GAZI UNIVERSITY
GRADUATE SCHOOL OF HEALTH SCIENCES

Ph. D.
THESIS

# EVALUATION OF SEMEN PARAMETERS AND SPERM MORPHOLOGY IN INDIVIDUALS WITH DIFFERENT BODY MASS INDEX (BMI)

Khadeejah Sead Mohammed ABRAHEEM

HISTOLOGY AND EMBRYOLOGY DEPARTMENT

FEBRUARY 2022


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**FEBRUARY 2022**

## ETHICAL STATEMENT

I hereby declare that in this thesis study I prepared in accordance with thesis writing rules of Gazi University Graduate School of Health Sciences

- All data information and documents presented in this thesis have been obtained within the scope of academic rules and ethical conduct.
- All the information, documents, assessments, and results have been presented in accordance with scientific ethical conduct and moral rules.
- All material used in this thesis that is not original to this work has been fully cited and referenced.
- No change has been made in the data used.
- The work presented in this thesis is original,

Or else, I admit all loss of rights to be incurred against me.

Khadeejah Sead Mohammed ABRAHEEM

10/03/2022

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(Ph. D. Thesis)

Khadeejah Sead Mohammed ABRAHEEM

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February 2022

## ABSTRACT

Infertility is defined as the inability of couples to achieve pregnancy despite having unprotected and regular sexual intercourse for at least one year (WHO, 2010). Infertility affects about 15% of the population. Obesity may be measured with a simple alternative metric: body mass index(BMI). This study aimed to understand the relationship between BMI and semen parameters of infertile men. In our study, a total of 60 infertile men were divided into three groups BMI, normal weight (18.5-24.99 kg/m2), overweight (25-29.99 kg/m2) and obese (>30.0). kg/m2). Semen parameters (volume, concentration, total semen count, morphology, and motility) were compared between the three BMI groups. In addition, Terminal Uridine Nick-End Labeling (TUNEL) test to determine DNA fragmentation, along with using oxidative stress values (TOS, TAS) were determined, whereas sperm chromatin condensation was analyzed with aniline blue. In the obese and overweight groups, semen volume, total concentration, total motility, and motility at different rates were shown to be reduced. However, these reductions were not statistically significant for all parameters. Although there was no statistical difference between the overweight and obese groups percentage of sperm with abnormal morphology, percentage of chromatin dense cells as assessed by aniline blue, and TUNEL positive sperm percentage was higher in the overweight and obese groups than in the normal group. This difference was statistically significant. Although TAS values were higher in the normal group, when compared to the overweight and obese groups did not show a statistically significant difference. Although TOS values were higher in the obese group results compared to normal, overweight, and obese groups, were not statistically significant. This study clarified the link between obesity and sperm quality, morphology, chromatin structure, DNA integrity, and antioxidant/oxidant balance. Therefore, increased body mass index BMI can negatively affect normal sperm.

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# FARKLI VÜCUT KİTLE İNDEKSİNE (BMI) SAHİP BİREYLERDE SEMEN PARAMETRELERİ VE SPERM MORFOLOJİSİNİN DEĞERLENDİRİLMESİ

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Şubat 2022

## ÖZET

İnfertilite, çiftlerin en az bir yıl boyunca korunmasız ve düzenli cinsel ilişkiye girmesine rağmen gebelik elde edememesi olarak tanımlanmaktadır (WHO, 2010). İnfertilite toplumun yaklaşık %15'ini etkiler. Vücut Kitle İndeksi (BMI), obezitenin kolayca hesaplanabilen bir belirtecidir. Bu çalışma, infertil erkeklerin BMI ile semen parametreleri arasındaki ilişkiyi ortaya koymayı amaçlamıştır. Çalışmamızda, toplam 60 infertil erkek, çalışma popülasyonu BMI, normal kilolu (18,5-24,99 kg/m2), fazla kilolu (25-29.99 kg/m2) ve obez (>30,0) olmak üzere üç gruba ayrıldı. kg/m2). Semen parametreleri (hacim, konsantrasyon, toplam semen sayısı, morfoloji ve motilite) üç BMI grubu arasında karşılaştırıldı. Ayrıca, Terminal Üridin Nick-End Etiketleme (TUNEL) testi kullanılarak DNA fragmantasyonu belirlendi, oksidatif stres değerleri (TOS, TAS) da ölçüldü, sperm kromatin yoğunlaşması ise anilin mavisi ile analiz edildi. Obez grupta ve fazla kilolu grupta normal gruba göre semen hacmi, konsantrasyon, toplam sperm sayısı, morfoloji ve motilitenin farklı oranlarda azaldığı belirlendi. Ancak, bu azalma tüm parametreler için istatistiksel olarak anlamlı değildi. Fazla kilolu ve obez gruplar arasında istatistiksel bir fark olmamasına rağmen anormal morfolojiye sahip sperm yüzdesi, anilin mavisi ile değerlendirilen kromatin yoğun hücre yüzdesi ve TUNEL pozitif sperm yüzdesi aşırı kilolu ve obez gruplarda normal gruba göre daha yüksekti. Bu fark istatistiksel olarak anlamlıydı. TAS değerleri normal grupta daha yüksek olmasına rağmen fazla kilolu ve obez gruplerdan elde edilen sonuçlarla karşılaştırıldığında istatistiksel olarak anlamlı bir fark göstermedi. Obez grupta TOS değerleri daha yüksek olmasına rağmen normal, fazla kilolu ve obez gruplardan elde edilen TOS sonuçlarının istatistiksel olarak değerlendirilmesinde de anlamlı bir fark görülmedi. Bu çalışma, obezite ile sperm kalitesi, morfolojisi, kromatin yapısı, DNA bütünlüğü ve antioksidan/oksidan dengesi arasındaki bağlantıyı netleştirdi. Bu nedenle artan vücut kitle indeksi BMI normal spermi olumsuz etkileyebilir.

| | | |
|---|---|---|
| Bilim Kodu | : | 1033 |
| Anahtar Kelimeler | : | İnfertilite,vücut kitle indeksi, Obezite,TUNEL,TAS,TOS. |
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## ABBREVIATIONS

The abbreviations used in this study are presented below, along with their explanations.

| Abbreviations | Descriptions |
|---|---|
| **FSH** | Follicle-Stimulating Hormone |
| **LH** | Luteinizing Hormone |
| **PAS** | Periodic Acid-Schiff |
| **H** | Hematoxylin |
| **E** | Eosin |
| **µm** | Micrometer |
| **ml** | Milliliter |
| **DNA** | Deoxyribonucleic Acid |
| **ERC** | Excess Residual Cytoplasm |
| **WHO** | World Health Organization |
| **BMI** | Body Mass Index, Vücut kitle indeksi |
| **TSC** | Total Sperm Count |
| **kg/m$^2$** | Kilogram / Square Meter |
| **ART** | Assisted Reproductive Technology |
| **E2** | Estradiol |
| **ROS** | Reactive Oxygen Species |
| **$O_2$** | Superoxide Anions |
| **$H_2O_2$** | Hydrogen Peroxide |
| **ROO•** | Peroxyl |
| **OH•** | Hydroxyl |
| **ATP** | Adenosine Triphosphate |
| **RPL** | Recurrent Pregnancy Loss |
| **TUNEL** | Terminal Uridine Nick-End Labeling |
| **TAS/TOS** | Total Antioxidant/ Total Oxidant |
| **Min** | Minute |
| **TdT** | Nucleotidyl Transferase |
| **PBS** | Phosphate Buffer Solution |
| **µl/$cm^2$** | Microliter/ Square Centimeter |

| | |
|---|---|
| **UMI** | Idiopathic Male Infertility |
| **SDF** | Sperm DNA Fragmentation |
| **ICSI** | Intracytoplasmic Sperm Injectiction |

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# 1. INTRODUCTION

An infertile pair is defined by the World Health Organization (WHO) (2010) as a sexually active couple who are unable to conceive naturally within one year of being sexually active. Nearly 15% of all couples are affected by infertility. Male infertility affects 50% of couples who are unable to conceive a child. Some of the most common reasons of male infertility are: anomalies of the urogenital system, including congenital and acquired problems, different cancers, sperm-duct obstructions, urogenital system infections, and the use of certain medicines. Other causes include: testicular trauma and varicocele. Anomalies in the genetic code. Idiopathic male infertility, on the other hand, is a disease in which the cause of male infertility cannot be determined in 30% to 40% of instances (1). Body mass index (BMI), which is an inexpensive and easy-to-apply measure, is an accepted criterion in the definition of obesity. In BMI measurement, the person's total weight (in kilograms) is divided by the square of the height (in meters) (kg/m2) (2). BMI level that is not in the normal range has been shown to be associated with many diseases (3). Today, the relationship between obesity and reproductive biology has been studied in different models. For example, decreased blastocyst formation and decrease in live birth rates were determined in couples with obesity problems who applied to assisted reproductive techniques centers and were shared in the literature (4). The undesirable effects of obesity on the male reproductive system have also been investigated in many studies (5, 6). In addition to the imbalance that obesity may create in the male hormonal level, the negative consequences of impaired erectile function, semen quality and sperm mitochondrial activity, decrease in sperm count, increase in sperm DNA damage and negative results in seminal oxidative stress levels can be given as examples of these effects (7). Widely accepted scientific evidence supports the role of oxidative stress (OS) as a causal factor in many pathological processes. The consequences of the irregularities in OS levels, whose systemic effects are known, are also parallel to the reproductive system microenvironment. The increase in OS levels in female and/or male individuals can result in pathology in many processes from zygote formation to the continuation of pregnancy (8). The presence of moderate amounts of reactive oxygen (ROS) in the male reproductive system does not adversely affect sperm capacitation, acrosome reaction, and motility activation. However, an increase in oxidative stress load and/or a decrease in antioxidant defense creates pathological results and constitutes an important factor of infertility (9). Studies have shown the relationship between oxidative stress level in the ejaculate and increased BMI. It has been stated that increased

oxidative stress causes changes in sperm morphology and leads to DNA fragmentation (10). It has been reported that DNA damage caused by increased seminal oxidative stress level in sperm hinders natural pregnancy and also causes a decrease in pregnancy rates that can be achieved with assisted reproductive techniques (8).

Studies conducted in obese infertile patients with a BMI of 30kg/m2 are frequently included in the literature. However, data on low-weight infertile male patients with a BMI of less than 25kg/m2 are scarcely shared in the literature (11).

In this study ( infertile men with BMI 18.5-24.9 kg/m2, overweight infertile men with BMI 25.0-29.9 kg/m2, and obese infertile men with BMI >30kg/m2). The semen samples taken from the patients were evaluated and compared in terms of sperm morphology, oxidative stress parameters, DNA fragmentation, which is one of the cell death markers, sperm nucleus morphology and general semen parameters**.**

## 2. GENERAL INFORMATIONS

A man's reproductive framework which remains located outside of his body which is different with a woman. Furthermore, these main external structures contain several elements, for instance, the penis, the scrotum, as well as the testicles. There are a number of organs that make up the male reproductive system that presents inside the body , such as the vas deferens, ejaculatory ducts, urethra, seminal vesicles, prostate, and bulbourethral organs.

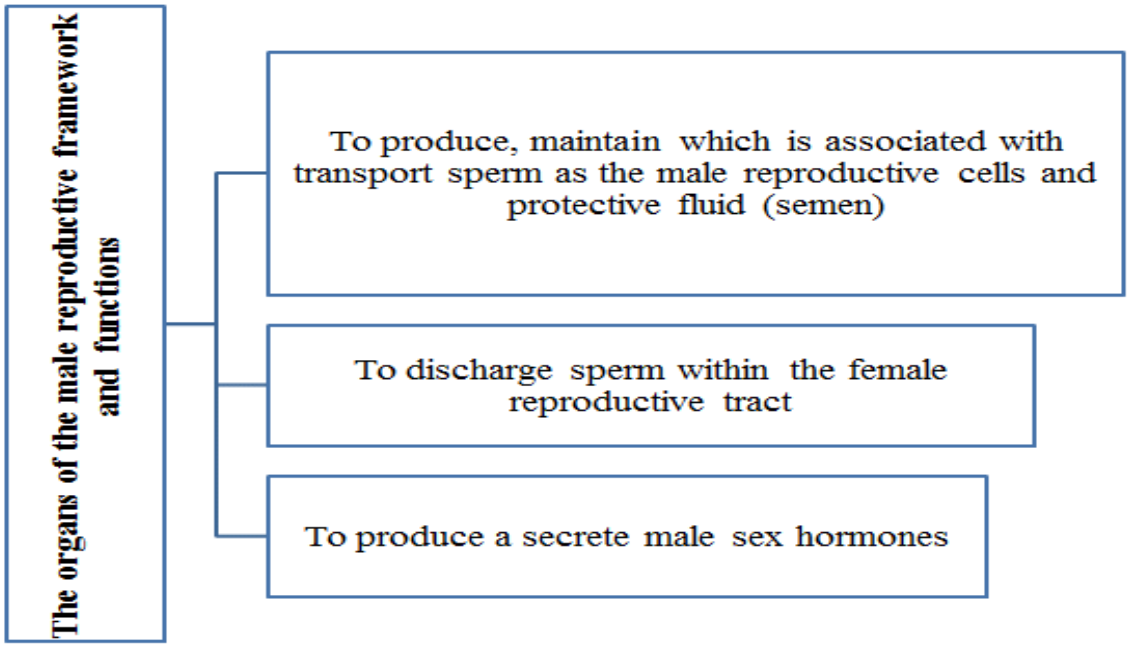



Figure 2.1. The organs of the male reproductive framework and functions.

Hormones, which are chemicals that promote the functioning of cells, are the primary male reproductive framework that is reliant on it. Aside from testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are key players in the male reproductive system's smooth operation.

### 2.1. Spermatogenesis

It remains a process where primordial germ cells mature into spermatozoa. Furthermore, spermatogenesis is influenced by growing levels of pituitary gonadotropin and begins soon before puberty and continues throughout life, including a complicated process that is linked to an unprecedented sequence of events.

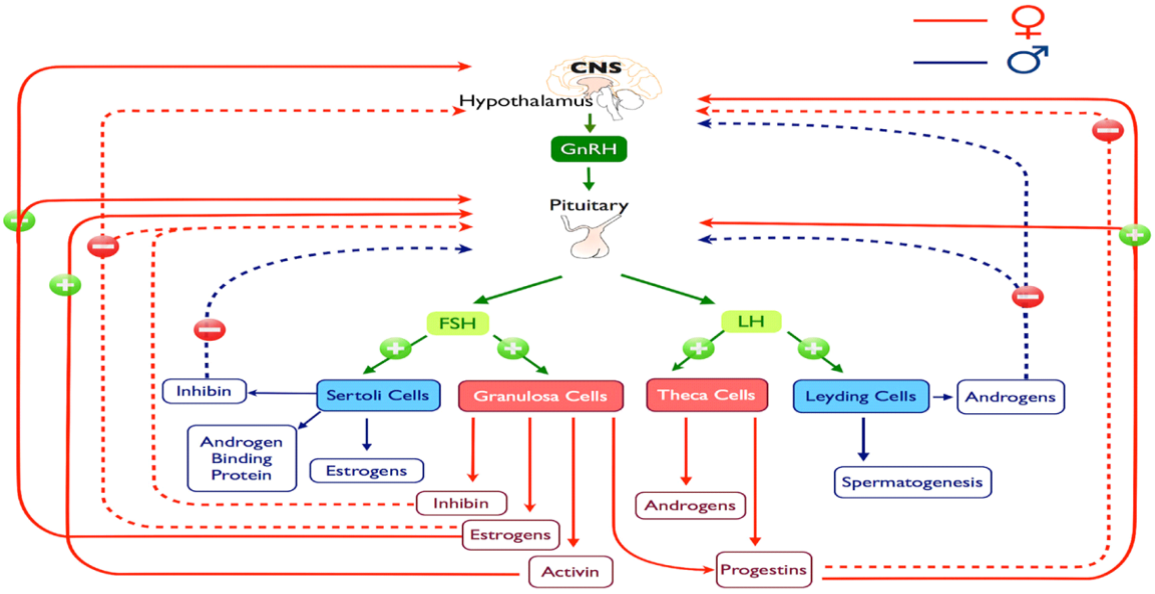



Figure 2.2. The axis of the hypothalamus-pituitary-gonads, as well as positive and negative feedback loops and products, are all shown. (35)

Testes; presented as a pair of oval-shaped tubuloalveos located in the scrotum, it is a combined type gland. Take place outside the abdominal cavity that is necessary to provide the required temperature of 34-35° C for spermatogenesis (96).

The testicles are also covered and surrounded externally by a connective tissue sheath named the tunica albuginea. Loose connective tissue containing blood vessels inside this sheath, tunica vasculosa is found. Thickening in the mediastinum region where the rete testis is located fibrous septa formed by the connective tissue capsule by entering into the organ divides the organ into 250-300 lobules. Seminiferous tubule ranging from 1 to 4 in each lobule includes (12,96).

Seminiferous tubules are approximately 150 µm in diameter and 80 cm in length. It is in the structure of a "U" shaped channel that opens to the rete testis. Seminiferous tubule epithelium, a specialized stratified epithelium composed of Sertoli cells and spermatogenic cells in its structure. Tunica propria has a special connective tissue structure. The basal lamina surrounding the tubules is a connective tissue rich in myoid cells and collagen fibrils (12,96).

Sertoli cells, functional and metabolic support for spermatogenesis are columnar shaped cells. Sertoli cell continues to have the ability to reduce spermatogenesis before puberty and at aging. They do not divide by mitosis after puberty. The enormous nucleolus may be

seen in the nucleus, which is situated at the cell's base. Nucleolus from the sides, in a unique three-part structure called karyosome, RNA and DNA surrounded by a pair of bodies containing.

Blood –testis barrier remains to be result from junctional complex between adjacent two sertoli cells. Blood testicular barrier, zonula occludens, macula adherens includes side-face junctions. On the lateral surfaces of Sertoli cells spermatogenic cells are located in the recesses found (96 ,97).

Sertoli cells It also has functions such as secreting seminiferous tubule fluid and phagocytosis are available. Limiting the number of germ cells Sertoli cells task and is species specific.it also provide a hormonal control for spermatogesis responsible cells (12, 96, 98).

Spermatogenesis occurs in 3 stages:

1-Spermatogonial stage.

2-Spermatocyte stage (meiosis).

3-Spermatid stage (spermiogenesis).

All spermatogenesis practices as well as stages occur in period of 64 days. In addition, end via maturation as well as release of the produced spermatid in process which is termed as spermation.

### 2.1.1. Spermatogonial stage

Stem cells of the spermatogonia undergo periodic cell divisions while retaining the lineage of spermatogonia that will eventually replace them as a primary spermatocyte. As shown in Figure 2.4. below, spermatogonia are categorised based on their nuclear appearance in conventional (H) and extracellular (E) preparations.

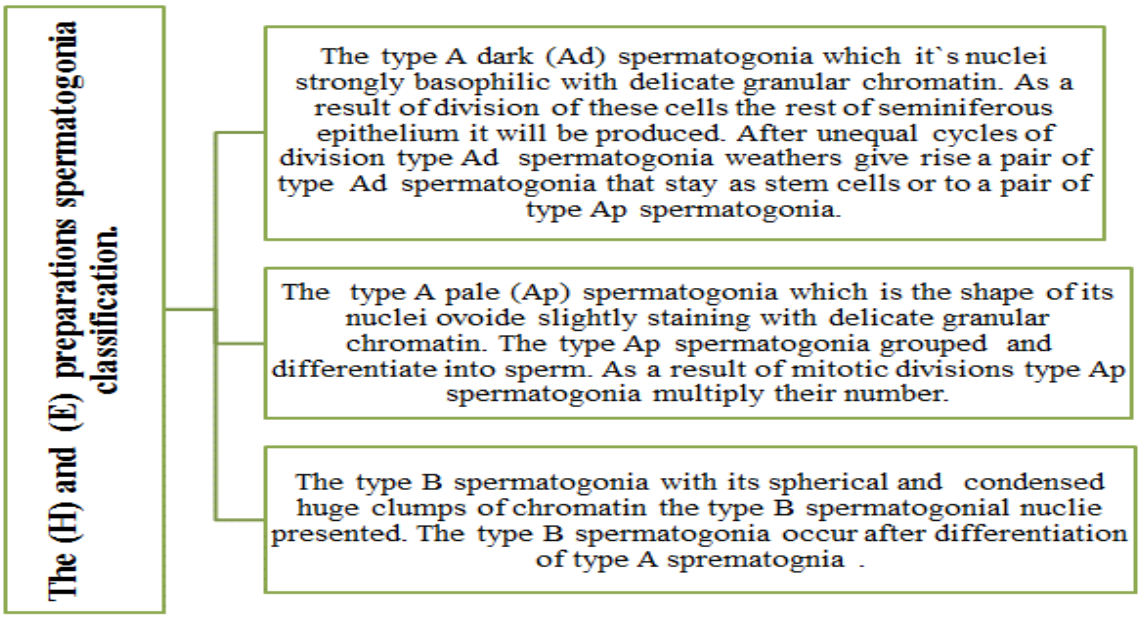



Figure 2.3. The (H) and (E) preparations spermatogonia classification.

Despite the fact that the sort Ad spermatogonia which is associated with the sort Ap spermatogonia enter in successive cycles of the mitotic and meiotic division all the progeny connected to each other via very fine cytoplasmic processes. Consequently, all the stages of spermatid production, as well as the development, will be synchronous. In addition, at the time of appearance of the sort B spermatogonia, this explains the full meaning of the end of the spermatogonial stage.

### 2.1.2. Spermatocyte stage (meiosis)

Subsequently, after several divisions of sort B spermatogonia primary spermatocyte, it will produce. Additionally, the normal chromosomal number (2n) and double DNA total value are present in initial spermatocytes as a result of DNA replication (4d). The goal is to decrease the number of chromosomes from 2n to 1n; a reduction of a factor of two. meiosis this study outcome in the production of secondary spermatocyte with haploid number of chromosomes (1n) as well as (2d) total value of DNA has been required. On the other hand, for the reason that of no replication of DNA total value meiosis ıı it will produce spermatid with haploid number of chromosoms (1n) as well as (1d) chromatid.

Furthermore, in a process of crossing-over, a paired of homologous chromosomes exchange their genetic characteristics as well as to yield genetic diversity.

### 2.1.3. Spermatid stage (spermiogenesis)

Consequential resulting from second meiotic division each spermatid contains haploid DNA content (1d) which is associated with (1n) chromosome number as twenty-two autosomes as well as an X or Y chromosome. Therefore, without cell division, on the other hand, in the process of cellular differentiation, the haploid spermatid undergoes in the spermiogenesis process which it will out came in the production of mature sperm. Consequently, after the sperm fertilizing the oocyte, the normal diploid content will be restored. Despite the fact that spermatids are linked to the plasma membrane of Sertoli cells through specific cellular junctions, this will occur throughout the course of spermiogenesis.

Spermiogenesis occur in four stages:

1. Golgi stage remains characterized via the presence of proacrosomal granules that is rich in glycoproteins subsequently, it a periodic acid-Schiff (PAS) positive granules. Moreover, these granules unite to acrosomal vesicle which is membrane-bounded and take a position at the anterior pole of the developing sperm. Furthermore, at a similar stage after taking a position at the posterior pole of the spermatid the centriole migrates and start assembly of 9 peripheral microtubule doublets as well as two central microtubules that form axoneme of the sperm tail.

2. Cap stage is the acrosomal vesicle present additional spreading over the anterior half of the nucleus and form acrosomal cap at a similar time the nuclear membrane that is lie bellow acrosomal cap which turned to be thicker and loses its pores as long as condensing of nuclear materials.

3. Acrosome stage in relation to Sertoli cell the spermatid changes the direction of its location as the head of the spermatid deeply embedded in the Sertoli cell which is associated with the direction toward the basal lamina. Furthermore, the flagellum of the seminiferous tubule points toward the tubule's lumen. Consequently, it exhibits elongation and flattening at the level of the nucleus's condensed state. By moving closer to anterior plasma membrane, nucleus, with its lengthy acrosome, and posteriorly showing its displacement. Moreover, the posterior pole of the spermatid the cytoplasmic microtubules regulate to form manchette. Similarly, the flagellum

developed from several modifications of the centrioles, afterward moving of the centrioles to the posterior aspect of the nucleus and its attachment to the shallow groove in the nucleus. The neck portion of the sperm is also well-known to be the connecting component. The outer dense fiber's periphery to the microtubules of the axoneme is made up of nine coarse fibers that extend from the centrioles linked to the nucleus. Additionally, for reason that it's connecting the nucleus with flagellum these fibers which are termed as connecting piece. Furthermore, the manchette and plasma membrane vanish and the flagellum expands while being covered by the plasma membrane. A helically coiled sheath forms around the rough fibers in the neck and posteriorly by migration of the mitochondria as well. A fibrous sheath with a double of longitudinal columns, connected to the number of connective ribs surrounding the 9 longitudinal fibers that extend to the flagellum's distal end, is also present in this part of the sperm. As a result, the lowest portion of the fibrous sheath, which is known as the end piece, is retained.

4. Maturation stage: Mature spermatozoa are formed when the excess cytoplasm around the flagella of the spermatid is removed during the maturation stage. Furthermore, this extra quantity from cytoplasm phagocytosed via Sertoli cells which is termed as the residual body. Moreover, the intercellular bridges that remained connect all the stages of spermatid development disappear as well as the spermatids loss its connection to each other belong to the Sertoli cells. It is also believed that after the spermatids have reached their final length and have been liberated from Sertoli cells to enter the seminiferous tubule lumen, they have completed the maturation stage of spermatogenesis. via the action of several enzymes similar to integrin-linked kinase on Sertoli-to-spermatid junctions the spermatid release at the period of spermiation.

**2.2. Structure of the Mature Sperm**

5. There are several unusual aspects to this cell, including the fact that it is around 60 micrometers long, and the sperm head is flattened, pointed, and measures 4.5 micrometers long by three micrometers broad by one micrometer thick. There are also other enzymes similar to trypsin found in the acrosomal cap that protects the nucleus's anterior two-thirds, including hyaluronidase, neuroaminidase, acrosin, and acid phosphatase. They are also crucial for breaking through the ovum's outer membrane,

which is why these enzymes are so important. It is also important to note that the acrosome response begins when the sperm contacts the egg. Sperm penetration and subsequent fertilization are also made easier by this intricate process, which is linked to the prevention of any extra sperm being let in.

6. However, it is still separated into a neck and a central portion known as the primary portion. Similarly, the centrioles and the origin of the coarse fibers are located in the short neck. As a result, the central section is seven micrometers long and includes the mitochondria and the axonemal complex. Similarly, these mitochondria power the sperm's movement and, as a result, the sperm's motility is dependent on them. It's also almost 40 nm long and includes the fibrous sheath that surrounds the coarse fibers as well as the axonemal structure. Axonemal complexes are only found in the last five micrometers of the flagellum in mature sperm (12).

## 2.3. Semen

### 2.3.1. Semen volume

Fluid discharges from the accessory sex organs are combined with concentrated spermatozoa in the epididymides after ejaculation to generate the semen remnants. In addition, it continues to be released in many boluses. As a result, pre- and post-vasectomy semen quantities show that the accessory organs, namely prostate and seminal vesicles, provide approximately 90% of semen volume, with modest contributions from the bulbourethral (Cowper's) gland, which is connected with epididymis  In addition, the World Health Organization's (2010) semen volume cut-off value is 1.5 ml (13).

### 2.3.2. Semen ph

The pH of the various accessory gland secretions, particularly the alkaline seminal vesicular secretion that is part of the acidic prostatic secretion, is reflected in the pH balance. Another thing to keep in mind is that it's important to keep an eye on the pH as soon as possible after liquefaction since it's affected by CO2 loss that happens after manufacturing. Semen from fertile males, on the other hand, has a limited number of reference points for its pH.

Additionally, this manual keeps the consensus value of 7.2 as a lower threshold value until further information is available.

### 2.3.3. Sperm motility

Progress in sperm motility and the likelihood of becoming pregnant are still linked, according to research (14). After liquefaction of the sample, the sperm motility inside the semen should be measured as soon as possible after ejaculation, ideally within half an hour, but in any event within one hour, to reduce the detrimental effects of dehydration, pH, or temperature changes on motility.

Sperm movement Categories

Spermatozoa with progressive or non-progressive motility may be distinguished from those that remain immotile using a basic framework for grading motility. Each spermatozoon's motility stays graded as shown in the figure 2.5. below:

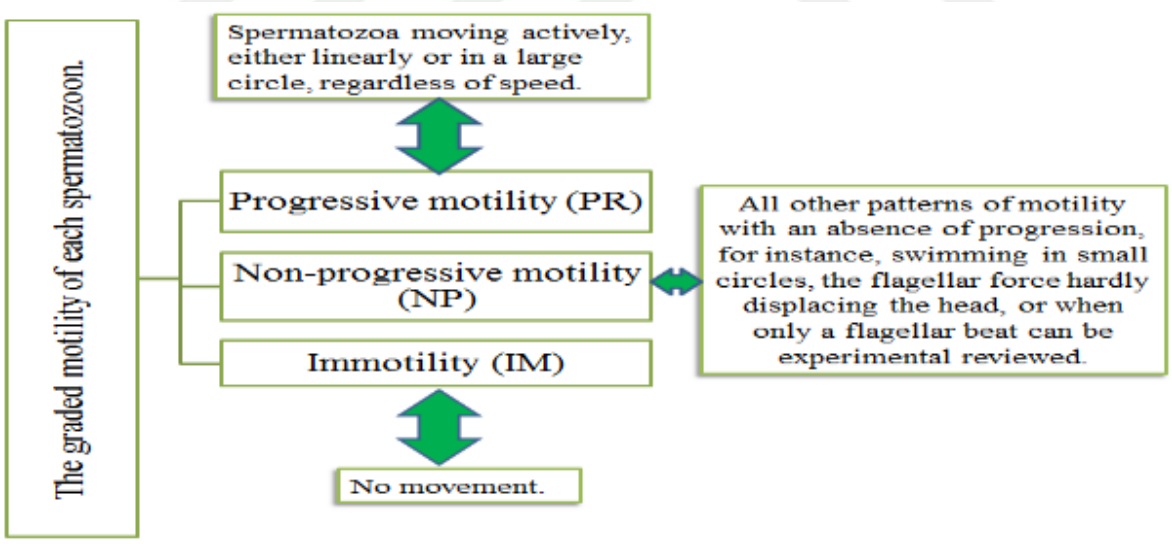



Figure 2.4. The graded motility of each spermatozoon.

It's also crucial to include sperm motility when considering total motility (PR + NP) or progressive motility (PR).

The lowest limit for motility total (PR + NP) is still set at 40%.

Progressive motility (PR) continues to have a lower reference limit of 32%.

### 2.3.4. Sperm vitality

On the contrary, it is critical for samples with fewer than 40% progressively motile spermatozoa to frequently examine the membrane integrity of the cells in order to ensure the integrity of the cells. In addition, the proportion of dead cells should not exceed (within sampling error) the percentage of immotile spermatozoa that can be determined by this test. A higher fraction of viable cells than motile cells is also common.

To determine the proportion of viable spermatozoa, look for those that have an unbroken cell membrane, either by dye exclusion or hypotonic swelling. Dyes stay permeable through damaged plasma membranes, which may be present in non-vital (dead) cells, which is why the dye exclusion method is still used. As a result, the hypo-osmotic swelling test assumes that only cells with intact membranes, which are referred to as "living cells," would swell when placed in hypotonic solutions.

The viability of the sperm should also be tested within 30 minutes of the semen sample being liquefied. Dehydration and fluctuations in temperature may have detrimental effects on vitality, therefore it's best to avoid testing them for an hour after ejaculation. Furthermore, the presence of a considerable proportion of viable and non-viable cells (necrozoospermia) may suggest epididymal disease (15). As a result, the lowest reference level for viability (membrane-intact spermatozoa) remains 58% (16).

### 2.3.5 Sperm numbers

Both time to pregnancy and pregnancy rates are affected by sperm count and concentration. In addition, the concentration of spermatozoa in the ejaculate is used to compute the number of spermatozoa in the ejaculate. The total quantity of spermatozoa in the ejaculate remains correlated with testicular volume (17) for normal ejaculates when the male tract is unobstructed and the abstinence time is short. As a result, the measurement of the examination's ability to produce spermatozoa belongs to the patency of the male tract. Furthermore, the concentration of spermatozoa in the semen, which is associated to

fertilization and pregnancy rates, is influenced by the amount of the seminal vesicles' secretions, which is linked to the prostate and is not a specific measure of testicular activity.

Total sperm count and sperm concentration are two distinct concepts. Sperm concentration, on the other hand, refers to the quantity of viable spermatozoa per unit volume of semen, and it is a function of both the number of spermatozoa released and the amount of fluid used to dilute them. It's also worth noting that the total quantity of sperm refers to the total number of spermatozoa in the whole ejaculate and is calculated by multiplying the sperm concentration by the semen volume.

There is still a lower reference limit of spermatozoa concentration of $15 \times 10^6$ cells per milliliter.

The bottom limit for the total number of sperm in an ejaculate is $39 \times 10^6$ spermatozoa.

**2.3.6. The sperm morphology**

Spermatozoa are made up of a head, neck, middle piece (midpiece), primary piece, and end piece, among other components. To further complicate things, the end piece is still difficult to arrange and exhibit using a light microscope. The cell may be thought to consist a head (and neck) that belongs to the tail (the middle component) (principal piece). A spermatozoon's head and tail must be normal in order for it to be considered normal. Additionally, borderline forms should be regarded as aberrant as well.

The ideal form for the head is smooth, normal, and oval in shape. A well-defined acrosomal region should also be present, encompassing between forty and seventy percent of the head surface. Vacuoles in the acrosomal region should not exceed 20% of the sperm head, and should not include any big vacuoles. There should also be no vacuoles in the post-acrosomal area.

Slender, regular, and about the same in length as the sperm head, the midpiece serves as the main structural support for the device. There should be an equal distance between the principal axis of the midpiece and that of the head. One-third or more of the sperm head size is considered an abnormal amount of residual cytoplasm.

In general, the main piece should have a consistent caliber throughout its length, be thinner than the middle piece, and be around 45 nm long (about 10 times the head length). If there is no acute angle that indicates a flagellar break, it may be looped back on itself.

Sperm abnormalities are categorized into three main groups based on their morphology:

Spermatozoa from various human sperm samples have been shown to be malformed in various ways. In addition, aberrant spermatozoa are often seen in patients with faulty spermatogenesis and epididymal diseases. In most cases, there are a variety of morphological flaws. It's also worth noting that sperm with aberrant DNA tends to have a lower fertility potential than normal sperm. Genetic abnormalities like as DNA fragmentation, chromosomal aberrations and aneuploidy have all been linked to morphological malformations. The shape of the sperm tail (the midpiece and the major piece) is also taken into consideration, although less so than the shape of the head.

The defects classifications

- Vacuolated more than two vacuoles or more than 20% of the head area occupied by unstained vacuolar areas (including vacuoles in the post-acrosomal area), small or large acrosomal areas (less than 40% or more than 70% of the head area), double heads, or any combination of these are all types of defects in the head that can be seen.

- An asymmetrical insertion of the midpiece into the head, thick or uneven, abruptly curved, excessively thin, or any integration of these.

- Short, repeated, broken, smooth hairpin bends, highly angulated bends of variable width, as well as coiled, are the most common flaws in a piece.

- ERC is a sign of aberrant spermatozoa that have accumulated as a result of a faulty spermatogenic process. Spermatozoa with abnormally high amounts of irregularly discolored cytoplasm, one-third or more of the sperm head size, and faulty midpieces are still abnormal, according to this study. Furthermore, this abnormally large clump of cytoplasm is not a cytoplasmic droplet (18).

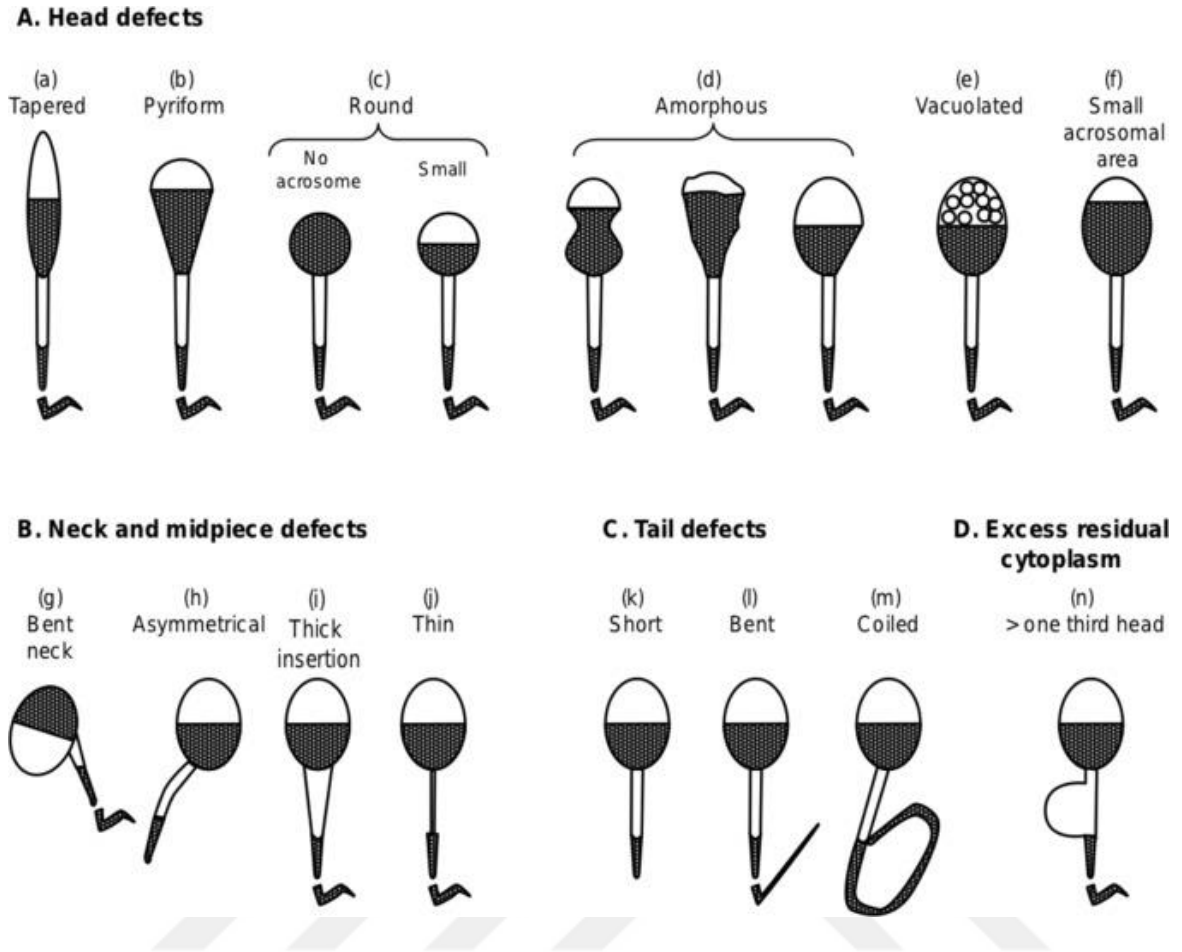



Figure 2.5. Detailed diagrams of several aberrant human spermatozoa types. (2)

## 2.4. Infertility

As long as the fertility considered as the main fact base of the reproductive health, in contrast, all over the world as reported via WHO infertility considered as major health problem (19) the couple who fails to conceive after one year of regular and unproductive sexual intercourse according to WHO considered as infertile couple, about 48.5 million couples worldwide remain infertile couples which represent fifteen percent of the couples at reproductive age (19, 20). In addition, in almost half of these couples, a male fertility factor remains involved.(21)

Infertility may also be caused by a variety of disorders, including varicocele, cryptorchidism, hypogonadism, testicular tumor, and systemic illnesses. Idiopathic infertility, on the other hand, affects around 40 percent of all couples, and is the most common kind of infertility (20, 22).

## 2.5. What Means of BMI (Body Mass Index) as Well as How to be Calculated

A patient's BMI is still calculated in the scientific community using their weight in kilograms divided by their height in meters squared (in meters squared). It was also justified as underweight (18.5), normal (18.5-24.9), overweight (25-29.9) or obese more than 30 based on (WHO) categories of BMI at that time (23,24,25,26). Men's reproductive potential may be assessed using their BMI and sperm parameters, which are considered to be the gold standard. Many studies have shown an inverse link between BMI and sperm concentration or total sperm count (TSC), but others have failed to show this correlation (1,27,28). Many original investigations have shown that overweight and obese people were more likely than normal weight people to have oligozoospermia or azoospermia (27) a higher, although non-significant, chance of aberrant sperm count was connected with obesity. There was a correlation between sperm count and BMI, with overweight males having a lower sperm count than slender ones, regardless of their BMI.

## 2.6. The Obesity as Well as Underweight Effect on Semen Quality

Non-communicable disease risk factors associated with obesity, defined by the World Health Organization (WHO) as a body mass index (BMI) greater than or equal to 30 (kg/m2), remain a medical condition of excess body fat that adversely impacts morbidity and mortality (29). Class I (between 30 and 34.9), class II (between 35 and 39.9), and class III (beyond 40) of obesity continue to exist (30,31). A global obesity epidemic has also taken hold, with more than 1.4 billion people being considered overweight or obese, according to data from the World Health Organization (WHO) as released at 2008 (23). Diabetes, hypertension, hyperlipidemia, and sleep apnea are all conditions associated with obesity and may be used to diagnose the metabolic syndrome. The obesity and comorbidities have been related to higher mortality and a decrease in life expectancy as well (32). As new data from the Centers for Disease Control shows, the metabolic alterations caused by obesity may significantly disrupt reproductive function and so influence the fecundity of a large proportion of the population. Ovulation problems and unexplained reproductive difficulties are still associated to fat in women (32,33). Over the last five decades, fertility rates have steadily decreased, which has coincided with an increase in obesity rates (32).

Obesity in women is well-documented, but the impact on male fertility and the characteristics of the sperm is less clear (20,23). When it comes to males, there's been a worrying trend of deteriorating sperm quality that reflects the rising obesity rates (32). The hypothalamic-pituitary-gonadal axis, which regulates spermatogenesis, must be intact in order for sperm to be formed in the testicles. The testicular environment may be altered by obesity in such a manner that sperm production and health may be affected (20,32). As a result, the research shows that the associations between semen parameters and obesity have been inconclusive in the past. A connection has been shown between obesity and reduced sperm motility, shape, and DNA integrity in multiple investigations (20,27,30,32). Obesity has been linked to changes in reproductive hormones, with several studies showing negative connections with testosterone (T) levels and a few finding favorable associations with estradiol (E2) levels (32). With regard to assisted reproductive technology (ART), male obesity is linked to an extended time to conception, a lower rate of pregnancy and an increase in the incidence of pregnancy loss (20,34,35).

### 2.7. Reactive Oxygen Species (ROS)

Hydroxyl (OH•), peroxyl (ROO•), hydrogen peroxide (H2O2) and superoxide anions (O2•) radicals are among the most reactive free radicals that are known to be oxidizing. Activated leukocytes in the seminal plasma, which are linked to mitochondria in the spermatozoa, are the primary source of ROS in sperm. Many studies show that ROS-mediated damage to spermatozoa contributes to infertility pathophysiology in 30 to 80 percent of men with infertility (36,37). Free radicals are needed for cell functions, but they may also interfere with these processes when they are present in high quantities. This is known as oxidative stress, which occurs when the antioxidant scavenging framework of the cell is overwhelmed by the overproduction of ROS (36,38). Furthermore, oxidative stress is a significant contributor to male infertility, both as a cause of sperm cell malfunction and as an underlying cause (39). High levels of ROS and oxidative stress are also linked to male infertility's pathogenesis, according to previous research (22). due to spermatozoa's structural and functional integrity being compromised (39 ,40). As a result, the exact mechanism and method of oxidative stress-induced decline in sperm functionality remains a mystery, but it is widely accepted that it is caused by peroxidative damage to the axoneme and depletion of intracellular ATP levels, which is followed by the generation of 4-hydroxynonenal and malondialdehyde due to oxidation of lipid membrane components (41,42). Infertility,

recurrent pregnancy loss (RPL), childhood mortality, and high rates of morbidity in the offspring have been linked to high levels of DNA damage in human spermatozoa (36,43).

### 2.8. Chromatin Condensation

However, it has been a well reported that the maturity based on sperm chromatin which remains an important issue for fertilizing capacity of spermatozoa as well as embryonic development processes (44,45). Prognoses for pregnancy and fertilization might be made more accurate by using the primary mechanism of proportionation that relies only on sperm and is related with aberrant chromatin condensation in (ejaculate). Therefore, in the time duration of spermiogenesis, sperm nuclear remains completely documented as well as undergoes factors and characteristic which is associated with rearrangements also is an important compaction (46,47). Additional nucleoproteins, such as histones, are replaced by transition proteins before being replaced by an additional basic protein, which is referred to as protamine P1 and P2 in the context of chromatin condensation (46,48). As a result of nuclear compaction, spermatozoa's passage through the male and female genital tracts as well as its contact with the egg is protected by the paternal genome. Male infertility is often linked to anomalies in chromatin condensation as a consequence of nuclear damage as a result of DNA denaturation or fragmentation (49).

# 3. MATERIALS AND METHODS

By the decision of Gazi University Clinical Research Ethics Committee and number 364 dated 14.05.2018 the thesis was carried out with permission.

In our study, semen samples taken from couples who applied to Gazi University Medical Faculty Assisted Reproductive Treatment Center (ÜYTEM) and male patients diagnosed with male-borne infertility will be used from 07 2018- 07 2020. Each patient had his informed consent. In this study the sample group consisted of 60 infertile Turkish men between the ages of 22 – 54 years. Medical and infertility history of each patient was recorded.

**Inclusion Criteria for Volunteers:**

- Between the ages of 22 and 54 (50).

**Volunteers Exclusion Criteria (50-51):**

- Smoking, Alcohol use
- Regular sauna use
- Diagnosis of diabetes Mellitus, hypothyroidism, hypertension, chronic kidney-liver disease, asthma, epilepsy, atherosclerosis, orchidectomy, steroid use, hypogonadism in need of medical treatment, thromboembolism diagnosis, testicular cancer, chronic inflammatory bowel disease.
- Having a history of cerebrovascular disease
- Detection of bacterial contamination in semen
- Diagnosed azoospermia (~ 1 × 106 ml − 1 sperm concentration) each patient´s weight and height with a professional calibrated device were measured. BMI was calculated by dividing the weight to height squared. Patients were stratified according to their BMI

into three groups. The evaluation process included a total of 60 patients with infertility symptoms.

- 20 normal (BMI 18.5-24.9 kg/$m^2$).
- 20 overweight (25.0-29.9 kg/$m^2$).
- 20 (BMI≥30 kg/m2) obese men were eligible for the study (52).

the patients asked to refrain from sexual activity for a period of 3 days. Semen samples were obtained from patients by masturbation in a special room close to the laboratory. The collected semen specimens were assessed for conventional semen parameters including sperm motility and sperm concentration after liquefaction (experiment 1). Chromatin condensation assessment (experiment 2). Evaluation of DNA fragmentation by TUNEL method (experiment 3). Oxidant – antioxident parameters by use of TAS/TOS analyzer was performed (experiment 4).

### 3.1. Experiment 1: Semen Analysis

Semen samples were collected by masturbation after a recommended 3-5 days of abstinence. After collection, specimens were allowed to liquefy at room temperature for 30 min before using for analysis. On microscopic examination, sperm count and the percentage of motility and morphology were objectively evaluated according to the recommendation of World Health Organization (WHO) (13). Criteria determined by WHO (World Health Organization, 2010):

- Volume: 1.5 m
- Sperm concentration: 15x106 / ml
- Total sperm count: 39 x 106
- Total mobility 40%
- Progressive motility 32%

- Morphology 4%

For evaluation of sperm morphology 10µl of semen dropped over slides. A thin smear of semen was created by pulling it with the help of anther clean slide. Air dried smear was fixed with 4% paraformaldehyde solution. After fixation the smear washed with distilled water for 10 minutes then stained with spermac dye (LOT: FP12S05, FertilPro)(53).following the manufacturer's instructions.

1. Smears were stained with solution A (red) for one minute.
2. Excess dye was removed by washing with tap water.
3. Stained for one minute with solution B (light green ).
4. Repeated washing like second step.
5. stained for one minute with solution C (dark green).
6. Repeated washing like second step.
7. Air-dried smears were examined under light microscope with oil immersion at 1000x magnification .

### 3.2. Experiment 2: Chromatin Maturation Assessment

for acidic aniline blue staining 10 µl were taken from liquefied semen and smeared. Air-dried smears were fixed with 4% paraformaldehyde for thirty minutes at room temperature. After fixation the smears washed with distilled water for ten minutes. Stained with 2% acetic acid 2,5% anilin blue (Ph= 3,5) (B8563 LOT: SLBQ9814V, Sigma) solution for 5 minutes . the excess dya over the the smears removed by washing with tap water and then dried in the air. 200 sperms were evaluated at 1000x magnification with immersion oil under light microscope. Spermatozoa were classified according to their staining characteristics with aniline blue, completely stained (++), partially stained (+ -) and not stained sperms (-) by two different researchers with DM 4000 Leica Germany imaging analysis systems.(54)

### 3.3. Experiment 3: TUNEL Test for DNA Fragmantation

TUNEL, one of the most commonly used DNA fragmentation detection methods, is a clinically appropriate test with high sensitivity and specificity. TUNEL test is performed by determining fluorescently labeled dUTP, which is included in single and double strand DNA fragments, catalyzed by terminal nucleotidyl transferase (TdT), an enzyme that works independently of the mold.

(ApopTag plus peroxidase In Situ Apoptosis Detection kit) (S7101) (Millipore ) was used in the thesis. The protocol is as follows:

1. 10 µl were taken from liquefied semen and smeared. Air- dried smears were fixed with 4% paraformaldehyde for thirty minutes at room temperature. Areas to be examined were marked with hydrophobic pen .

2. The marked areas were washed with phosphate buffer solution PBS (AP-90003-500 LOT:BBI120127,Thermo) 2 times for 5 minutes each wash.

3. Treated with 0.2% triton X-100 in PBS for 6 minutes on ice.(this solvent permeabilizes cells).

4. Washed in 2 changes of PBS for 5 minutes each wash.

5. Quench in 3.0% hydrogen peroxide in PBS for 5 minutes at room temperature.

6. Rinse the specimen twice with PBS or dH2O for 5 minutes each time , in coplin jar.

7. Equilibration buffer applied directly on the specimen .

8. After pipette onto the section 55µl/5$cm^2$ of working strength TdT enzyme, then incubated in humidified chamber at 37ºc for 1 hour.

9. Working strength stop /wash buffer applied on the specimenand incubated for 10 minutes at room temperature.

10. the specimens were Washed in 3 changes of PBS for 1 minute each wash.

11. Anti- Digoxigenin peroxidase conjugate applied at room temperature and incubated in a humidified chamber for 30 minutes .

12. the specimens were Washed in 4 changes of PBS in coplin jar for 2 minutes per wash at room temperature.

13. Peroxidase substrate was applied to completely cover the specimen for 15 minutes.

14. The specimen washed in 3 changes of d H2O in coplin jar for 1 minute each wash.

15. The slides incubated in d H2O in a coplin jar for 5 minutes at room temperature.

16. Counterstained in 0.5%(w:v) methyl green in coplin jar for 10 minutes at room temperature.

17. The specimen washed in 3 changes of d H20 in coplin jar.

18. The specimen washed in 100% N-BUTANOL in coplin jar.

19. The specimen dehydrated by moving the slide through jar of XYLENE.

20. Mounting the specimen(55,56).*

All these operations were carried out with the DM 4000 Leica Germany visual analysis system available in our department, 200 sperms were evaluated as sperm positive or negative for TUNEL .

### 3.4. Experiment 4: TAS/TOS Analysis

Semen 700G was centrifuged for 10 minutes. After the supernatant part was separated it was stored at -20 c in Eppendorf tubes (1-1.5 ml).

TAS (REL Assay Diagnostics, LOT: EL18091A) and TOS (REL Assay Diagnostics, LOT: MT181050) levels were measured calorimetrically using the commercial kit. During the experiment, it was applied as stated in the kit contents. Color change was determined using a SHIMADZU UV-1601 spectrophotometer.

### 3.5. Statistical Methods

The conformity of continuous variables to the normal distribution was examined graphically and using the Shapiro-Wilk test. Mean ± standard deviation, median, minimum and maximum values were used to describe the variables.

One-way analysis of variance (ANOVA) was applied to compare the variables that provided the parametric test assumptions. When a difference was found in the ANOVA result, the source of the difference was investigated with the Bonferroni post-hoc test.

Kruskal-Wallis test was applied to compare skewed data (nonparametric) variables. When a difference was found, post-hoc pairwise comparisons were made with the Mann-Whitney test with Bonferroni correction to identify the different group.

Statistical analysis and calculations were done with IBM SPSS Statistics 21 (IBM Corp, NA, USA). In statistical decisions, $p \leq 0.05$ value was accepted as an indicator of significant difference.

# 4. RESULTS

## 4.1. General Sperm Parameter Findings

The semen volume values of the subjects in the groups were 3.1±1.4 in the “normal” group; It was found to be 3.2±1.4 in the “overweight” group and 2.5±0.9 in the “obese” group (Table 1, Graphic 4.1). Although a decrease was observed in semen volume values in the obese group, the Kruskal-Wallis test result was not statistically significant between all groups (p=0.347).

The concentration values of the subjects in the groups were 30±24% in the “normal” group; It was found to be 24±25% in the “overweight” group and 31±29% in the “obese” group (Table 1, Graphic 4.2). The Kruskal-Wallis test result was not statistically significant between all groups (p=0.468).

The total concentration values of the subjects in the groups were 79±53% in the “normal” group; It was found to be 80±111 in the “overweight” group and 76±71 in the “obese” group (Table 1, Graphic 4.3). Although a decrease was observed in total concentration values in the obese group, the Kruskal-Wallis test result was not statistically significant between all groups (p=0.515).

Total motility values of the subjects in the groups were 62±16% in the “normal” group; It was found to be 56±23% in the “overweight” group and 60±18% in the “obese” group (Table 1, Graph 4.4). There was no statistical significance among all groups with the result of the Kruskal-Wallis test (p = 0.591).

The motility sperm count of 4% of the subjects in the groups was 4±7% in the “normal” group; It was found to be 4±7% in the “overweight” group and 2±5% in the “obese” group (Table 1, Graph 4.5). No statistical significance was found among all groups with the result of the Kruskal-Wallis test (p = 0.703).

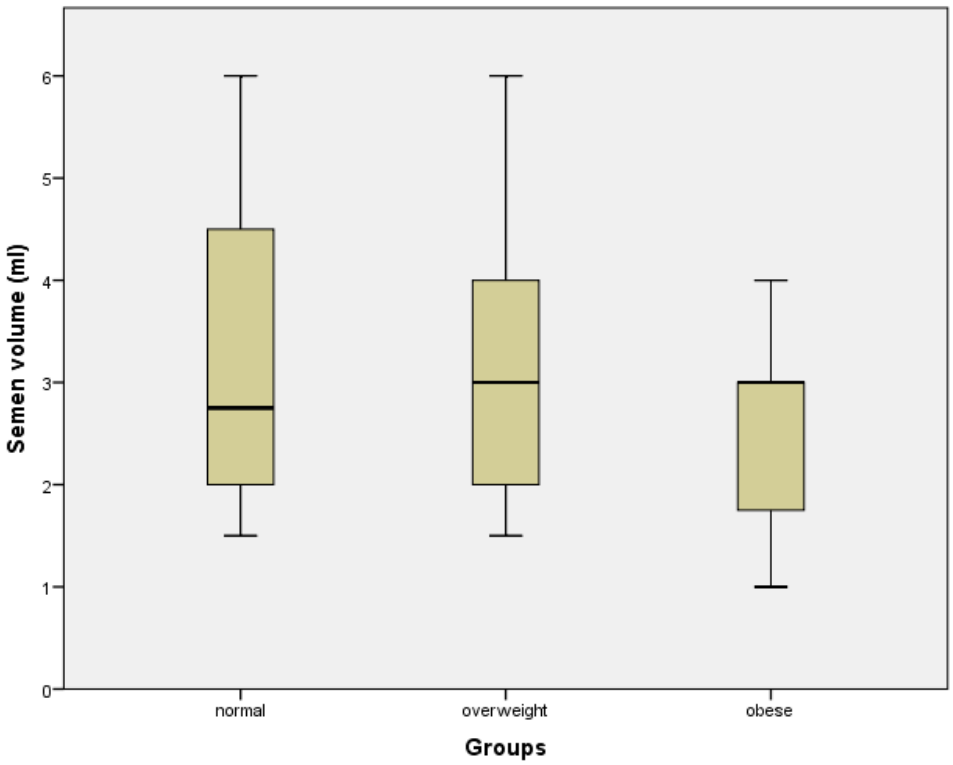
3% motility sperm count of the subjects in the groups was 48±13% in the “normal” group; It was found to be 43±19% in the “overweight” group and 47±17% in the “obese” group

(Table 1, Graph 4.6). There was no statistically significant difference between all groups in the result of the Kruskal-Wallis test (p = 0.520).

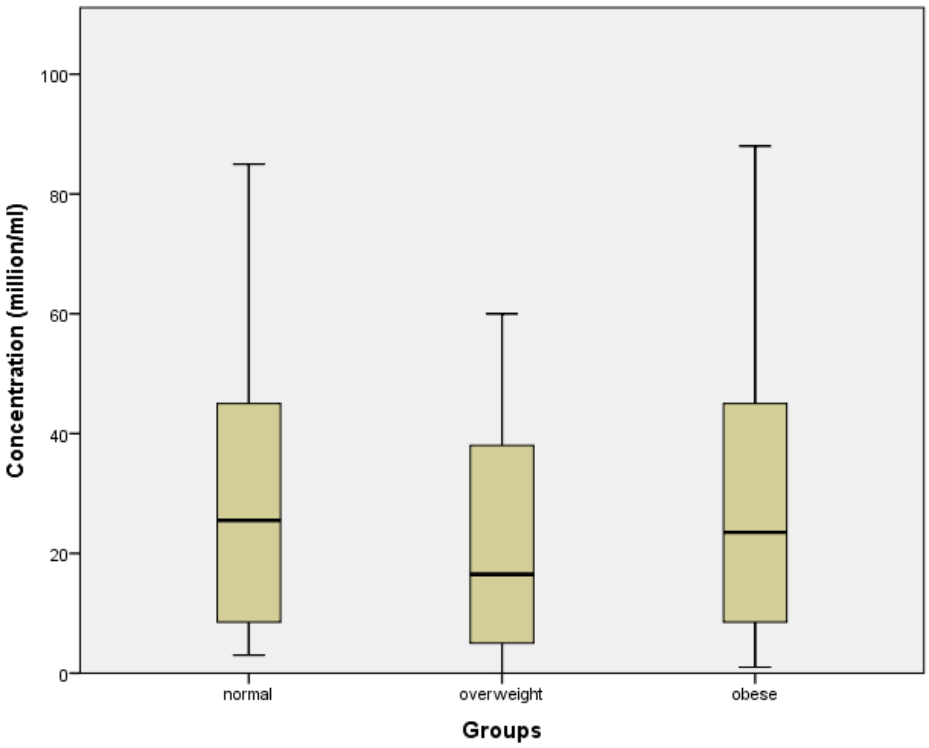
The 2% motility sperm count of the subjects in the groups was 10±5% in the "normal" group; It was found to be 9±5% in the "overweight" group and 11±5% in the "obese" group (Table 4.1, Graph 4.7). The Kruskal-Wallis test result was not statistically significant between all groups (p=0.409).

Table 4.1. Descriptive data on general sperm parameters

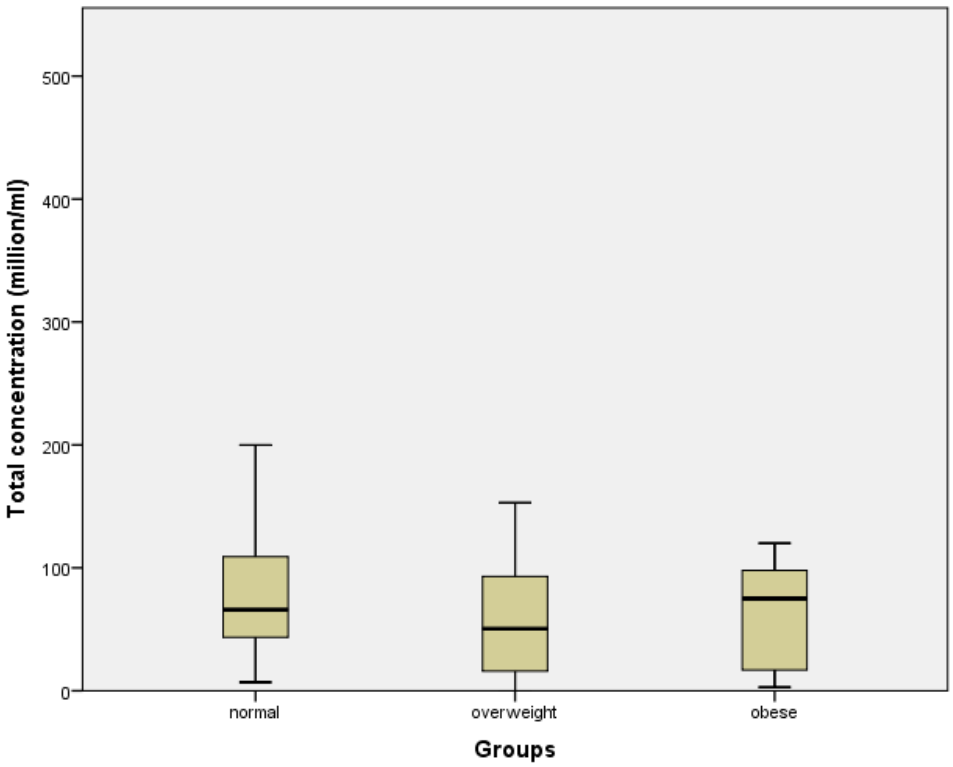
| | | Groups | | |
|---|---|---|---|---|
| | | Normal | overweight | Obese |
| Semen volume (ml) | Mean | 3,1 | 3,2 | 2,5 |
| | Standard Deviation | 1,4 | 1,4 | 0,9 |
| | Median | 2,8 | 3,0 | 3,0 |
| | Minimum | 1,5 | 1,5 | 1,0 |
| | Maximum | 6,0 | 6,0 | 4,0 |
| Concentration (million/ml) | Mean | 30 | 24 | 31 |
| | Standard Deviation | 24 | 25 | 29 |
| | Median | 26 | 17 | 24 |
| | Minimum | 3 | 0 | 1 |
| | Maximum | 85 | 95 | 88 |
| Total concentration (%) | Mean | 79 | 80 | 76 |
| | Standard Deviation | 53 | 111 | 71 |
| | Median | 66 | 51 | 75 |
| | Minimum | 7 | 0 | 3 |
| | Maximum | 200 | 475 | 264 |
| Total motility (%) | Mean | 62 | 56 | 60 |
| | Standard Deviation | 16 | 23 | 18 |
| | Median | 61 | 60 | 60 |
| | Minimum | 10 | 0 | 0 |
| | Maximum | 84 | 90 | 85 |
| Motility %4 (%) | Mean | 4 | 4 | 2 |
| | Standard Deviation | 7 | 7 | 5 |
| | Median | 0 | 0 | 0 |
| | Minimum | 0 | 0 | 0 |
| | Maximum | 26 | 21 | 20 |
| Motility %3 (%) | Mean | 48 | 43 | 47 |
| | Standard Deviation | 13 | 19 | 17 |
| | Median | 50 | 45 | 50 |
| | Minimum | 10 | 0 | 0 |
| | Maximum | 68 | 80 | 77 |
| Motility %2 (%) | Mean | 10 | 9 | 11 |
| | Standard Deviation | 5 | 5 | 5 |
| | Median | 10 | 10 | 10 |
| | Minimum | 0 | 0 | 0 |
| | Maximum | 20 | 21 | 20 |



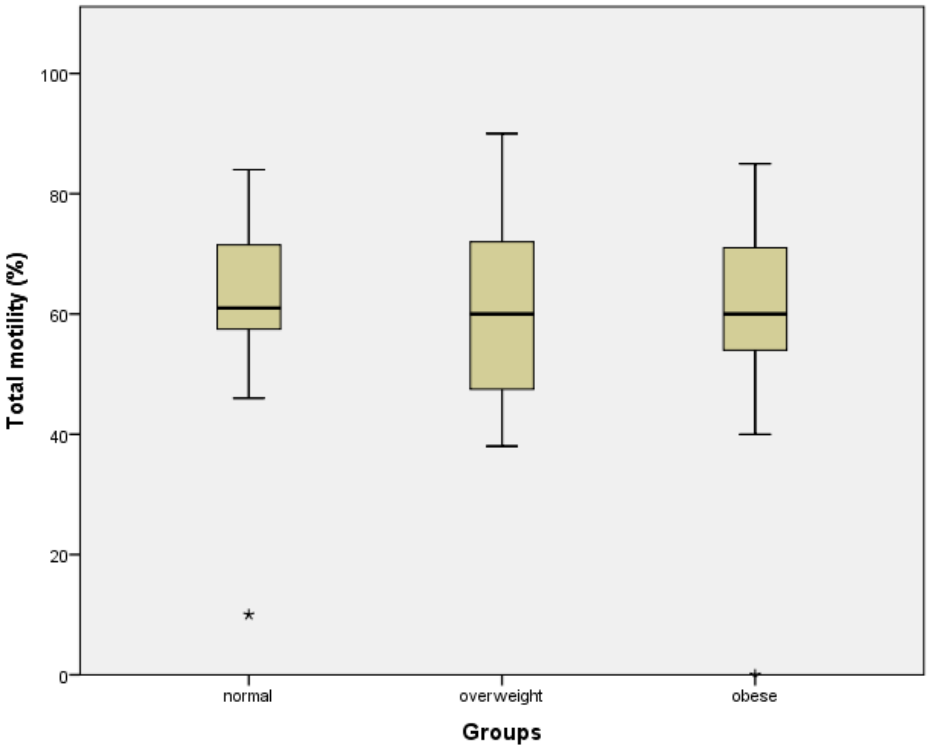

Graphic 4.1. Descriptive data graph of semen volume values of all groups



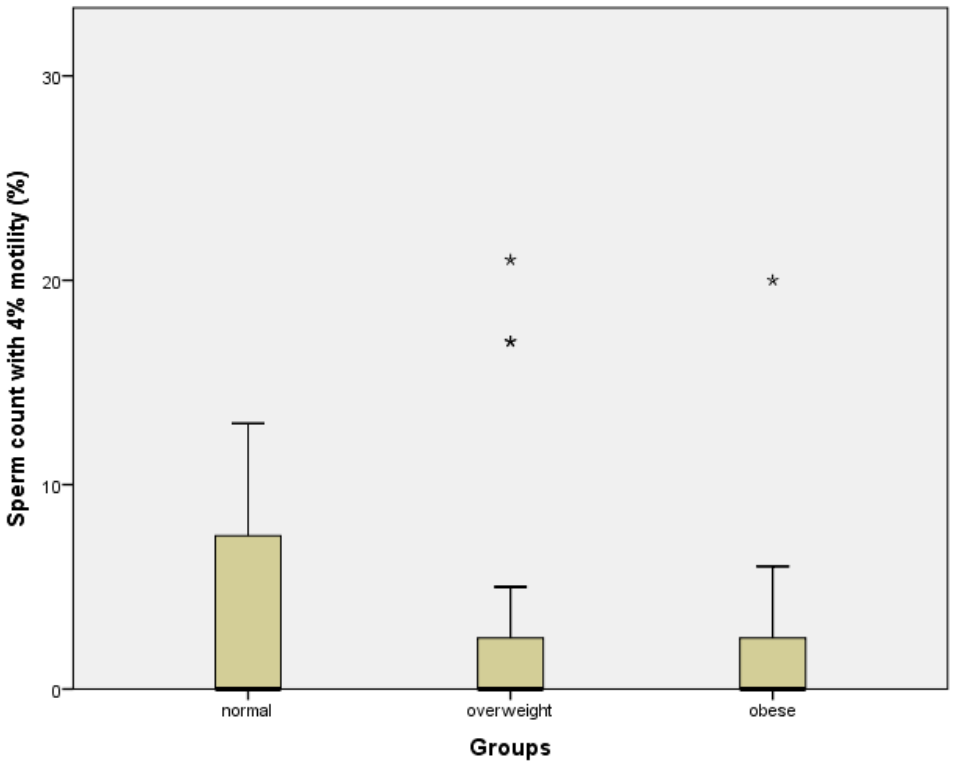

Graphic 4.2. Descriptive data graph of concentration values of all groups



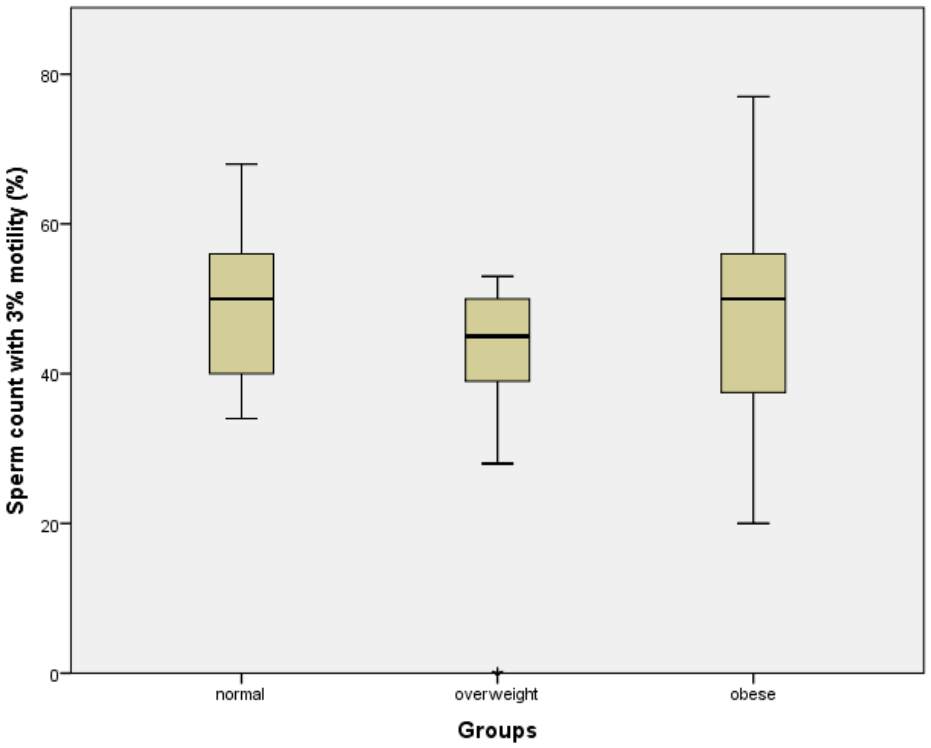

Graphic 4.3. Descriptive data graph of total concentration values for all groups



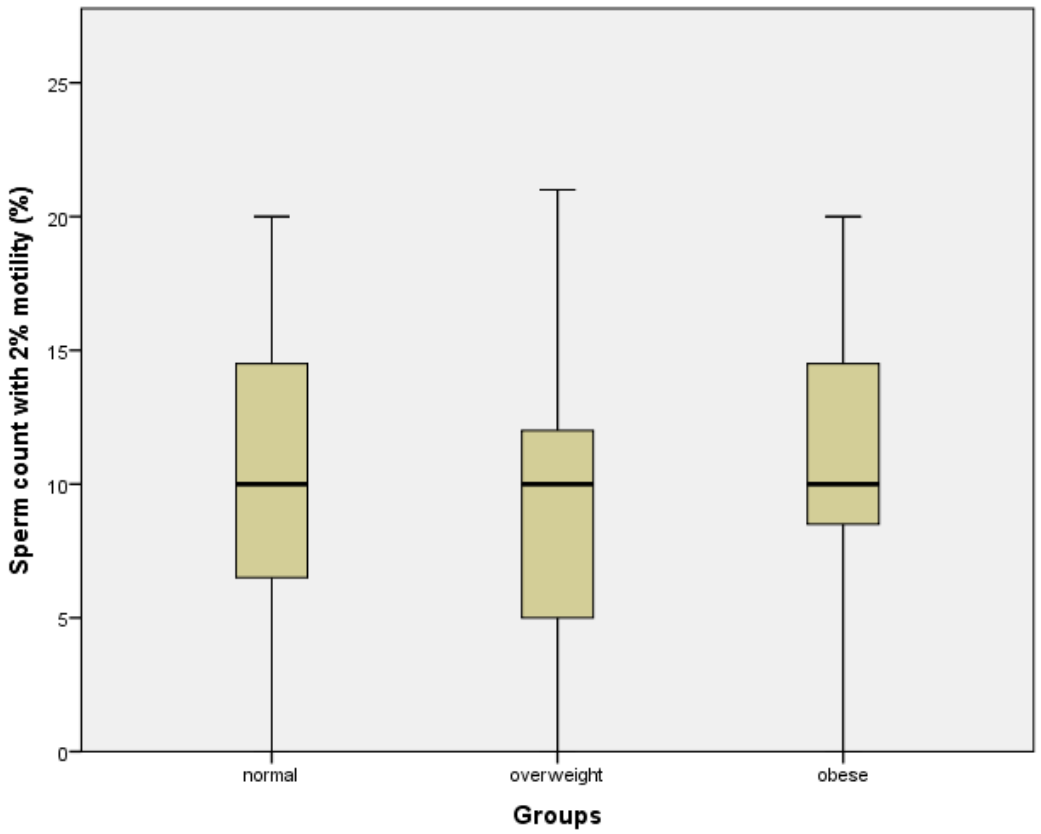

Graphic 4.4. Descriptive data graph of total motility values of all groups




Graphic 4.5. Descriptive data graph of 4% motility sperm counts values of all groups




Graphic 4.6. Descriptive data graph of 3% motility sperm counts values of all groups




Graphic 4.7. Descriptive data graph of 2% motility sperm counts values of all groups

## 4.2 Sperm Morphology Findings

Sperm morphologies were evaluated in the samples of the subjects in the groups. The number of sperm with normal morphology was 27.20±13.30% in the normal group; It was found to be 16.25±5.61% in the overweight group and 15.30±5.97% in the obese group (Figure 4.1).

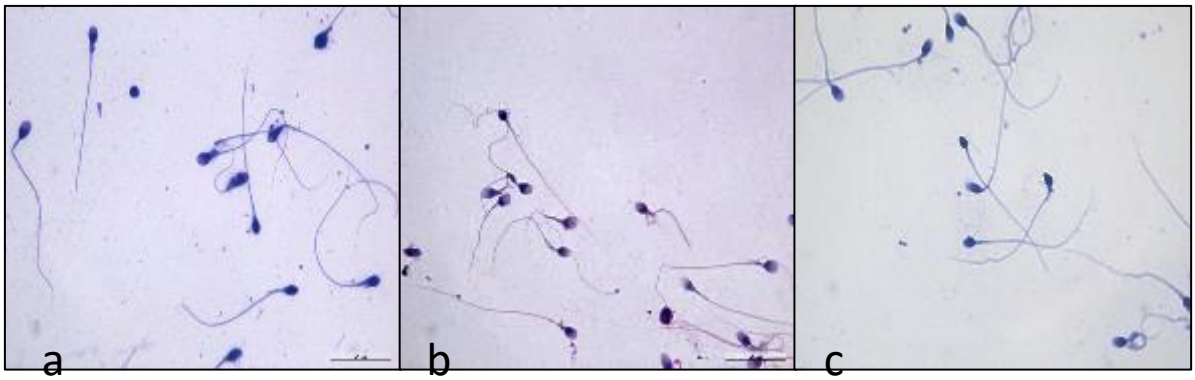



Figure 4.1. A view of sperm samples obtained from normal (a), overweight (b) and obese (c) men. X1000 Spermac

Sperm count with head anomaly was 10.15±5.51% in the normal group; 9.40±6.18% in the overweight group and 11.35±6.06% in the obese group.

Sperm count with neck anomali was 9.6±6.05% in the normal group; It was determined as 10.45±6.76% in the overweight group and 11.85±4.52% in the obese group.

The number of sperm with tail anomali was 5.05±4.19% in the normal group; It was found to be 4.85±4.04% in the overweight group and 8.20±5.84% in the obese group.

Sperm count with head and neck anomali was 19.55±8.20% in the normal group; It was found to be 24.20±14.10% in the overweight group and 21.10±7.86% in the obese group.

Sperm count with head and tail anomalies was 0.10±0.31% in the normal group; 0.50±1.28% in the overweight group and 0.10±0.45% in the obese group.

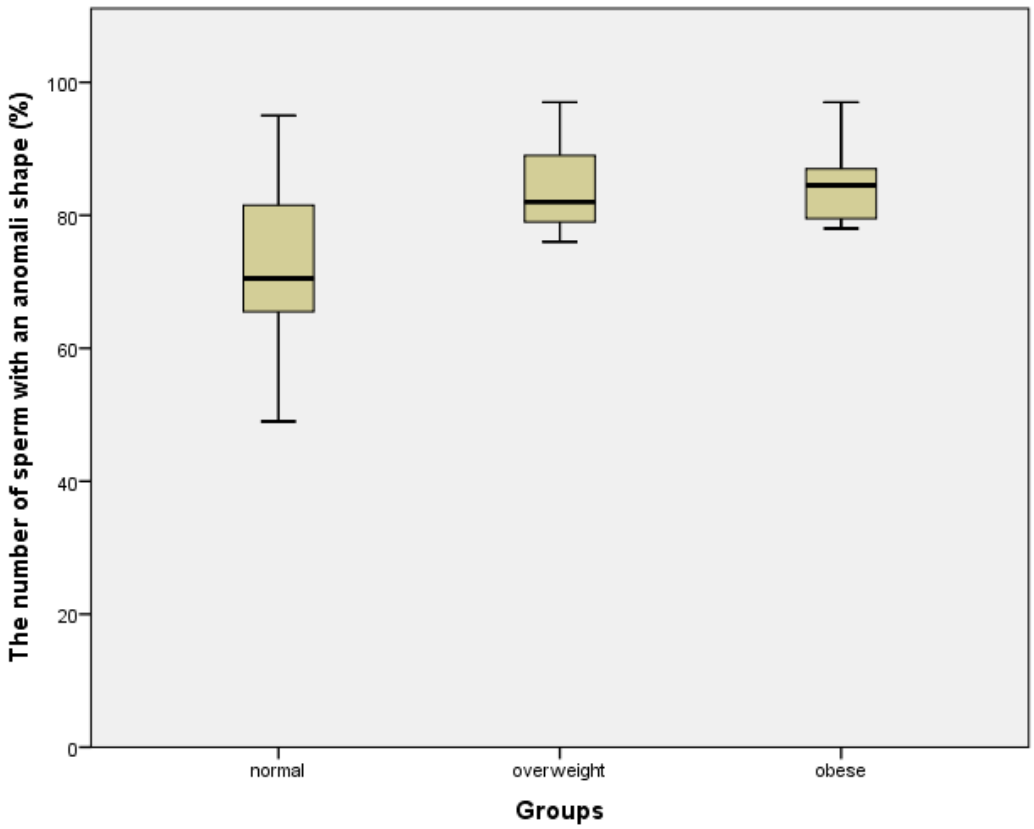
Sperm count with head, neck and tail anomalies was 28.35±11.88% in the normal group; It was found to be 34.50±10.16% in the overweight group and 32.20±15.68% in the obese group (Table 2).

Briefly, the total sperm count with anomali was 72.8±13.30% in the normal group; It was found to be 83.9±5.93% in the overweight group and 84.8±6.07% in the obese group (Table 4.2, Graph 5.8). When the groups were compared in terms of total sperm count with anomali, the Kruskal-Wallis test result was statistically significant between all groups (p=0.004).

Although there was no statistical difference between the overweight and obese groups (p=1,000); The percentage of sperm with abnormal morphology was higher in the overweight and obese groups compared to the normal group. This difference was statistically significant according to the Mann Whitney U test with Bonferoni correction (p= 0.021; 0.006, respectively)

Table 4.2. Descriptive data of sperm morphology parameters

| | | Groups | | |
|---|---|---|---|---|
| | | normal | overweight | Obese |
| Normal shape (%) | Mean | 27,20 | 16,25 | 15,30 |
| | Standard Deviation | 13,30 | 5,61 | 5,97 |
| | Median | 29,50 | 18,00 | 15,50 |
| | Minimum | 5,00 | 6,00 | 3,00 |
| | Maximum | 51,00 | 24,00 | 22,00 |
| Total anomali shape (%) | Mean | 72,80 | 83,90 | 84,80 |
| | Standard Deviation | 13,30 | 5,93 | 6,07 |
| | Median | 70,50 | 82,00 | 84,50 |
| | Minimum | 49,00 | 76,00 | 78,00 |
| | Maximum | 95,00 | 97,00 | 97,00 |
| Head anomali (%) | Mean | 10,15 | 9,40 | 11,35 |
| | Standard Deviation | 5,51 | 6,18 | 6,06 |
| | Median | 8,50 | 7,50 | 12,00 |
| | Minimum | 0,00 | 1,00 | 2,00 |
| | Maximum | 20,00 | 20,00 | 22,00 |
| Neck anomali (%) | Mean | 9,60 | 10,45 | 11,85 |
| | Standard Deviation | 6,05 | 6,76 | 4,52 |
| | Median | 9,50 | 9,00 | 12,00 |
| | Minimum | 2,00 | 2,00 | 5,00 |
| | Maximum | 26,00 | 27,00 | 20,00 |
| Tail anomali (%) | Mean | 5,05 | 4,85 | 8,20 |
| | Standard Deviation | 4,19 | 4,04 | 5,84 |
| | Median | 4,50 | 4,50 | 7,50 |
| | Minimum | 0,00 | 0,00 | 0,00 |
| | Maximum | 15,00 | 17,00 | 20,00 |
| Head and neck anomali (%) | Mean | 19,55 | 24,20 | 21,10 |
| | Standard Deviation | 8,20 | 10,14 | 7,86 |
| | Median | 20,00 | 24,00 | 21,50 |
| | Minimum | 9,00 | 7,00 | 8,00 |
| | Maximum | 40,00 | 43,00 | 36,00 |
| Head and tail anomali (%) | Mean | 0,10 | 0,50 | 0,10 |
| | Standard Deviation | 0,31 | 1,28 | 0,45 |
| | Median | 0,00 | 0,00 | 0,00 |
| | Minimum | 0,00 | 0,00 | 0,00 |
| | Maximum | 1,00 | 5,00 | 2,00 |
| Head, neck and tail anomali (%) | Mean | 28,35 | 34,50 | 32,20 |
| | Standard Deviation | 11,88 | 10,16 | 15,68 |
| | Median | 25,00 | 36,00 | 30,00 |
| | Minimum | 15,00 | 16,00 | 11,00 |
| | Maximum | 63,00 | 49,00 | 65,00 |




Graphic 4.8. Anomali shape sperm count values of all groups descriptive data graph

### 4.3. Chromatin Condensation Findings

Sperm chromatin condensation were evaluated by aniline blue. Mainly, sperm with unstained nuclei were evaluated as mature sperm, while sperm with blue stained nuclei were evaluated as immature sperm.

Aniline blue stained (partially stained and stained) sperm count was 47.45±20.02% in the normal group; 62.65 ± 16.11% in the overweight group and 62.05 ± 5.52% in the obese group (Table 4.3, Figure 4.2, Graph 4.9).

When the groups were compared in terms of the number of aniline blue stained sperm, the ANOVA test result was statistically significant between all groups (p=0.005). Though there was no statistical significant difference between the overweight and obese groups (p=1,000); In the overweight and obese groups, the percentage of dyed sperm increased compared to the normal group. This increase was statistically significant compared to the post hoc test with Bonferoni correction (Respectively, p= 0.011; 0.015).

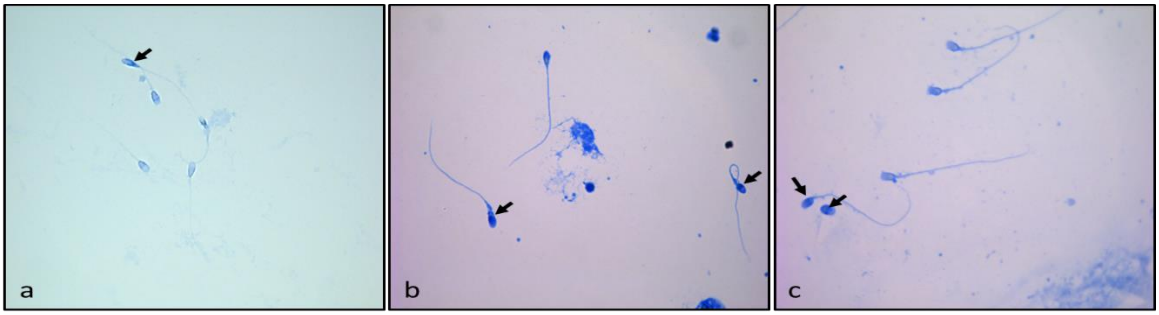
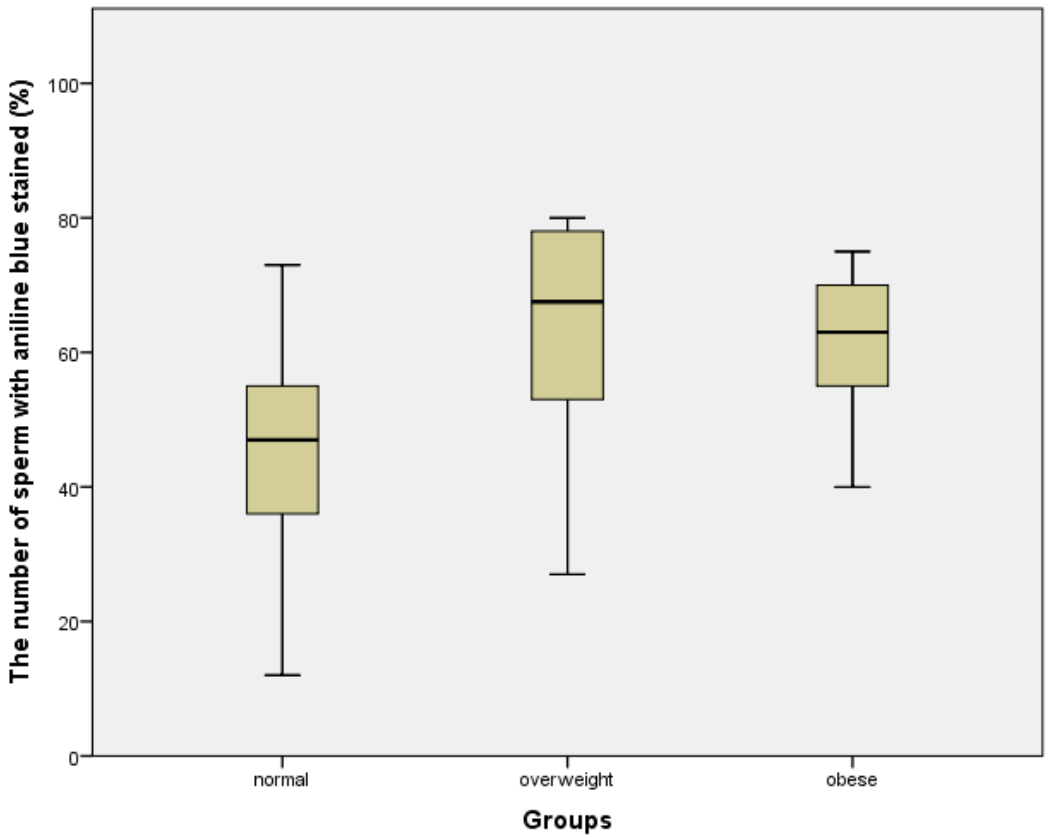



Figure 4.2. Representative view of aniline blue stained sperm samples of the groups (normal (a), overweight (b) and obese (c)). Arrow: aniline blue dyed sperm nuclei, X1000, Aniline blue

Table 4.3. Descriptive data of aniline blue staining

| | | Groups | | |
|---|---|---|---|---|
| | | normal | overweight | obese |
| Aniline blue stained cell (%) | Mean | 47,45 | 62,65 | 62,05 |
| | Standard Deviation | 20,02 | 16,11 | 9,52 |
| | Median | 48,50 | 67,50 | 63,00 |
| | Minimum | 12,00 | 27,00 | 40,00 |
| | Maximum | 93,00 | 80,00 | 75,00 |




Graphic 4.9. Descriptive data graph of aniline blue positive sperm count values of all groups

### 4.4. TUNEL Findings

TUNEL positive sperms were evaluated in the samples belonging to the subjects in the groups. TUNEL positive sperm count was 21±18% in the normal group; 52 ± 13% in the overweight group and 54 ± 34% in the obese group (Table 4.4, Figure 4.3, Graph 4.10).

When the groups were compared in terms of TUNEL positive sperm count, the Kruskal-Wallis test result was statistically significant between all groups ($p<0.001$). Although there was no statistical difference between the overweight and obese groups ($p=1,000$); TUNEL positive sperm percentage increased in the overweight and obese groups compared to the normal group. This increase was statistically significant according to the Bonferoni-corrected Mann Whitney U test ($p<0.001$; $<0.001$, respectively).

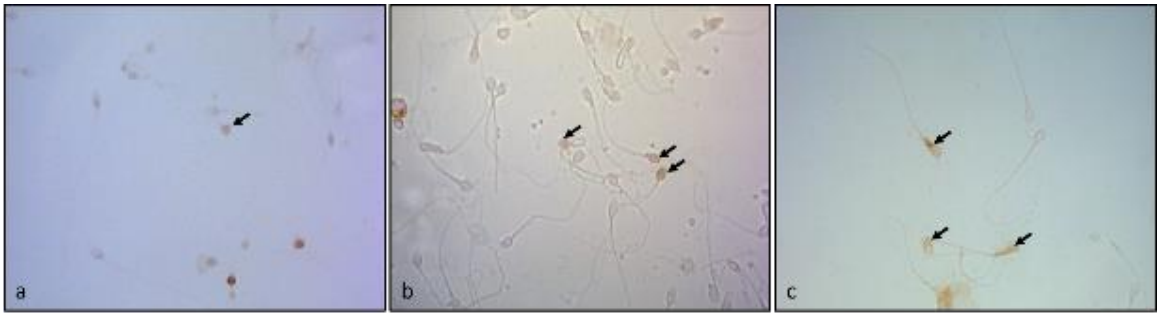
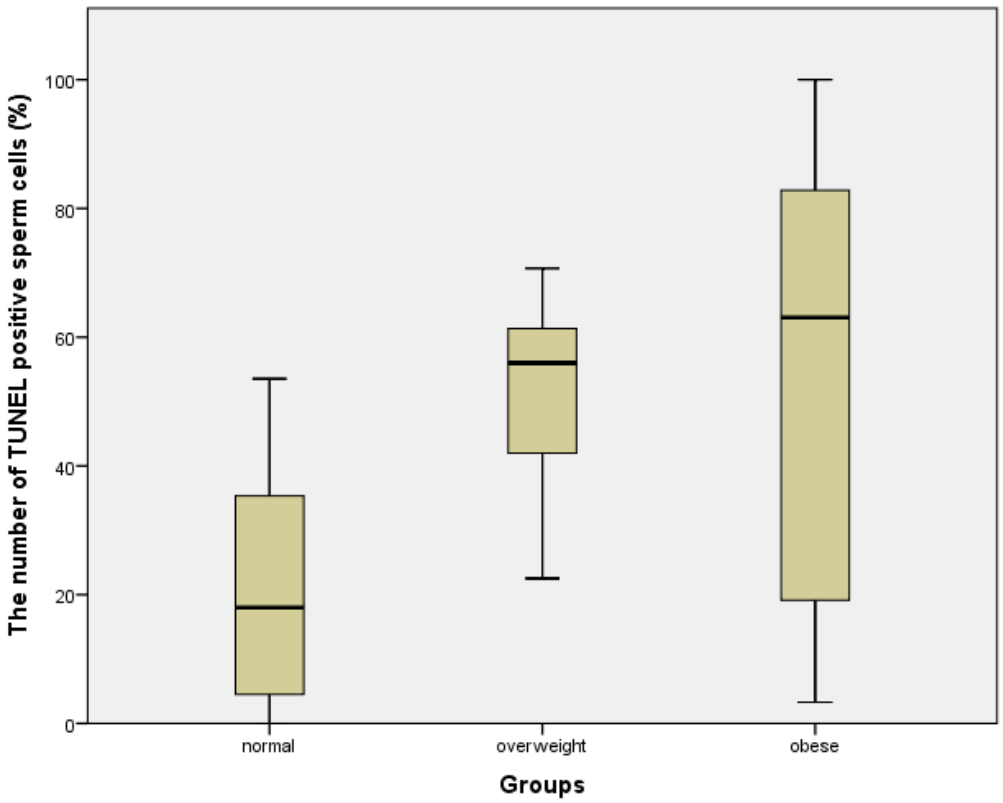



Figure 4.3. Representative view of TUNEL positive sperms of the groups (normal (a), overweight (b) and obese (c)). Arrow: TUNEL positive sperm, X1000

Table 4.4. Descriptive data of TUNEL analysis

| | | Groups | | |
|---|---|---|---|---|
| | | normal | overweight | obese |
| TUNEL positive cells (%) | Mean | 21 | 52 | 54 |
| | Standard Deviation | 18 | 13 | 34 |
| | Median | 18 | 56 | 63 |
| | Minimum | 0 | 23 | 3 |
| | Maximum | 54 | 71 | 100 |




Graphic 4.10. Descriptive data graph of TUNEL positive sperm count values for all groups

## 4.5. Evaluations for TAS and TOS Parameters

### 4.5.1. TAS values

TAS results were obtained as 1,40±0,33 for 'Normal' group, 1,37±0,34 for 'Overweighted' group and 1,34±0,31 for 'Obese' group (Table 4.5). When the obtained results were evaluated statistically by Kruskal Wallis test, no significant difference was found. Pairwise comparison of groups was applied with Mann Whitney test. When the obtained results were evaluated statistically, no significant difference was found between Normal and Overweighted groups (p=0.824) even TAS values were higher at Normal group. The statistically evaluation of results obtained from Normal and Obese groups also showed no significant difference (p=0.448) even TAS values were higher at Normal group. Similarly obtained results of Overweighted and Obese groups were evaluated statistically, again no significant difference was found between groups (p=0.706), even TAS values were higher at Overweighted group.

Table 4.5. Descriptive statistical values obtained from biochemical values of TAS parameter

| | | TAS | | | | |
|---|---|---|---|---|---|---|
| | | Mean | Standard Deviation | Median | Minimum | Maximum |
| Groups | normal | 1,40 | ,33 | 1,50 | ,65 | 1,79 |
| | overweight | 1,37 | ,34 | 1,38 | ,69 | 1,81 |
| | obese | 1,34 | ,31 | 1,35 | ,67 | 1,82 |

### 4.5.2. TOS values

TOS results were obtained as 18,11±11,96 for 'Normal' group, 19,22±15,22 for 'Overweighted' group and 21,68±23,79 for 'Obese' group (Table 5.6). When the obtained results were evaluated statistically by Kruskal Wallis test, no significant difference was found (p=0.308). Pairwise comparison of groups was applied with Mann Whitney test. When the obtained results were evaluated statistically, no significant difference was found between Normal and Overweighted groups (p=0.937), even TOS values were higher at Overweighted group. The statistically evaluation of results obtained from Normal and Obese groups also showed no significant difference (p=0.136) even TOS values were higher at Obese group. Similarly obtained results of Overweighted and Obese groups were evaluated statistically, again no significant difference was found between groups (p=0.273) even TOS values were higher at Obese group.

Table 4.6. Descriptive statistical values obtained from biochemical values of TOS parameter

| | | TOS | | | | |
|---|---|---|---|---|---|---|
| | | Mean | Standard Deviation | Median | Minimum | Maximum |
| Groups | normal | 18,11 | 11,96 | 15,64 | 4,89 | 58,94 |
| | overweight | 19,22 | 15,22 | 14,89 | 2,77 | 73,40 |
| | obese | 21,68 | 23,79 | 11,70 | 4,89 | 98,51 |

# 5. DISCUSSION

Male infertility is caused by a variety of factors, but it accounts for around 40% of all infertility cases (57). A number of factors, including the existence of anti-sperm antibodies, sperm production abnormalities, hormonal imbalances, obstruction of sperm ducts due to testicular trauma or varicocele, anatomical issues, and even some drugs, might contribute to male infertility. As a result, certain variables that might have an impact on the semen parameters with BMI were eliminated. In addition, some variables, including as smoking, varicocele, hormonal changes, and other factors that impact fertility, were omitted from the most recent study. According to the World Health Organization (WHO), more than 1.4 billion people were overweight or obese in 2008. (58). When it comes to sperm parameters, studies have been mixed. The WHO has released the semen parameters, and they have been used in various research. The data obtained on the influence of obesity on sperm parameters differed from one another, as well (50). Furthermore, this study found no statistically significant differences in the semen parameters between non-obesity, overweight, and obese infertile patients. In addition, the medical community is currently debating the impact of obesity on sperm quality.

Men's infertility is also known as idiopathic male infertility and is also attributed to unknown reasons (UMI). Furthermore, an aberrant semen parameter has been associated to idiopathic male infertility (59). Male infertility is first evaluated in the laboratory using conservative semen analysis, which measures important sperm characteristics such as morphology and viability as well as sperm concentration and motility (13).

It is still difficult to diagnose the malfunctioning of the spermatozoon using routine semen analysis, since the spermatozoon is a very specific cell that displays an extensive variety of biological features in order to achieve fertilization (60). In males, aberrant semen parameters are thought to be a sign of reproductive problems. Nevertheless, up to thirty percent of men with normal semen characteristics (normozoospermic) are diagnosed with UMI since the explanation for infertility is uncertain (59,61,62). (59,61,62). Idiopathic infertility has proven to be a challenging condition for andrologists, with no major success reported with any treatment.

In light of the fact that men with subnormal semen analysis might also be fertile a while, however, men with normal semen parameters continue to be infertile (63). (63). This has led to the development of enhanced sperm function and seminal fluid quality evaluations, such as oxidative stress and sperm DNA fragmentation (SDF), that can better guide diagnosis, treatment, as well as the prediction of male fertility outcomes (64, 65). According to this study, there were no significant differences in the conventional seminal parameters among the three groups studied.. Many investigations have come to the same conclusion as this one, with guys being infertile despite having normal semen parameters (60).

According to a WHO surveillance study, a higher BMI is linked to lower sperm quality, which affects the sperm's volume, concentration, and motility (66, 67). The study also looked at the sperm counts of men who were pregnant with their partners and found that the total sperm counts of obese men were significantly lower than those of non-obese men (mean 231106 vs. 324106, respectively), despite the fact that the sperm parameters do not show any changes (68).

Our research looked at sperm metrics such as volume of sperm, concentration of sperm, total concentration of sperm and various percentages of sperm motility. In comparison to the normal weight group, the obese individuals had lower levels of semen volume, total concentration, total motility, and 3 percent and 4 percent motility, respectively. All of these decreases, however, were not statistically significant.

Furthermore, Mah and Wittert (69) have observed that sperm concentration and total sperm count are lower in obese men than in lean men, although motility associated with morphology is unaffected by obesity. Overweight and obese infertile men were shown to have a greater frequency of oligozoospermia and poor progressive motile sperm concentration compared to men of normal weight in this research, which was done retrospectively (70) It has been shown that overweight and obese men have a reduced sperm volume, which is linked to an increase in the percentage of degenerated spermatozoa, which is linked to an increase in sperm DNA fragmentation (71).

In contrast, multivariate research has shown a negative correlation between progressive motility, BMI, motility, and the neutral alphaglucosidase levels reported as an epididymal functional marker (72). In contrast, other human populations have shown that no such

correlation exists (1,28). A recent meta-analysis by Macdonald et al. has shown that there is no correlation between BMI and other seminal variables. There was no statistical link between (BMI) and sperm concentration, sperm motility, ejaculate volume, or the shape and size of sperm. Similarly, (74) Alahmar et al., (2018) reported that obesity had no impact on sperm concentration, motility, or morphology (20). Furthermore, the most recent study is in line with previously published findings, since no statistical association was found between the three groups.

Due to a lack of objective measuring criteria, it is possible to dispute the appraisal of sperm morphology since it is still vulnerable to the subjective impacts of the observer. body mass index (BMI) have been shown to be statistically significant predictors of male sperm morphology. Weight reduction has been shown to increase semen quality, including sperm count, which is linked to proper sperm morphology, according to small-sample studies (75). According to studies, aberrant sperm morphology and (BMI) still have a strong positive link, according to findings (76).

The sperm morphology of the participants in the groups were examined in this research. In the normal group, there were 27.20 ± 13.30 percent of sperm with normal morphology; in the overweight and obese groups, it was 16.25 ± 5.61 percent and 15.30 ± 5.97 percent, respectively. For the overweight and obese groups, the total sperm count with abnormalities was 72.8 ± 13.30%, 83.9 ± 5.93%, and 84.8 ± 6.07%, respectively.

Although the overweight and obese categories were statistically indistinguishable. Overweight and obese men had a larger proportion of abnormally shaped sperm than normal men. This was a statistically significant difference.

Cellular aging, hormonal and metabolic alterations are all linked to aging. In addition, the number of times a cell has reproduced influences its age. Telomeres, on the other hand, get shorter as we get older, which means that our genetic information can no longer be precisely replicated. In addition, since hormone levels change throughout time, they may influence cellular activity and behavior. The body's capacity to heal cells, tissues, and organs is hampered by exposure to toxins, the sun, dietary pollutants, and smoking, all of which may cause tissue damage. Similarly, as time passes, metabolic activities and cellular energy

generation suffer a similar fate (77, 78). In addition, the age of a man might have a detrimental impact on his ability to reproduce.

However, sperm production declines with age, as do other aspects of a man's sex life, such as his hormone levels and his ability to engage in sexual activity (79). Furthermore, as stated in, aging has been linked to substantial reductions in sperm volume, motility, morphology, and count (80, 81). The sperm chromatin, which is linked to DNA integrity, has also been shown to be adversely affected by aging and obesity (67, 82). According to studies, male aging has resulted in an increase in genomic instability within the sperm DNA in many forms such as epigenetic alterations and DNA fragmentation as well as gene mutations as well as other chromosomal abnormalities (76,83). As a result, paternal age remained a significant factor in both cleavage and blastocyst stage embryo aneuploidy as well as mosaicism (84). Instead of finding a relationship between male age and aneuploidy in transplanted embryos, other studies found none (85).

For example, increased cell disintegration processes and shorter lifespans are linked to genetic instability and disruption of homeostatic pathways as stated by various research papers on the effects of ageing and obesity on metabolic imbalances (86). In fact, it has been shown that sperm chromatin condensation is still a crucial factor in protecting sperm DNA throughout its passage through the female reproductive canal and in managing epigenetic reprogramming in the pre-implantation period of time (87).

Sperm chromatin integrity has a similar effect on reproductive outcomes, as stated in (88), provided that father obesity affects the molecular components of motile spermatozoa. As a result, sperm chromatin integrity may be detrimental to embryo morphology, as stated in (89), which is linked to blastocyst formation. In addition, it may result in embryo implantation failure or many spontaneous abortions, as shown in the study (90). As RAAD, Georges, et al. discovered, paternal obesity as evaluated by (BMI and WC), which is associated to modifications in the molecular composition of the motile sperm-enriched fraction and pre-implantation embryo morphokinetics, as disclosed in (91). The quality of sperm is also crucial in determining the quality of the growing embryo when it is injected intracytoplasmically (ICSI) (92). For the opposite situation when only "abnormal-sperm embryos" are still accessible, the injection of individual, aberrant sperm cells may diminish both fertilization and implantation rates (93).

Aniline blue was used to examine sperm chromatin condensation in our investigation. Mature sperm were considered to have unstained nuclei, whereas immature sperm had blue stained nuclei. A total of 47.45 ± 20.02 percent of sperm in the normal group, 62.65 ± 16.11 percent in the overweight and 62.05 ± 5.52 percent in the obese group were stained with aniline blue. However, despite the lack of a statistically significant difference between the obese and overweight groups (p=1,000), the proportion of coloured sperm was higher in the latter two than in the former group.

During the last phases of spermiogenesis and epididymal transport, nuclear chromatin condenses. One of the most notable alterations is the substitution of histone lysine-rich proteins with protamine high in arginine. Bisulfidic linkages between cysteine residues are also produced at the same time. Nuclear chromatin is densely packed in order to preserve it from both natural and chemical damage. There may be a higher DNA fragmentation rate due to anomalies in the condensation of the sperm chromatin (91). According to, oxidative stress, aberrant chromatin packing, and apoptosis that fails to terminate properly are all three potential causes of DNA fragmentation (92). Similar to the oxidation of DNA bases known as guanosine, excessive production of (ROS) may lead to an increase in DNA damage, either through the production of byproducts from lipid degradation or through direct interaction with the DNA strand. This leads to non-specific single and double-strand breaks as stated in the study (93).

In an experiment of Chavarro et al. (94) including (483) males, researchers found that sperm with severe DNA damage were more common in the obese men's group than in the men's group with normal weight. According to Kort et al. (95), an increase in (BMI) was associated with an increase in the sperm chromatin structure assay's DNA fragmentation index. Obese males had a higher DNA fragmentation index, which was associated with a greater risk of developing diabetes (27 percent and 25.8 percent , respectively). Overweight and obese men are more likely to have sperm with DNA damage than their normal-weight counterparts.

The following are the TUNEL results from our investigation, which are in line with previous findings. There was a TUNEL positive sperm count of 21% ± 18% in the normal group, 52% ± 13% of overweight men, and 54% ± 35% of obese men, respectively. although no statistically significant difference between overweight and obese groups .

Overweight and obese men had a higher proportion of TUNEL positive sperm than the normal group, this was a significant statistically .

Reactive oxygen species (ROS) have been linked to male infertility for a long time now, and a diminished antioxidant capacity may play a role in this condition as well (84). Furthermore, oxidative stress-induced spermatozoa destruction may be a mechanism through which obesity impairs male fertility. Likewise, as reported by, an increase in body mass index (BMI) has been linked to a modest increase in sperm DNA damage (85). A better understanding of how obesity affects sperm oxidative stress is also emerging. The primary cause of (ROS) formation in infertile men's sperm is morphologically defective, immature sperm with excessive cytoplasm, as reported in the study by (86). Infertility patients have a high (ROS) production, whereas only a small percentage of fertile men have elevated (ROS) production in their seminal fluid (84).

According to this study, seminal plasma TAS and TOS levels in infertile and fertile males did not vary, contrary to several other research that found no difference (87).

In addition, research from throughout the globe shows that infertile males have higher levels of free oxygen radicals than fertile men (88). Similarly, Sharma et al. (1999) found that normozoospermic infertile men with lower levels of TAS had greater levels of TOS, while Shiva et al. (2011) found that azoospermic individuals with lower levels of oxidative stress had stronger antioxidant capacities (88,89). While the normal group had higher TAS levels than the overweight group, the researchers found that there was no statistically significant difference between the two groups ($p = 0.824$). Even though the normal group's TAS was greater than that of the obese group ($p = 0.448$), no statistically significant difference was found.

While TAS levels in the overweight group were higher than in the obese group ($p = 0.706$), the difference between the two groups was not statistically significant ($p = 0.706$). Even though (TOS) values were greater in the overweight group, statistically significant differences between the groups ($p = 0.937$) were found when the normal and overweight populations were compared. A statistically insignificant difference was found when (TOS) values for normal and obese subjects were compared ($p = 0.136$), despite obese subjects having significantly higher (TOS) values. There was no statistical difference in (TOS) values

between overweight and obese groups, even though obese individuals had higher (TOS) values ($p = 0.273$). Because this was a small-scale investigation, it is likely that if the sample size had been bigger, the negative trend identified between (BMI) and sperm oxidative stress would have been statistically significant (90). It was also shown that DNA in human sperm was still densely packed.

When the antioxidant level obtained from the samples of the groups belonging to our study was evaluated with the TAS parameter, TAS results were obtained as 1,40±0,33 for 'Normal' group, 1,37±0,34 for 'Overweighted' group and 1,34±0,31 for 'Obese' group. When comparing the Normal and Overweighted groups, no statistically significant differences could be identified. In fact, TAS values were higher at Normal group. The statistically evaluation of results obtained from Normal and Obese groups also showed no significant difference. Also, TAS values were higher at Normal group. Similarly obtained results of Overweighted and Obese groups were evaluated statistically, again no significant difference was found between groups, even TAS values were higher at overweighted group.

When the oxidant level obtained from the samples of the groups belonging to our study is evaluated with the TOS parameter, TOS results were obtained as 18,11±11,96 for 'Normal' group, 19,22±15,22 for 'Overweighted' group and 21,68±23,79 for 'Obese' group. Even though TOS values were greater in the Overweighted group, there was no statistically significant difference between the two groups. The statistically evaluation of results obtained from Normal and Obese groups also showed no significant difference, even TOS values were higher at Obese group. Similarly obtained results of Overweighted and Obese groups were evaluated statistically, again no significant difference was found between groups, even TOS values were higher at Obese group.

# 6. CONCLUSION

When sperm samples were obtained from normal weight, overweight and obese people, general sperm parameters were compared and studied, according to this research. Weight gain was shown to have an effect on the amount of sperm in the body, as well as the pace at which the sperm moved throughout the ducts. The proportion of abnormal sperm in the overweight and obese categories rose dramatically compared to the normal group in the research, which analyzed sperm morphology. Analyzing the aniline blue staining of sperm nuclear morphologies, we discovered that the number of immature sperm in the overweight and obese group was higher than in the normal weight group. This research also found that the number of sperm with DNA fragmentation increased in the overweight and obese categories compared to the normal group. TAS and TOS experiments, which were conducted to reveal the antioxidant/oxidant relationship in cells, showed that the antioxidant level decreased with increasing body weight; The oxidant level was shown to increase.

With rising body mass index, sperm parameters decreased, sperm morphology deteriorated, the number of immature sperm grew, and the number of sperm with DNA fragmentation increased, decrease in antioxidant level and increase in oxidant level were found. An association between obesity and sperm quality, chromatin structure, DNA integrity and antioxidant/oxidant balance was discovered in this investigation. As a result, an elevated BMI may have an impact on the presence of healthy sperm in a male partner.

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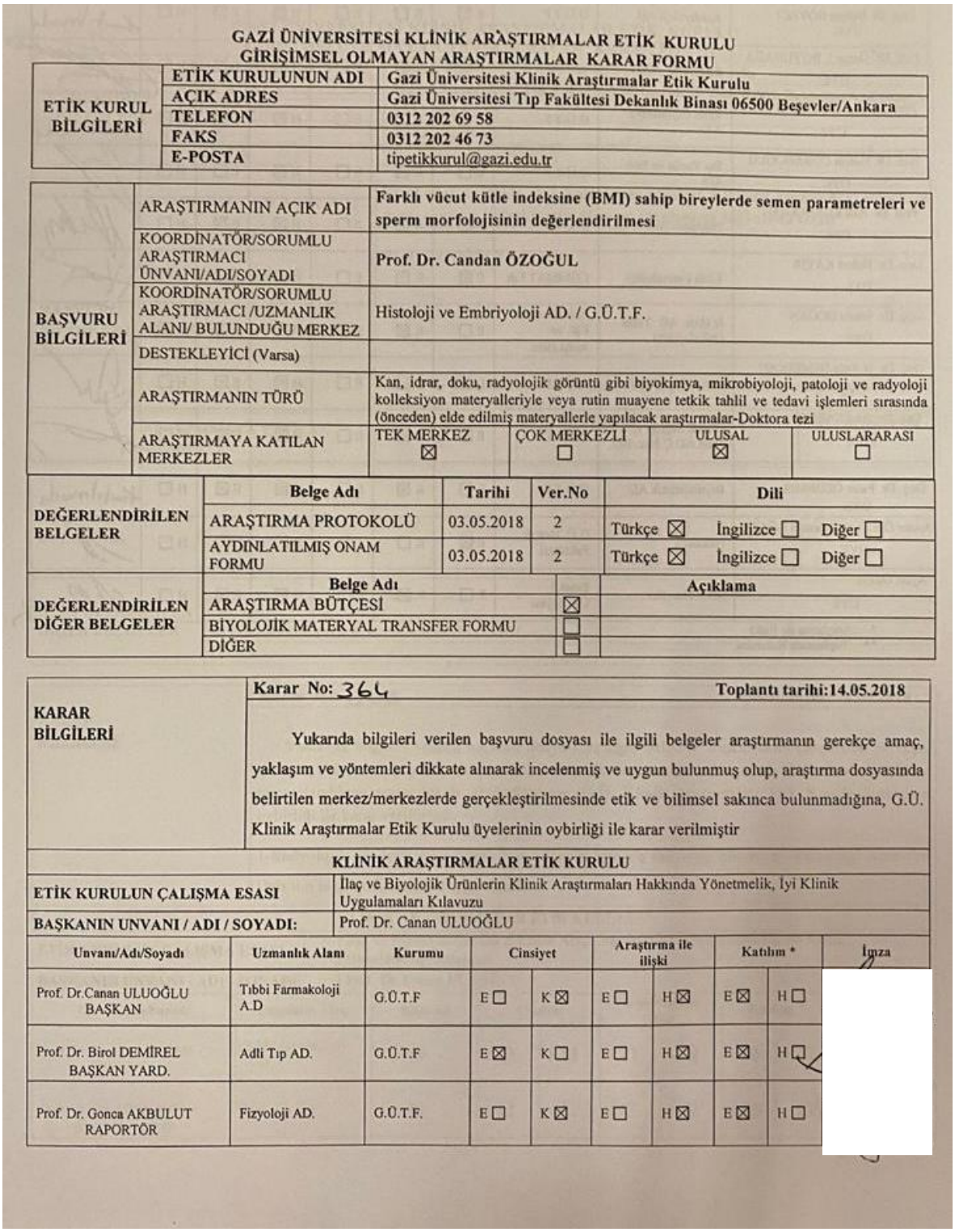
# APPENDICES

## APPENDICE-1. Ethics Committee Permission Letter



**GAZİ ÜNİVERSİTESİ KLİNİK ARAŞTIRMALAR ETİK KURULU**
**GİRİŞİMSEL OLMAYAN ARAŞTIRMALAR KARAR FORMU**

| | | |
|---|---|---|
| ETİK KURUL BİLGİLERİ | ETİK KURULUNUN ADI | Gazi Üniversitesi Klinik Araştırmalar Etik Kurulu |
| | AÇIK ADRES | Gazi Üniversitesi Tıp Fakültesi Dekanlık Binası 06500 Beşevler/Ankara |
| | TELEFON | 0312 202 69 58 |
| | FAKS | 0312 202 46 73 |
| | E-POSTA | tipetikkurul@gazi.edu.tr |

| | | | | | |
|---|---|---|---|---|---|
| BAŞVURU BİLGİLERİ | ARAŞTIRMANIN AÇIK ADI | Farklı vücut kütle indeksine (BMI) sahip bireylerde semen parametreleri ve sperm morfolojisinin değerlendirilmesi | | | |
| | KOORDİNATÖR/SORUMLU ARAŞTIRMACI ÜNVANI/ADI/SOYADI | Prof. Dr. Candan ÖZOĞUL | | | |
| | KOORDİNATÖR/SORUMLU ARAŞTIRMACI /UZMANLIK ALANI/ BULUNDUĞU MERKEZ | Histoloji ve Embriyoloji AD. / G.Ü.T.F. | | | |
| | DESTEKLEYİCİ (Varsa) | | | | |
| | ARAŞTIRMANIN TÜRÜ | Kan, idrar, doku, radyolojik görüntü gibi biyokimya, mikrobiyoloji, patoloji ve radyoloji kolleksiyon materyalleriyle veya rutin muayene tetkik tahlil ve tedavi işlemleri sırasında (önceden) elde edilmiş materyallerle yapılacak araştırmalar-Doktora tezi | | | |
| | ARAŞTIRMAYA KATILAN MERKEZLER | TEK MERKEZ ☒ | ÇOK MERKEZLİ ☐ | ULUSAL ☒ | ULUSLARARASI ☐ |

| | Belge Adı | Tarihi | Ver.No | Dili |
|---|---|---|---|---|
| DEĞERLENDİRİLEN BELGELER | ARAŞTIRMA PROTOKOLÜ | 03.05.2018 | 2 | Türkçe ☒ İngilizce ☐ Diğer ☐ |
| | AYDINLATILMIŞ ONAM FORMU | 03.05.2018 | 2 | Türkçe ☒ İngilizce ☐ Diğer ☐ |

| | Belge Adı | | Açıklama |
|---|---|---|---|
| DEĞERLENDİRİLEN DİĞER BELGELER | ARAŞTIRMA BÜTÇESİ | ☒ | |
| | BİYOLOJİK MATERYAL TRANSFER FORMU | ☐ | |
| | DİĞER | ☐ | |

**KARAR BİLGİLERİ**

**Karar No:** 364 **Toplantı tarihi:14.05.2018**

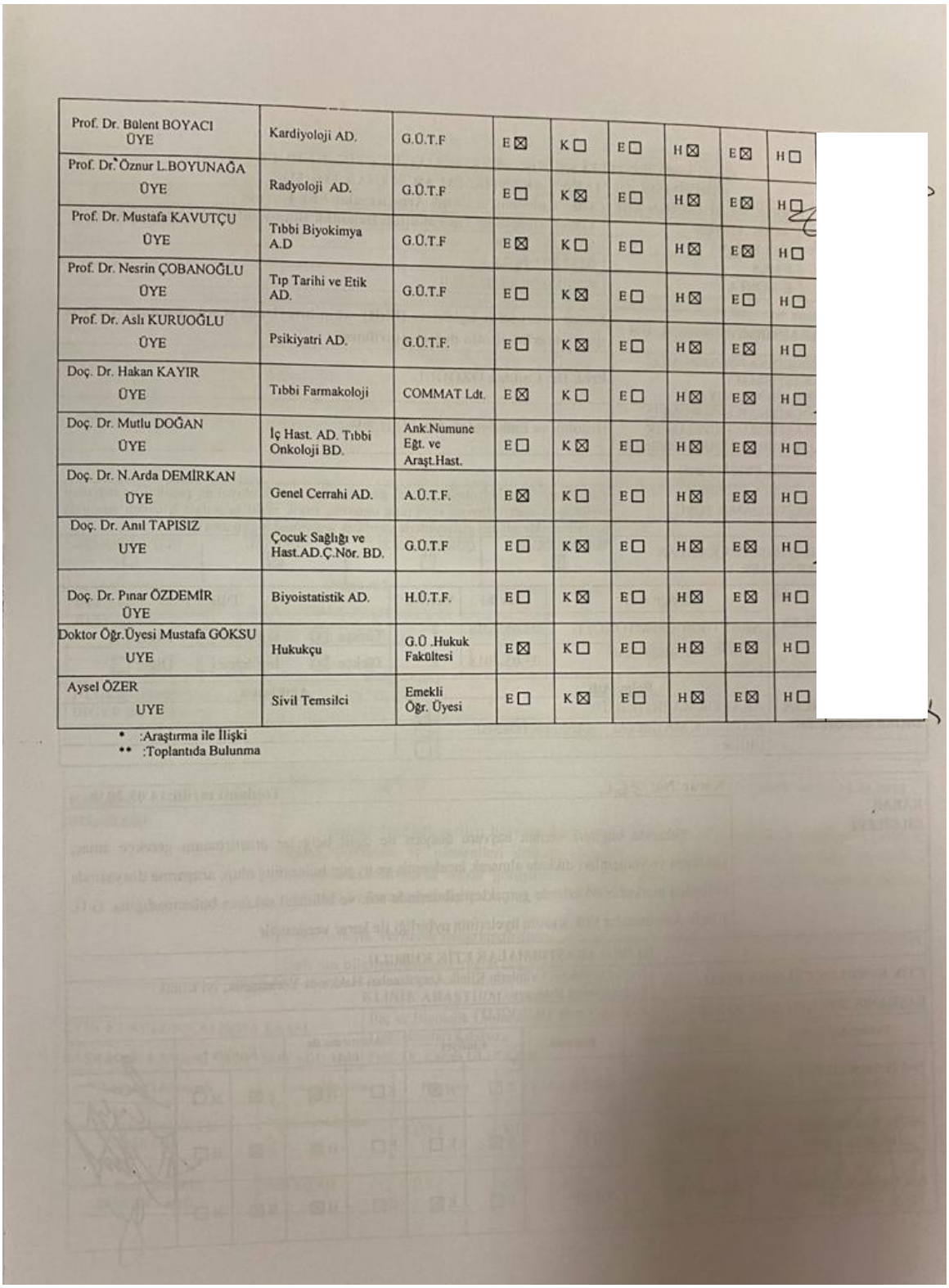
Yukarıda bilgileri verilen başvuru dosyası ile ilgili belgeler araştırmanın gerekçe amaç, yaklaşım ve yöntemleri dikkate alınarak incelenmiş ve uygun bulunmuş olup, araştırma dosyasında belirtilen merkez/merkezlerde gerçekleştirilmesinde etik ve bilimsel sakınca bulunmadığına, G.Ü. Klinik Araştırmalar Etik Kurulu üyelerinin oybirliği ile karar verilmiştir

**KLİNİK ARAŞTIRMALAR ETİK KURULU**

| | |
|---|---|
| ETİK KURULUN ÇALIŞMA ESASI | İlaç ve Biyolojik Ürünlerin Klinik Araştırmaları Hakkında Yönetmelik, İyi Klinik Uygulamaları Kılavuzu |
| BAŞKANIN UNVANI / ADI / SOYADI: | Prof. Dr. Canan ULUOĞLU |

| Unvanı/Adı/Soyadı | Uzmanlık Alanı | Kurumu | Cinsiyet | | Araştırma ile ilişki | | Katılım * | | İmza |
|---|---|---|---|---|---|---|---|---|---|
| Prof. Dr.Canan ULUOĞLU BAŞKAN | Tıbbi Farmakoloji A.D | G.Ü.T.F | E ☐ | K ☒ | E ☐ | H ☒ | E ☒ | H ☐ | |
| Prof. Dr. Birol DEMİREL BAŞKAN YARD. | Adli Tıp AD. | G.Ü.T.F | E ☒ | K ☐ | E ☐ | H ☒ | E ☒ | H ☐ | |
| Prof. Dr. Gonca AKBULUT RAPORTÖR | Fizyoloji AD. | G.Ü.T.F. | E ☐ | K ☒ | E ☐ | H ☒ | E ☒ | H ☐ | |

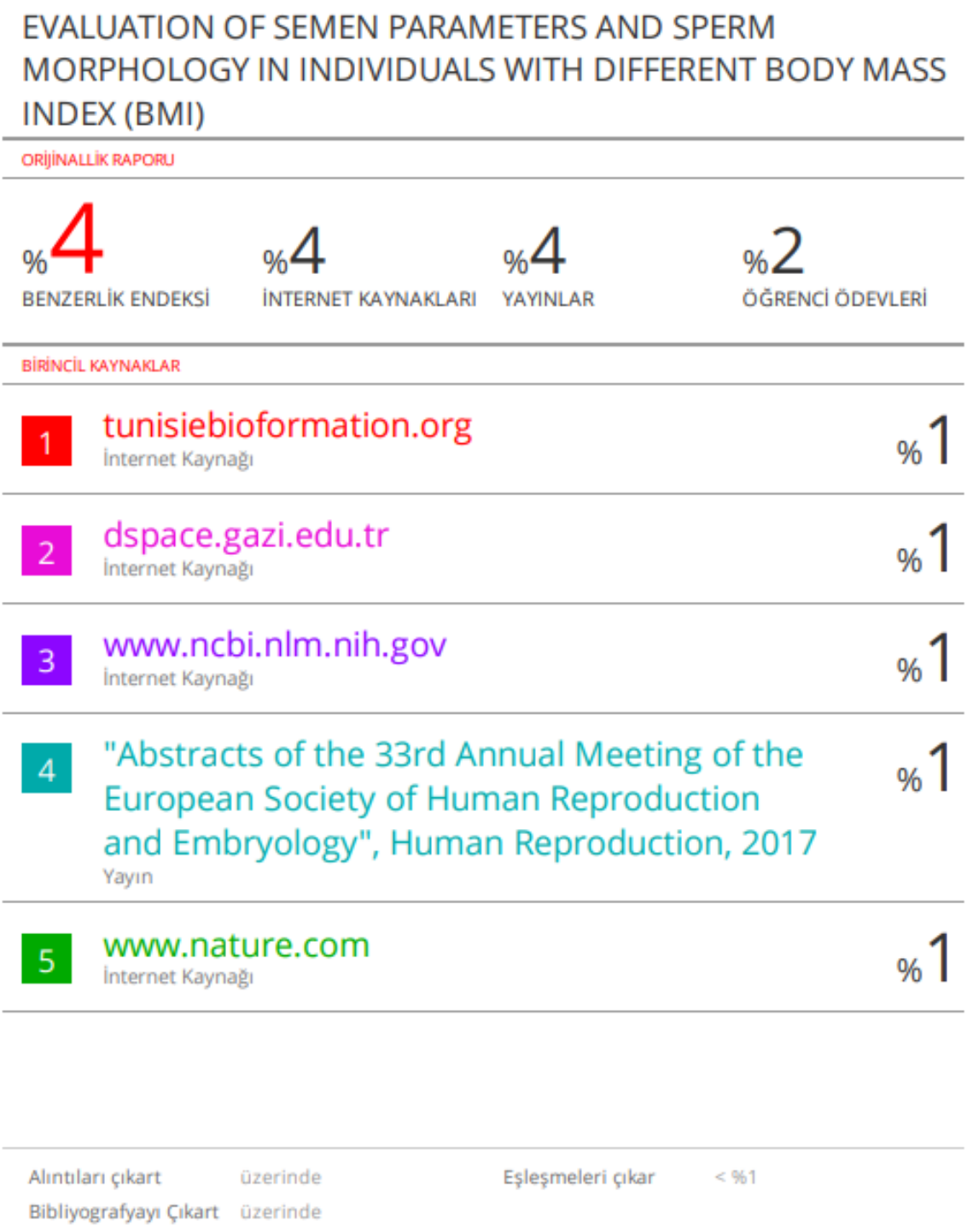
## APPENDICE-1. (continue) Ethics Committee Permission Letter



| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Prof. Dr. Bülent BOYACI ÜYE | Kardiyoloji AD. | G.Ü.T.F | E☒ | K☐ | E☐ | H☒ | E☒ | H☐ |
| Prof. Dr. Öznur L.BOYUNAĞA ÜYE | Radyoloji AD. | G.Ü.T.F | E☐ | K☒ | E☐ | H☒ | E☒ | H☐ |
| Prof. Dr. Mustafa KAVUTÇU ÜYE | Tıbbi Biyokimya A.D | G.Ü.T.F | E☒ | K☐ | E☐ | H☒ | E☒ | H☐ |
| Prof. Dr. Nesrin ÇOBANOĞLU ÜYE | Tıp Tarihi ve Etik AD. | G.Ü.T.F | E☐ | K☒ | E☐ | H☒ | E☐ | H☐ |
| Prof. Dr. Aslı KURUOĞLU ÜYE | Psikiyatri AD. | G.Ü.T.F. | E☐ | K☒ | E☐ | H☒ | E☒ | H☐ |
| Doç. Dr. Hakan KAYIR ÜYE | Tıbbi Farmakoloji | COMMAT Ldt. | E☒ | K☐ | E☐ | H☒ | E☒ | H☐ |
| Doç. Dr. Mutlu DOĞAN ÜYE | İç Hast. AD. Tıbbi Onkoloji BD. | Ank.Numune Eğt. ve Araşt.Hast. | E☐ | K☒ | E☐ | H☒ | E☒ | H☐ |
| Doç. Dr. N.Arda DEMİRKAN ÜYE | Genel Cerrahi AD. | A.Ü.T.F. | E☒ | K☐ | E☐ | H☒ | E☒ | H☐ |
| Doç. Dr. Anıl TAPISIZ UYE | Çocuk Sağlığı ve Hast.AD.Ç.Nör. BD. | G.Ü.T.F | E☐ | K☒ | E☐ | H☒ | E☒ | H☐ |
| Doç. Dr. Pınar ÖZDEMİR ÜYE | Biyoistatistik AD. | H.Ü.T.F. | E☐ | K☒ | E☐ | H☒ | E☒ | H☐ |
| Doktor Öğr.Üyesi Mustafa GÖKSU UYE | Hukukçu | G.Ü .Hukuk Fakültesi | E☒ | K☐ | E☐ | H☒ | E☒ | H☐ |
| Aysel ÖZER UYE | Sivil Temsilci | Emekli Öğr. Üyesi | E☐ | K☒ | E☐ | H☒ | E☒ | H☐ |

* :Araştırma ile İlişki
** :Toplantıda Bulunma

**APPENDICE-2. Result of Plagiarism**



EVALUATION OF SEMEN PARAMETERS AND SPERM MORPHOLOGY IN INDIVIDUALS WITH DIFFERENT BODY MASS INDEX (BMI)

ORİJİNALLİK RAPORU

| %4 | %4 | %4 | %2 |
|---|---|---|---|
| BENZERLİK ENDEKSİ | İNTERNET KAYNAKLARI | YAYINLAR | ÖĞRENCİ ÖDEVLERİ |

BİRİNCİL KAYNAKLAR

| | | |
|---|---|---|
| 1 | tunisiebioformation.org<br>İnternet Kaynağı | %1 |
| 2 | dspace.gazi.edu.tr<br>İnternet Kaynağı | %1 |
| 3 | www.ncbi.nlm.nih.gov<br>İnternet Kaynağı | %1 |
| 4 | "Abstracts of the 33rd Annual Meeting of the European Society of Human Reproduction and Embryology", Human Reproduction, 2017<br>Yayın | %1 |
| 5 | www.nature.com<br>İnternet Kaynağı | %1 |

| | | | |
|---|---|---|---|
| Alıntıları çıkart | üzerinde | Eşleşmeleri çıkar | < %1 |
| Bibliyografyayı Çıkart | üzerinde | | |

# CURRICULUM VITAE

Surname, Name : Khadeejah Sead Mohammed ABRAHEEM

Nationality : Libyan

**Education**

| Degree | Education Unit | Date of Graduation |
|---|---|---|
| Ph. D Thesis | Gazi University / Histology and Embryology | Continues |
| Bachelors' Degree | Faculty Of Medicin –Omer Al-Mukhtar | 2010/2011 |

**Education**

High school certificate: life sciences high school- 2004- nusseibeh school of specialized sciences, Al-baida-libya.

**Work Experience**

Work as an ophthalmologist in the ophthalmology department of al-thawra teaching hospital from 2012-2014, Al-baida-libya.

Internship at al-thawra hospital from :01.08.2010 to: 31.07.2011, Al-baida-libya.

**Certifications**

Intellectual elements symposium 21-23 06 2019 – neuroscience application and research center selçuk university – konya- turkey.

XX the LABORATORY ANIMAL PRACTICE AND ETHICS 32-31 may 2017 ,Gazi university, Ankara, Turkey.

Turkish course – Ankara university Turkish and foreign language application and research center TÖMER 2015.

English language course –IBT – 2014.

Complementary perspectives on ART: in memory of the 95th anniversaty 04 – 05 November 2021.

I received a PARTICIPATION CERTIFICATE for those who participated in the "Scientific Ar-Ge Project in Health Sciences (TÜBİTAK-ARDEB) Writing Applied Training" organized by our Institute on November 12, 2021

*GAZİLİ OLMAK AYRICALIKTIR...*