. and Chen, S. C. 2017. Identification and expression analysis of two pro-inflammatory cytokines, TNF-α and IL-8, in cobia (Rachycentron canadum L.) in response to Streptococcus dysgalactiae infection. Fish & Shellfish Immunology, 67: 159-171.

Nicolai, C. J., Wolf, N., Chang, I. C., Kirn, G., Marcus, A., Ndubaku, C. O. and Raulet, D. H. 2020. NK cells mediate clearance of CD8+ T cell–resistant tumors in response to STING agonists. Science Immunology, 5(45): eaaz2738.

Noordermeer, D. and Feil, R. 2020. Differential 3D chromatin organization and gene activity in genomic imprinting. Current Opinion in Genetics & Development, 61: 17-24.

Novak, T., Hamedi, M., Bergmeier, L. A., Fortune, F. and Hagi-Pavli, E. 2021. Saliva and serum cytokine profiles during oral ulceration in Behçet's disease. Frontiers in Immunology, 12: 724900.

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

Olmos-Ortiz, A., Flores-Espinosa, P., Mancilla-Herrera, I., Vega-Sánchez, R., Díaz, L. and Zaga-Clavellina, V. 2019. Innate immune cells and toll-like receptor–dependent responses at the maternal–fetal interface. International Journal of Molecular Sciences, 20(15): 3654.

Olojede, S. O., Lawal, S. K., Aladeyelu, O. S., Olaniyi, K. S., Moodley, R., Rennie, C. O. and Azu, O. O. 2022. Studies on testicular ultrastructural and hormonal changes in type-2 diabetic rats treated with highly active antiretroviral therapy conjugated silver nanoparticles. Life Sciences, 298: 120498.

Owen, T. 2017. From cell to muscle: development of new ways to study inherited cardiomyopathies based on engineered heart tissue. PhD. Thesis, Imperial College of London, 258 page, London.

Padilla, A., Keating, P., Hartmann, J. X. and Marí, F. 2017. Effects of α-conotoxin ImI on TNF-α, IL-8 and TGF-β expression by human macrophage-like cells derived from THP-1 pre-monocytic leukemic cells. Scientific Reports, 7(1): 1-11.

Pan, Y., Wen, X., Hao, D., Wang, Y., Wang, L., He, G. and Jiang, X. 2020. The role of IL-37 in skin and connective tissue diseases. Biomedicine & Pharmacotherapy, 122: 109705.

Park, J. 2017. Reduced Level of Serum Insulin-like Growth Factor-1 and Male Infertility. Reproductive & Developmental Biology, 41(2): 5-5.

Park, Y. S., Lee, S. H., Choi, H. W., Lee, H. S., Lee, J. S. and Seo, J. T. 2018. Abnormal human sperm parameters contribute to sperm DNA fragmentation in men with varicocele. The World Journal of Men's Health, 36(3): 239-247.

Parrish, J. J., Willenburg, K. L., Gibbs, K. M., Yagoda, K. B., Krautkramer, M. M., Loether, T. M. and Melo, F. C. 2017. Scrotal insulation and sperm production in the boar. Molecular Reproduction and Development, 84(9): 969-978.

Pedrana, G., Larrañaga, C., Diaz, A., Viotti, H., Lombide, P., Cavestany, D. and Sloboda, D. M. 2021. Maternal undernutrition during pregnancy and lactation increases transcription factors, ETV5 and GDNF, and alters regulation of apoptosis and heat shock proteins in the testis of adult offspring in the rat. Reproduction, Fertility and Development, 33(7): 484-496.

Persson, H., Potrzebowski, W., Potrzebowska, K. and Svensson, L. M. 2019. Spatial mapping of affinity changes for the integrin LFA-1 during cell migration using clusters identified based on local density. Journal of Biophotonics, 12(3): e201800080.

Perussello, C. 2022. Food for thought: Planetary healing begins on our plate. Lantern Books, 22(1).

Petersen, C. G., Mauri, A. L., Vagnini, L. D., Renzi, A., Petersen, B., Mattila, M. and Franco, J. G. 2018. The effects of male age on sperm DNA damage: an evaluation of 2,178 semen samples. JBRA Assisted Reproduction, 22(4): 323.

Peterson, Y. K. and Luttrell, L. M. 2017. The diverse roles of arrestin scaffolds in G protein–coupled receptor signaling. Pharmacological Reviews, 69(3): 256-297.

Petri, B. and Sanz, M. J. 2018. Neutrophil chemotaxis. Cell and Tissue Research, 371: 425-436.

Press, W. H., Hawkins, J. A., Jones Jr, S. K., Schaub, J. M. and Finkelstein, I. J. 2020. HEDGES error-correcting code for DNA storage corrects indels and allows sequence constraints. Proceedings of the National Academy of Sciences, 117(31): 18489-18496.

Qin, J. and Xu, J. 2022. Arginine methylation in the epithelial-to-mesenchymal transition. The FEBS Journal, 289(23): 7292-7303.

Rahman, M. B., Schellander, K., Luceño, N. L. and Van Soom, A. 2018. Heat stress responses in spermatozoa: Mechanisms and consequences for cattle fertility. Theriogenology, 113: 102-112.

Ramal-Sanchez, M., Valbonetti, L., Tsikis, G., Dubuisson, F., Blache, M. C., Labas, V. and Bernabo, N. 2019. Graphene oxide: A glimmer of hope for Assisted Reproductive Technology. Carbon, 150: 518-530.

Rees, R. C. 1992. Cytokines as biological response modifiers. Journal of Clinical Pathology, 45(2): 93.

Romauch, M. 2020. Zinc-α2-glycoprotein as an inhibitor of amine oxidase copper-containing 3. Open Biology, 10(4): 190035.

Ruiz, A. R. J. and Granja, F. S. 2017. Comparing ubisense, bespoon, and decawave uwb location systems: Indoor performance analysis. IEEE Transactions on instrumentation and Measurement, 66(8): 2106-2117.

Rutanen, E. M. 1993. Cytokines in reproduction. Annals of Medicine, 25(4): 343-347.

Sajid, H., Ayub, K., Arshad, M. and Mahmood, T. 2019. Highly selective acridinium based cyanine dyes for the detection of DNA base pairs (adenine, cytosine, guanine and thymine). Computational and Theoretical Chemistry, 1163: 112509.

Santi, D., Crépieux, P., Reiter, E., Spaggiari, G., Brigante, G., Casarini, L. and Simoni, M. 2020. Follicle-stimulating hormone (FSH) action on spermatogenesis: a focus on physiological and therapeutic roles. Journal of Clinical Medicine, 9(4): 1014.

Santos, J. C., Marques, C. C., Baptista, M. C., Pimenta, J., Teixeira, J., Montezinho, L. and Pereira, R. M. 2022. Effect of a Novel Hydroxybenzoic Acid Based Mitochondria Directed Antioxidant Molecule on Bovine Sperm Function and Embryo Production. Animals, 12(7): 804.

Sanz, M., Marco del Castillo, A., Jepsen, S., Gonzalez-Juanatey, J. R., D'Aiuto, F., Bouchard, P. and Wimmer, G. 2020. Periodontitis and cardiovascular diseases: Consensus report. Journal of Clinical Periodontology, 47(3): 268-288.

Satyavolu, N. S. R., Loh, K. Y., Tan, L. H. and Lu, Y. 2019. Discovery of and insights into DNA "codes" for tunable morphologies of metal nanoparticles. Small, 15(26): 1900975.

Saxena, S. and Singh, R. K. 2021. Chemokines orchestrate tumor cells and the microenvironment to achieve metastatic heterogeneity. Cancer and Metastasis Reviews, 40(2): 447-476.

Schumacher, B., Pothof, J., Vijg, J. and Hoeijmakers, J. H. 2021. The central role of DNA damage in the ageing process. Nature, 592(7856): 695-703.

Schünemann, H. J., Vist, G. E., Higgins, J. P., Santesso, N., Deeks, J. J., Glasziou, P. and Cochrane GRADEing Methods Group. 2019. Interpreting results and drawing conclusions. Cochrane Handbook for Systematic Reviews of Interventions, 403-431.

Setti, A. S., Braga, D. P. D. A. F., Provenza, R. R., Iaconelli Jr, A. and Borges, E. 2021. Oocyte ability to repair sperm DNA fragmentation: the impact of maternal age on intracytoplasmic sperm injection outcomes. Fertility and Sterility, 116(1): 123-129.

Sharkey, A. 1998. Cytokines and implantation. Reviews of Reproduction, 3: 52-61.

Sharma, I., Singh, A., Siraj, F. and Saxena, S. 2018. IL-8/CXCR1/2 signalling promotes tumor cell proliferation, invasion and vascular mimicry in glioblastoma. Journal of Biomedical Science, 25(1): 1-13.

Sharma, P., Ghanghas, P., Kaushal, N., Kaur, J. and Kaur, P. 2019. Epigenetics and oxidative stress: A twin-edged sword in spermatogenesis. Andrologia, 51(11): e13432.

Sharma, U., Sun, F., Conine, C. C., Reichholf, B., Kukreja, S., Herzog, V. A. and Rando, O. J. 2018. Small RNAs are trafficked from the epididymis to developing mammalian sperm. Developmental Cell, 46(4): 481-494.

Simons, J. E. and Olson, S. D. 2018. Sperm motility: Models for dynamic behavior in complex environments. Cell Movement: Modeling and Applications, 169-209.

Skinner, R., Mulder, R. L., Kremer, L. C., Hudson, M. M., Constine, L. S., Bardi, E. and Green, D. M. 2017. Recommendations for gonadotoxicity surveillance in male childhood, adolescent, and young adult cancer survivors: a report from the International Late Effects of Childhood Cancer Guideline Harmonization Group in collaboration with the PanCareSurFup Consortium. The Lancet Oncology, 18(2): e75-e90.

Śledziński, P., Zeyland, J., Słomski, R. and Nowak, A. 2018. The current state and future perspectives of cannabinoids in cancer biology. Cancer Medicine, 7(3): 765-775.

Sonawani, A., Kharche, S., Dasgupta, D. and Sengupta, D. 2022. Allosteric modulation of the chemokine receptor-chemokine CXCR4-CXCL12 complex by tyrosine sulfation. International Journal of Biological Macromolecules, 206: 812-822.

Spangler, C. J. A. 2022. Exploring the mechanistic basis of nucleosome interactions. PhD. Thesis, The University of North Carolina, 220 page, Chapel Hill.

Stadelman, W. J., Newkirk, D. and Newby, L. 2017. Egg science and technology. CRC Press, 608 page, Boca Raton.

Stappler, E., Dattenböck, C., Tisch, D. and Schmoll, M. 2017. Analysis of light-and carbon-specific transcriptomes implicates a class of G-protein-coupled receptors in cellulose sensing. Msphere, 2(3): e00089-17.

Takeshima, T., Usui, K., Mori, K., Asai, T., Yasuda, K., Kuroda, S. and Yumura, Y. 2021. Oxidative stress and male infertility. Reproductive Medicine and Biology, 20(1): 41-52.

Tang, G., He, J., Liu, J., Yan, X. and Fan, K. 2021. Nanozyme for tumor therapy: Surface modification matters. Exploration, 1(1): 75-89.

Van Doorn, J. 2020. Insulin-like growth factor-II and bioactive proteins containing a part of the E-domain of pro-insulin-like growth factor-II. Biofactors, 46(4): 563-578.

Velloso, F. J., Bianco, A. F., Farias, J. O., Torres, N. E., Ferruzo, P. Y., Anschau, V. and Correa, R. G. 2017. The crossroads of breast cancer progression: insights into the modulation of major signaling pathways. OncoTargets and Therapy, 10: 5491.

Vrba, L. and Futscher, B. W. 2018. A suite of DNA methylation markers that can detect most common human cancers. Epigenetics, 13(1): 61-72.

Wang, L., Long, H., Zheng, Q., Bo, X., Xiao, X. and Li, B. 2019. Circular RNA circRHOT1 promotes hepatocellular carcinoma progression by initiation of NR2F6 expression. Molecular Cancer, 18(1): 1-12.

Wang, Z., He, Z., Emara, A. M., Gan, X. and Li, H. 2019. Effects of malondialdehyde as a byproduct of lipid oxidation on protein oxidation in rabbit meat. Food Chemistry, 288: 405-412.

Westendorp, R. G., Langermans, J. A., Huizinga, T. W., Elouali, A. H., Verweij, C. L., Boomsma, D. I. and Vandenbrouke, J. P. 1997. Genetic influence on cytokine production and fatal meningococcal disease. The Lancet, 349(9046): 170-173.

Williamson, M. 2018. The role of competitive glycosaminoglycan binding in determining the inflammatory profile of the Cystic Fibrosis airway. PhD. Thesis, Royal College of Surgeons in Ireland, 204 page, Dublin.

Wolf, P., Schoeniger, A. and Edlich, F. 2022. Pro-apoptotic complexes of BAX and BAK on the outer mitochondrial membrane. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 119317.

Wolff, G., Melia, C. E., Snijder, E. J. and Bárcena, M. 2020. Double-membrane vesicles as platforms for viral replication. Trends in Microbiology, 28(12): 1022-1033.

Wu, D., Tang, H. X., Wu, Y., Qian, S. B., Xu, D. and Qi, J. 2020. The possible association between serum interleukin 8 and acute urinary retention in Chinese patients with benign prostatic hyperplasia. Andrologia, 52(11): e13763.

Wu, T., Lyu, R., You, Q. and He, C. 2020. Kethoxal-assisted single-stranded DNA sequencing captures global transcription dynamics and enhancer activity in situ. Nature Methods, 17(5): 515-523.

Wu, Y., Zhang, N., Li, Y. H., Zhao, L., Yang, M., Jin, Y. and Guo, H. 2017. Citrinin exposure affects oocyte maturation and embryo development by inducing oxidative stress-mediated apoptosis. Oncotarget, 8(21): 34525.

Xiong, F., Zhang, R., Meng, Z., Deng, K., Que, Y., Zhuo, F. and Ren, M. 2017. Brassinosteriod Insensitive 2 (BIN2) acts as a downstream effector of the Target of Rapamycin (TOR) signaling pathway to regulate photoautotrophic growth in Arabidopsis. New Phytologist, 213(1): 233-249.

Xu, J., Ma, H., Jin, J., Uttam, S., Fu, R., Huang, Y. and Liu, Y. 2018. Super-resolution imaging of higher-order chromatin structures at different epigenomic states in single mammalian cells. Cell Reports, 24(4): 873-882.

Yamaguchi, Y., Tayama, C., Tomikawa, J., Akaishi, R., Kamura, H., Matsuoka, K. and Hata, K. 2019. Placenta-specific epimutation at H19-DMR among common pregnancy complications: its frequency and effect on the expression patterns of H19 and IGF2. Clinical Epigenetics, 11(1): 1-13.

Yan, J. and Charles, J. F. 2018. Gut microbiota and IGF-1. Calcified Tissue International, 102: 406-414.

Yan, Z., Wang, M. and Ferry, J. G. 2017. A ferredoxin-and F420H2-dependent, electron-bifurcating, heterodisulfide reductase with homologs in the domains bacteria and archaea. MBio, 8(1): e02285-16.

Yang, H., Shen, Z., Luo, L., Gao, J., Chen, S., Che, J. and Dong, X. 2022. Molecular dynamics insights into the selectivity toward CXCR1 and CXCR2 antagonists. Chemical Physics Letters, 795: 139539.

Yang, M., Rito, T., Metzger, J., Naftaly, J., Soman, R., Hu, J. and Gleicher, N. 2021. Depletion of aneuploid cells in human embryos and gastruloids. Nature Cell Biology, 23(4): 314-321.

Yoshida, T. and Delafontaine, P. 2020. Mechanisms of IGF-1-mediated regulation of skeletal muscle hypertrophy and atrophy. Cells, 9(9): 1970.

Yusupov, M., Van der Paal, J., Neyts, E. C. and Bogaerts, A. 2017. Synergistic effect of electric field and lipid oxidation on the permeability of cell membranes. Biochimica et Biophysica Acta (BBA)-General Subjects, 1861(4): 839-847.

Zaferani, M., Suarez, S. S. and Abbaspourrad, A. 2021. Mammalian sperm hyperactivation regulates navigation via physical boundaries and promotes pseudo-chemotaxis. Proceedings of the National Academy of Sciences, 118(44): e2107500118.

Zandieh, Z., Vatannejad, A., Doosti, M., Zabihzadeh, S., Haddadi, M., Bajelan, L. and Amanpour, S. 2018. Comparing reactive oxygen species and DNA fragmentation in semen samples of unexplained infertile and healthy fertile men. Irish Journal of Medical Science, 187: 657-662.

Zeinali, M., Hadian Amree, A., Khorramdelazad, H., Karami, H. and Abedinzadeh, M. 2017. Inflammatory and anti-inflammatory cytokines in the seminal plasma of infertile men suffering from varicocele. Andrologia, 49(6): e12685.

Zeng, M. Y., Miralda, I., Armstrong, C. L., Uriarte, S. M. and Bagaitkar, J. 2019. The roles of NADPH oxidase in modulating neutrophil effector responses. Molecular Oral Microbiology, 34(2): 27-38.

Zha, C., Meng, X., Li, L., Mi, S., Qian, D., Li, Z. and Liu, Y. 2020. Neutrophil extracellular traps mediate the crosstalk between glioma progression and the tumor microenvironment via the HMGB1/RAGE/IL-8 axis. Cancer Biology & Medicine, 17(1): 154.

Zhang, B., Su, Y., Zhou, J., Zheng, Y. and Zhu, D. 2021. Toward a better regeneration through implant-mediated immunomodulation: harnessing the immune responses. Advanced Science, 8(16): 2100446.

Zhang, X., Yu, D., Sun, J., Wu, Y., Gong, J., Li, X. and Wang, T. 2020. Visualization of ligand-bound ectodomain assembly in the full-length human IGF-1 receptor by cryo-EM single-particle analysis. Structure, 28(5): 555-561.

Zheng, W. W., Song, G. E., Wang, Q. L., Liu, S. W., Zhu, X. L., Deng, S. M. and Tan, Y. 2018. Sperm DNA damage has a negative effect on early embryonic development following in vitro fertilization. Asian Journal of Andrology, 20(1): 75.

Zhou, X., Xie, F., Wang, L., Zhang, L., Zhang, S., Fang, M. and Zhou, F. 2020. The function and clinical application of extracellular vesicles in innate immune regulation. Cellular & Molecular Immunology, 17(4): 323-334.

Zore, T., Palafox, M. and Reue, K. 2018. Sex differences in obesity, lipid metabolism, and inflammation—a role for the sex chromosomes?. Molecular Metabolism, 15: 35-44.

Zulaikhah, S. T. 2017. The role of antioxidant to prevent free radicals in the body. Sains Medika, 8(1): 39-45.