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Impact of body mass index on various semen parameters and sex hormones in some infertile Libyan men.

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DECLARATION

I am Mohammed Abdalwafi Abdalsalam Amhaisen the undersigned hereby confirm that the work contained in this thesis, unless otherwise referenced is the researcher's own work, and has not been previously submitted to meet requirements of an award at this University or any other higher education or research institution, I furthermore, cede copyright of this thesis in favor of University of Tripoli.

Student name: Mohammed Amhaisen

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DEDICATION

This work is dedicated to my beloved parents

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Abstract

Overweight and obese entail health risks with potential effects on the social and economic well-being of an individual and the community at large. Poor health conditions resulting from being overweight and obesity may lead to a disruption of male sex hormones and affect semen parameters.

The aim of this study is to investigate the effect of body mass index (BMI) on semen parameters and sex hormones level in infertile males. stratified samples and simple random samples were used in selecting 100 infertile men between 20-45 years old with a duration of infertility of more than one year. The patients with risk factors, besides obesity, that could affect semen parameters or reproductive hormones were evaluated. Patients were separated into the following three groups: normal weight (<25 kg/m^2), overweight (25–29.9 kg/m^2), and obese (≥30 kg/m^2). The BMI was determined using weight and height measurements, in addition, measurement of skinfold to determine body fat percentage. Semen parameters and reproductive hormones were evaluated and compared among the groups. The results revealed a significant relationship between sperm total motility and BMI of the studied participants (P= 0.01) the obese participants had significantly higher sperm motility as compared to the overweight ones (P= 0.015) but had no different motility when compared to normal weight patients. Overweight participants had significantly lower sperm motility compared to the normal ones (P= 0.03). The prolactin was the only sex hormone associated with increase BMI (P value= 0.011), it being significantly lower in overweight and obese participants compared to participants with normal weight (P value=0.031, 0.016 respectively), while both groups with overweight and obesity had no statistically significant difference in prolactin level. The spearman's Rank Correlation confirmed that LH (r= -0.212, P= 0.034), testosterone (r= -0.298, P= 0.003), prolactin (r= -0.201, P= 0.045), T/E2 ratio (r= -0.198, P= 0.049), and abnormal sperm morphology (r= -0.203, P= 0.043) had a weak yet significant negative correlation with BMI (they worsened as BMI increased).

Regarding the association between waist circumference (WC) with both semen parameters and sex hormones profile, it found that there is no statistically significant relation between sex hormones represented by (FSH, LH, estradiol, testosterone, prolactin, and T/E2 ratio) and waist circumference. In addition, there is a statistically significant association between waist circumference and abnormal sperm morphology and total motility (P= 0.045, 0.021 respectively). Participants with WC ≥102 cm had a significantly higher percentage of abnormal sperm morphology as compared to those with lower WC (≤94 cm) (P= 0.034), and about the total motility, it was significantly higher in participants with WC ≥102 cm when compared to those with WC ≥95 – 101 cm as (P= 0.016). It also found that there was no relation between waist circumference and progressive sperm motility.

In conclusion, no obvious effect of BMI on semen parameters and men's fertility, whereas, increased waist circumference affects sperm morphology, which can lead to men's infertility.

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LIST OF ABBREVIATION

Abbreviation	Meaning
AMA	American Medical Association
AUA	American Urological Association
BAX	Bcl-2 Associated X-protein
Bcl-2	B-cell lymphoma 2
BCL-X	B-cell lymphoma-extra large
BMI	Body Mass Index
CM	Centimeter
CVD	Cardiovascular Disease
DFI	DNA Fragmentation Index
DNA	Deoxyribonucleic Acid
DTWS	Dial Type Weighing Scale
E2	Estradiol
EAU	European Association of Urology
ECL	Electrochemiluminescence
ED	Erectile Dysfunction
FSH	Follicular Stimulating Hormone
GnRH	Gonadotropins Releasing Hormone
HDL	High-Density Lipoprotein
HPG	Hypothalamic Pituitary Gonadal
HSD	Hydroxysteroid Dehydrogenase
IL	Interleukin
INF-γ	Interferon Gama
IU	International Unit
KG	Kilogram
L	Litter
LDL	Low-Density Lipoprotein
LH	Latinizing Hormone
MCP-1	Monocyte Chemotactic Protein
MIP-1	Macrophage Inflammatory Protein
ML	Milliliter
NAFLD	Non-Alcoholic Fatty Disease
Nmol/l	Nano-moles per litter
NPY	Neuropeptide Y
OS	Oxidative Stress
ROS	Reactive Oxygen Species
SD	Standard Deviation
SDF	Sperm DNA Fragmentation
SNPs	Single Nucleotide Polymorphisms
StAR	Steroid Genic Acute Regularity
STDs	Sexual Transmitted Diseases
T2DM	Type two Diabetes Mellitus
T/E2	Testosterone Estradiol Ratio

Abbreviation	Meaning
TGFβ	Transforming Growth Factor Beta
TLR	Toll-like Receptor
TNFα	Tumor Necrosis Factor Alpha
TSYP	Testis-Specific Protein Y
WC	Waist Circumference
WHO	World Health Organization
βHSD	Beta Hydroxysteroid Dehydrogenase

1. INTRODUCTION

1.1 Background and Context of Study

Obesity is considered a complex disease that can affect entire body processes. Obesity is a condition defined by an abnormal accumulation of fat or white adipose tissue in the body which can adversely affect health and shorten life span (Du Plessis *et al.*, 2010), obesity and overweight are increasingly recognized as major public health problems worldwide, It is a problem because it can lead to many other health problems, according to WHO estimates in 2016, about 1.9 billion people were overweight, and more than 650 million were obese (Mousa *et al*., 2016; Chung 2016; Baydilli *et al.*, 2020; Ogden *et al.*, 2006; Chavarro *et al.*, 2010; Maghsoumi-Norouzabad *et al.*, 2020).

Obesity has been related to a variety of medical disorders, including depression, diabetes, osteoporosis, liver and kidney disease, sleep apnea, and heart disease (Bieniek *et al*, 2016; Dubeux *et al.*, 2016; Ahmed *et al.*, 2018), men who are overweight are more liable to erectile dysfunction, decreased spermatogenesis, and hypogonadism, any of the aforementioned factors may increase the risk of male infertility in patients (Hammoud *et al*., 2008 a,b).

Infertility is defined as the inability to become pregnant after one year of unprotected sexual activity, It affects about 15% of couples and 48.5 million people around the world (Alahmar,*et al*, 2018). At least 10% of the people in developed countries are thought to have this serious health problem (Boivin *et al.*, 2007; Taylor, 2003; MacDonald *et al.*, 2013). In a study by (Hammoud *et al.*, 2006), male factors alone have been shown to account for 25-30% of all cases of infertility, and combined with female factors account for an additional 30% of all cases, male infertility is caused by a variety of conditions, including cryptorchidism, testicular torsion or trauma, varicocele, anti-sperm antibodies, hypogonadotropic hypogonadism, gonadal dysgenesis, and reproductive channel obstruction. This is not an exhaustive list of the reasons why men cannot have children. People have lately discussed adding obesity to this list (Sekhavat and Moein, 2010).

Obesity-induced alterations in the hypothalamic-pituitary-gonadal axis lead to hypogonadotropic-hyperestrogenic hypoandrogenism. This effect is clear in women who present with several reproductive diseases and who are at either of the two extremes of obesity or weight reduction experience this effect. (Giagulli *et al.*, 1994; Hammoud *et al.*, 2008). There are not many studies on this link in men. Hormonal changes due to obesity can make it difficult for men to have children. This may lead to elevated serum estradiol levels, decreased gonadotropin and testosterone levels, and decreased ability of sex hormone-binding

globulin to bind hormones (Kaufmann and Vermeulen, 2005; Aggerholm *et al*., 2008; Alshahran *et al*., 2015). Therefore, endocrine problems are more likely to cause changes in sperm quality in obese men.

1.2 Aims and objectives of study

Although being overweight and obese are identified as serious health and social problem with economic and academic impacts all over the world, studies on their prevalence amongst infertile men all over the country are quite limited in Libya. Thus, it has not been easy to establish statistics on causes, social problems, and health implications as well as the implication of overweight and obesity on the fertility of men.

Therefore, this study sought to know if obesity and overweight affect various semen parameters and sex hormones among some infertile Libyan men or not.

1.2.1 Aims of Study

This study aims to find out the status of endocrine dysregulation in overweight and obese men and its relationship with semen quality, findings of this study might help raise awareness among obese and overweight men towards the impacts that obesity has on their reproductive functions.

The study findings would enhance screening and treatments for obesity to increase the reproductive functions in men. Thus, understanding the causes of overweight and obesity will make preventive measures easier and hence help in minimizing the extent of endocrine function dysregulation and infertility.

1.2.2 Objectives of Study

The specific objectives of this study were the following:

I. To Estimate the sex hormones level in the blood (FSH, LH, testosterone prolactin, and estradiol), and to clarify the association between dysregulated sex hormones with sperm quality.
II. To investigate the relationship between body mass index, reproductive hormones, and semen quality for evaluation of infertility.
III. To determine the impacts of obesity and endocrine dysregulations on semen quality and male reproductive functions.

2. LITERATURE REVIEW AND THEORETICAL FRAMEWORK

2.1 Concept of Obesity

Obesity is defined as an accumulation of extra fat or white adipose tissue in the body that has a negative effect on health outcomes. Clinically defined based on the body mass index (BMI) which is calculated by dividing a person's weight in kilograms by their height in square meters. This number is used to figure out how much extra body fat is linked to bad health effects (WHO, 2000), the World Health Organization (WHO) recommends using BMI to clinically categorize weight and assess clinical risk, (Table 1) (McPherson & Lane, 2015), in this method, obesity is defined as a BMI of greater than 30 kg/m^2; whereas, the severe obesity is defined as a BMI of greater than 35 kg/m^2. However, the type and location of body fat accumulation are clinically significant in determining the risk of complications related to obesity. BMI doesn't take into account the relation between lean body mass and total body fat, nor does it look for dangerous visceral adiposity (white adipose tissue). It is thought that the BMI is not sensitive enough to accurately diagnose obesity in a large part of the population. This means that it could either underestimate or overestimate the risk of obesity (Okorodudu *et al*., 2010; Alberti *et al*., 2009),

Table (1): Body mass index categories and risk of co-morbidities

BMI	Category	Complication risk	Complication
18.5	underweight	Increased	Immunodeficiency; infectious disease; malignancies
18.5-24.9	Optimal weight	Low	Uncommon
25.0-29.9	Overweight	Mild	CVD; T2DM; neurodegeneration; malignancies
30.0-34.9	Obesity	Moderate	
35.0-39.9	Obesity	Severe	
>40	Obesity	Very severe	

Abbreviations: CVD, cardiovascular disease; T2DM, type two diabetes mellitus. (Zainudin *et al*., 2011)

Using body fat percentage, you can see the differences in the proportions of lean-to-fat mass between the genders. Men and women with adipose abnormalities had an increased risk of complication by >25% and >30%, respectively, on the other hand, waist circumference is the most accurate predictor of visceral fat, the ideal waist circumference range (88 to 94 cm, depending on race and gender) is determined by measuring the distance between the anterior superior iliac spine and the lowest region of the costal margin (Alberti *et al*., 2009; Adler et *al*., 2013).

Obesity-related disorders are considerably exacerbated by visceral adiposity. Among the most notable impacts are, type 2 diabetes (T2DM), cardiovascular disease (CVD), dementia,

several malignancies, and a deleterious influence on human reproduction. These are mediated by complex and poorly understood mechanisms, such as oxidative stress, hyperleptinemia, chronic systemic low-grade inflammation, and insulin resistance (hyperinsulinemia) As seen in Figure (1) (Leisegang *et al.*, 2019).

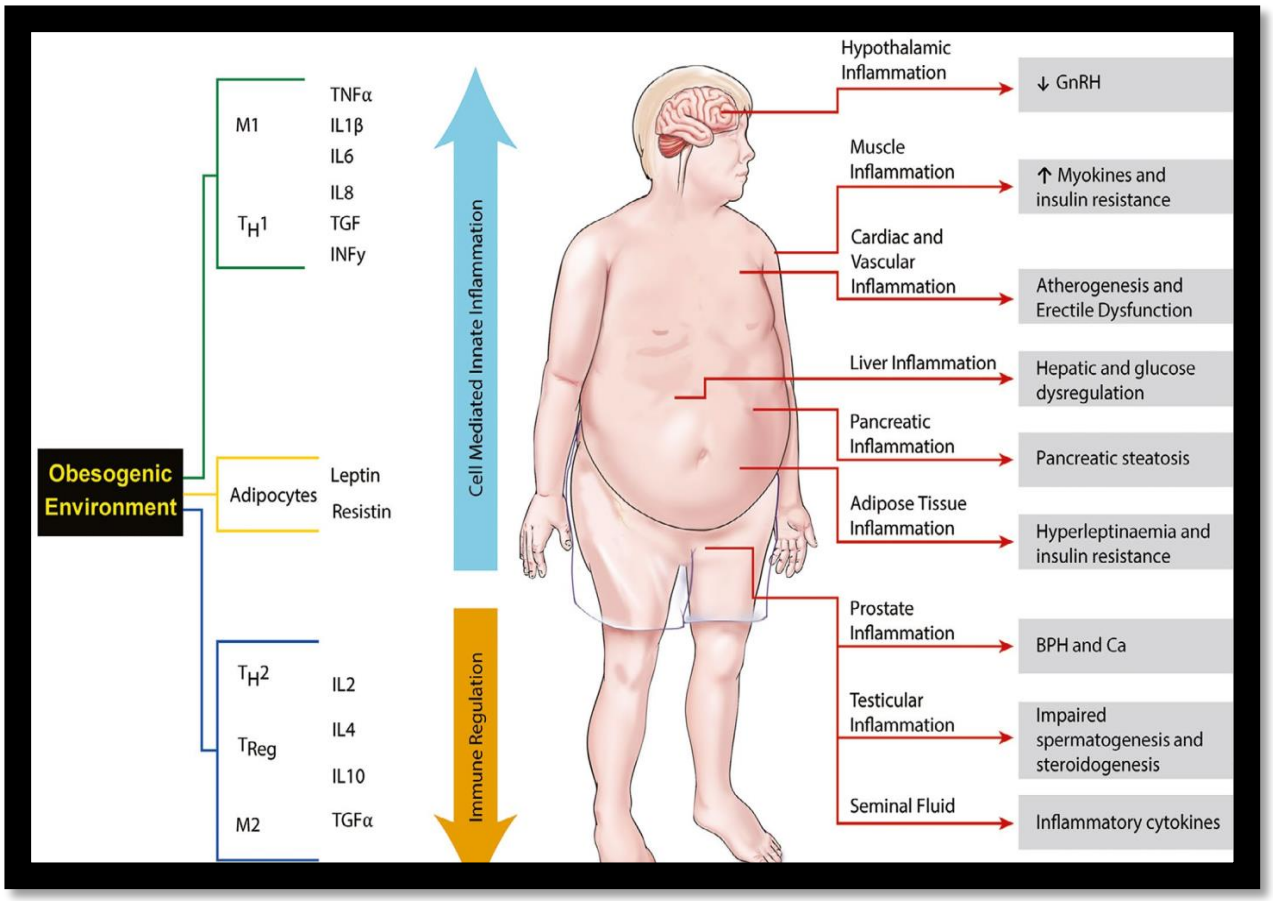

Figure (1): Complications of Obesity. Adapted from Leisegang *et al.*, 2019

2.2 Epidemiology of Obesity

Obesity is a global problem, and the number of obese men and women has risen quickly between 1980 and 2013 (28%–36%) and (29%–38%) respectively (Ng *et al.*, 2014). More than 200 million men out of the 1.4 billion people in the world are overweight or obese (Davidson *et al.*, 2015). Even though there was a lot of opposition, the American Medical Association (AMA) finally agreed in 2013 that obesity is a disorder. Most people agree that obesity is a worldwide problem that needs to be controlled and managed because of the diseases it can cause (Meldrum *et al.*, 2017).

2.3 Risk factors of Obesity

Significant non-modifiable risk factors for obesity include several genetic risk factors, like polygenic susceptibility, especially due to single nucleotide polymorphisms (SNPs) and

epigenetic inheritance, alongside aging (mainly due to oxidative stress), and female gender (Leisegang *et al*., 2017). Therefore, Low socioeconomic level, mental health issues, and sleep deprivation (as is the case with night shift workers) are also considered risk factors (Haslam & James, 2005). A more industrialized and obesogenic environment exacerbates the impact of the two primary causes of obesity, an increase in high-calorie food intake and a decrease in physical activity (Castro *et al*., 2017). Since there are often more overweight people in "Westernized" societies, this phenomenon is more widespread there (Haslam & James, 2005). Some medications like Tricyclics and Monoamine Oxidase Inhibitors (antidepressants), Beta-Blockers and Thiazide Diuretics that are used to control hypertension, Thiazolidinedione agents and Insulin, Anti-Psychotics, Oral Contraceptives, and glucocorticoids, all these drugs increase risk of obesity (Wofford *et al*., 2006). However, within an obesogenic environment, the mediators of obesity and associated consequences continue to be complicated (Leisegang *et al*., 2019).

2.4 Obesity: A key component of metabolic syndrome

The term "metabolic syndrome" refers to a cluster of metabolic disorders that, when present in an individual, increase that individual's risk of getting a variety of additional illnesses. Common diagnostic criteria require the presence of three of the following five identified features: increased waist circumference(obesity), high blood pressure, inadequate high-density lipoprotein (HDL) cholesterol, raised triglyceride levels, and glucose intolerance, since the obesity is only one of five diagnostic criteria, people of normal weight could have metabolic syndrome. On the other hand, patients who are considered to be overweight or obese but do not exhibit the phenotypic of metabolic syndrome may be at a lower risk for its complications (Alberti *et al*., 2009).

Obesity and metabolic syndrome share several co-morbidities and complications, Co-morbidities include obstructive sleep apnea, vasoconstriction, micro-vascular disorders, and non-alcoholic fatty liver disease (NAFLD). Complications include cardiovascular disease, diabetes type 2, cancer (including prostate cancer), neurotoxicity, and an acceleration of the aging process (McPherson & Lane, 2015). in males there are extra few complications include Erectile dysfunction, subclinical prostatitis, and low sperm quality (Leisegang *et al*., 2019). Male hypogonadism is strongly associated with obesity and metabolic syndrome, increasing the risk of male infertility and perpetuating the underlying mechanisms of obesity-induced complications. Hypogonadism is typically characterized by low levels of gonadotropin-

releasing hormone (GnRH) produced by the pituitary gland in men (Du Plessis *et al*., 2010; Huang, 2009).

2.5 Obesity and Hormonal imbalances

2.5.1 Hypothalamic-pituitary-gonadal axis

Obesity-related physiological changes can disrupt the hypothalamic-pituitary-gonadal (HPG) axis, a key reproductive regulatory system, this can affect the male reproductive endocrine system and leads to problems such as delayed puberty and reduced sperm production (Agarwal and Dutta, 2020). Figure 1 depicts these alterations along the HPG axis. Obesity is associated with adipocyte hypertrophy and hyperplasia and increased adipokines production, additionally to an abnormality in testicular function and endocrine regulation. These changes occur as a result of how obesity impacts adipocytes (Dutta *et al*., 2019 a,c).

It's likely that the increased levels of leptin that present in obese people limit the ability of Leydig cells to produce testosterone (Caprio *et al*., 1999), when there is more adipose tissue, more testosterone is turned into estradiol (Hammoud *et al*., 2006). White adipose tissue's overproduction and hyperactivity of the aromatase cytochrome P450 enzyme serve as an intermediary for this (Katib, 2015). Male obesity measures including BMI, total body fat percentage, subcutaneous fat percentage, and intra-abdominal fat have an inverse relationship with low testosterone and a direct relationship with high estrogen levels (Tsai *et al*., 2004).

Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are anterior pituitary gonadotropins that are secreted as a result of the pulsatile release of the hypothalamus gonadotropin-releasing hormone (GnRH) (Blache *et al*., 2003). The primary regulating hormones affecting the testicular cells are these gonadotropins. The Sertoli cell is stimulated to produce more inhibin by FSH, which indirectly controls spermatogenesis, Inhibin directly inhibits pituitary FSH secretion through a negative feedback mechanism. The LH stimulates steroidogenesis and testosterone production in Leydig cells, through its nuclear receptors in the Sertoli cell, testosterone further facilitates spermatogenesis (Dutta *et al*., 2019 d).

Both testosterone and estrogen may stop the production of kisspeptin and gonadotropin-releasing hormone by preventing the firing of KISS1 neurons in the arcuate nucleus of the hypothalamus (GnRH). It has been shown that the neuroendocrine pathways in the hypothalamus are affected by the hormone kisspeptin. The metabolic and reproductive processes are connected by these pathways (Wolfe & Hussain, 2018). Kisspeptin may

increase the amount of GnRH released, induce the breakdown of fat, and delay the synthesis of new fat. (Clarke *et al*., 2015).

2.5.2 Testicular steroidogenesis

Testicular functions are negatively affected when sex hormone levels are impaired by obesity. Since estrogen is physiologically more active than testosterone, a slight rise in estradiol levels compared to an increase in testosterone levels may have a greater inhibitory effect on testicular activity (Hammoud *et al.*, 2006).

By restricting the amount of active testosterone in males with normal weight, estrogen controls the overproduction of testosterone (Schulster *et al*., 2016). Oestrogen receptor expression in the male hypothalamus indicates that increased estrogen levels in obese men provoke the negative feedback mechanism to inhibit pulsatile GnRH release. This in turn leads to subsequent insufficiency of LH and FSH for steroidogenesis and spermatogenesis (Chimento *et al*., 2014). Elevated estrogen levels and increased systemic inflammation may have an impact on Leydig cell steroidogenesis. It is conceivable that rising levels of inflammatory cytokines have a deleterious impact on the proteins regulating cholesterol absorption into the mitochondria, including the steroidogenic acute regulatory (StAR) protein and important steroidogenic enzymes (P450scc, 3HSD, and 17HSD) (Leisegang & Henkel, 2018).

A growth-like substance released by Sertoli cells called inhibin B also prevents FSH from being produced. Obese males have repressed inhibin B production, breaking the HPG axis's feedback loop. This shows that obesity-mediated hormonal regulation not only impairs steroidogenesis but also negatively affects Sertoli cells directly (Salazar *et al*., 2018).

2.5.3 Metabolic Hormonal imbalances

Most cases of male infertility are caused by endocrine changes caused by excess white adipose tissue. This may have been averted if the individual had maintained a healthy weight. A high estrogen level and a rise in adipose tissue secretions interfere with the reproductive hormonal control over testicular functions (Bessesen *et al*., 2004). Adipose tissues serve as a source of Hormones, adipokines, pro-inflammatory cytokines, endogenous and exogenous toxins, in obese men, major adipokines include leptin, orexin, and adiponectin, ghrelin, and obestatin disrupt normal reproductive hormonal regulation. Leptin is predominantly produced and released by adipocytes and plays a crucial role in maintaining the homeostasis of energy intake and usage. Leptin is also necessary for optimal reproductive function, but at high

levels, it can negatively impact male fertility (Almabhouh *et al*., 2020 a,b; Ahima, 2008; Alvarez *et al*., 2013; Malik *et al.*, 2019; Sengupta *et al*., 2019; Perez *et al.*, 2013; Dutta *et al*., 2019).

leptin work through its receptors on the KISS1 neurons and increasing the production of GnRH from the hypothalamus. KISS1 neurons also connect to NPY neurons and GnRH neurons. Leptin working through KISS1, can thereby stop the NPY neurons from inhibiting GnRH. It is believed that obese males may develop leptin resistance, which may cause an inhibition of GnRH neurons by suppressing KISS1 neuron activity and elevating NPY levels (Ojeda *et al*., 2006). This gradually inhibits the pituitary's ability to produce gonadotropins, which reduces steroidogenesis and causes hypogonadotropic hypogonadism (Teerds *et al*., 2011). Additionally, it has been shown that exogenous leptin injection might dramatically increase fertility whereas a functional leptin gene deficit may lead to reduced gonadotropin production, which in turn affects fertility parameters. These evidences indicate that leptin is vital for maintaining normal testicular activities, however, increased leptin secretion in obese men affects testicular functioning perhaps by increasing endothelial cell mitochondrial fatty acid oxidation and causing uncontrolled reactive oxygen species (ROS) formation (Yamagishi *et al*., 2001; Bhat *et al*., 2006; Ramos & Zamoner, 2014; Mounzih *et al*., 1997).

in obese males the serum adiponectin levels are higher, and it has a negative relationship with testosterone (Page *et al*., 2005). In addition, it has been demonstrated that the non-classical metabolic hormone orexin (hypocretin), which increases the activity of the steroidogenic enzymes in Leydig cells, stimulates the synthesis of testosterone (Zheng *et al*., 2014).

Obesity affects orexin levels, which upsets the equilibrium between energy homeostasis and male reproductive activities (Sengupta *et al*., 2019). Obesity-related excess adipose tissues significantly raise resistin, an adipose tissue-specific secretory factor, according to reports, it lowers insulin sensitivity in obese men by raising blood glucose levels and reducing adipose cells' capacity to absorb glucose. (In insulin-resistant obese men, hyperinsulinemia may result in decreased SHBG and testosterone levels, which are essential for healthy spermatogenesis. As a result, obese men with type 2 diabetes may develop secondary hypogonadism. This may be a direct result of insulin resistance affecting the Leydig cells' ability to synthesize testosterone (Du Plessis *et al*., 2010; Flehmig *et al*., 2014).

The gastrointestinal tract produces the neuropeptide ghrelin, occasionally known as the" hunger hormone," It has been shown to be associated with low serum of testosterone in the

blood and a lack of steroidogenesis. Although ghrelin hasn't been directly linked to known effects on spermatogenesis, its participation in steroidogenesis is expected by the testis where its receptor is expressed (Ishikawa *et al.*, 2007; Dutta *et al*., 2019 b; Wang *et al*., 2011).

2.6 Obesity and spermatogenesis

The seminiferous tubules are in charge of keeping the germ cells' apoptosis, proliferation, and death processes in check, when the first stage of spermatogenesis is complete, the germ cells enter a new hormonal environment and begin to specialize. The Bcl-XL and Bax systems are triggered in conditions when there is an abundance of cells, which initiates the apoptotic process (Hikim & Swerdloff, 1999). A wide number of regulatory genes determine when and under what circumstances apoptosis occurs in the spermatogonial tissue. Obesity-related situations have been demonstrated to greatly increase the apoptosis rate in A1 spermatozoa. Recent studies suggest that spermatogenic cell death may underlie the vast majority of male infertility (Garolla *et al*., 2013).

Obesity may exacerbate apoptotic activation by elevating Bax gene expression while simultaneously lowering Bcl-2 gene activation. This triggers the activation of apoptosis-inducing enzymes known as caspases (Jia *et al*., 2018). Additionally, the condition of hyperlipidemia increases stress on the endoplasmic reticulum and promotes an increase in spermatogenic cell death by increasing binding immunoglobulin protein (BiP) production (Li *et al*., 2015).

2.7 Obesity and semen quality

Male fertility semen parameters primarily include sperm con- centration and total sperm count, motility, viability and morphology, among others. Human semen quality serves as a predictor of male fertility. (Carlsen *et al*., 1992; Dutta *et al.,* 2019; Krajewska-Kulak *et al*., 2017) there are new characteristics that might be developed, Poor sperm counts (oligozoospermia) and infertility (azoospermia) are more common in overweight or obese males (Sermondade *et al*., 2013). Obesity may cause physiological changes and hormonal adjustments that harm male fertility indicators (Bieniek *et al*., 2016). Although the relationship between BMI and sperm functions has been thoroughly investigated, the underlying processes are still not fully understood (Chavarro, *et al*., 2010; Hammiche *et al*., 2011; Sermondade *et al*., 2013).

Oligozoospermia, which is defined as having fewer than 15 million sperm per milliliter, is a complaint that is three times more likely to affect fat men than it is to affect men of normal

weight (WHO, 2010). Males with high body mass indices (more than 25 kg/m2) were shown to have lower total sperm counts than other types of men (Chavarro et al., 2010). Obesity has a detrimental influence not only on the volume of sperm but also on its motility and morphology, according to the findings of studies done by (Jensen *et al*., 2004) and (Kahn & Brannigan, 2017). Numerous studies have indicated that a person's weight has a negative impact on sperm function (Davidson *et al*., 2015; Aggerholm, *et al*., *2008;* MacDonald *et al*., 2010).

2.8 Obesity and sperm DNA integrity

Sperm DNA fragmentation (SDF) maybe strongly linked to obesity-induced changes in sperm functioning. Sperm DNA integrity is negatively impacted in obese males. According to reports, high BMI is associated with an increased DNA fragmentation index (DFI), which shows the connection between obesity and SDF (Panner *et al*., 2020).

Sperm DFI is high when BMI is above 25 kg/m2. A hypothetical explanation for this behavior is oxidative stress caused by increased testicular fat. In obese people, a variety of factors might play a role in the development of the testicular OS. The number of reactive oxygen species (ROS) generated by mitochondrial and peroxisomal fatty acid oxidation increases with increased adipose tissue mass. Elevated ROS levels can lead to oxidative damage to various biomolecules such as DNA, proteins, and lipids. These elements play a role in oxidizing the sperm's ability to fertilize an egg and maintain a pregnancy (Panner *et al*., 2020).

Maintaining integrity of sperm DNA is crucial for spermatozoa's capacity to fertilize and for optimal pregnancy outcomes (Benchaib *et al*., 2007). In addition to the conventional semen parameters, analysis of SDF offers a better knowledge of the male fertility status than. The American Urological Association (AUA) and the European Association of Urology (EAU) recommendations have also confirmed the significance of the SDF test in cases of male infertility (Jarow *et al*., 2011; Agarwal *et al*., 2016).

2.9 Obesity and erectile dysfunction

Obese men are 1.5 times more likely to experience erectile dysfunction (ED). (Sarwer *et al*,.2018), which has been linked to male infertility (Du Plessis *et al*., 2010). By lowering testosterone levels and causing a systemic inflammatory state through the production of inflammatory cytokines, obesity may cause erectile dysfunction (Seftel, 2006). These

proinflammatory cytokines directly cause the malfunction of endothelial cells, and they may cause male erectile dysfunction through the nitric oxide pathway (Yu *et al*., 2017).

Several pathogenic diseases, including diabetes, hypertension, and dyslipidemia, are associated with obesity and may contribute to the development of ED (Shamloul & Ghanem, 2013). A deeper knowledge of the precise process behind obesity-induced ED is necessary, particularly in light of issues including decreased libido, endocrine dysregulation, and psychiatric problems (Hammoud *et al*., 2008).

2.10 Mechanism implicated in obesity-induced male infertility

The chronic inflammatory responses and endocrine abnormalities, among other things, are part of the complicated pathophysiology of obesity. Along with increased visceral adipose tissue deposition, which causes pro-inflammatory responses from Th1 lymphocytes and M1 macrophages (Leisegang *et al*., 2019). This inflammatory response characterized by modulation of cytokines, adipokines and myokines that adversely affect numerous tissues including the hypothalamus, heart, liver, and the pancreas. It also has an adverse effect on the testes. Infertility and hypogonadism are two significant complications that have been related to obesity-induced systemic inflammation. These immune response's inflammatory mediators include interleukin (IL)-1, IL-6, IL-8, IL-12, tumor necrosis factor-alpha (TNF), interferon-gamma (IFN), transforming growth factor-beta (TGF), macrophage inflammatory protein (MIP-1), monocyte chemotactic protein (MCP-1), and neuroendocrine hormones leptin and resistin. (Leisegang *et al*., 2019).

The inflammatory responses are further elicited by leptin causing macrophage infiltration in tissues. They may also result in hypothalamic inflammation, which modulates the behavior of the HPG axis and alters the hypothalamus hormone release (Castro *et al*., 2017). Multiple pathways are responsible for the inflammation caused by obesity. These include endoplasmic reticulum stress, activation of serine/threonine kinase, and activation of toll-like receptor 4 (TLR4) (Thaler & Schwartz, 2010). Obese men's dyslipidemia status has been linked to testicular oxidative stress, a well-known route for impairing sperm function (Biswas *et al*., 2017).

LDL's ability to act as a ligand for macrophage pattern recognition receptors like the toll-like receptors may be modified by cellular activities (TLRs). In order to start the release of pro-inflammatory mediators, it directly activates pro-inflammatory signaling pathways. Additionally, monocytes, neutrophils, and other macrophages may take up the changed LDL,

which causes a buildup of cholesterol in those cells. This worsens the inflammatory situation by amplifying the TLR signaling pathways (Tall & Yvan-Charvet, 2015).

Obesity and metabolic syndrome lead to an increase in pro-inflammatory cytokines in the prostate, seminal vesicles, testicles, epididymis, and semen of obese men, contributing to inflammation of the male reproductive system. These cytokines are seen in lower amounts in normal men's reproductive organs. This is negatively related to signs of hypogonadism and worse semen quality (Leisegang *et al*., 2016) additionally, hyperglycemia is a separate component that can cause endoplasmic reticulum stress and oxidative stress. This may lead to inflammation, which might produce glycation end products and trigger SDF (Maresch *et al*., 2017). Inflammation of the reproductive tract caused by obesity is also dramatically increased in C-reactive protein, another important inflammatory marker (Leisegang *et al*., 2019).

Obesity-induced inflammation may have an effect on the HPG axis and the interactions it has with other hormones. This may affect how the endocrine system regulates reproductive functions. Obesity is related to decreased levels of testosterone, progesterone, and sex hormone-binding globulin (SHBG). And increase estrogen levels that severely block the HPG axis, inhibiting the production of hypothalamic GnRH and the consequent release of FSH and LH (Leisegang *et al*., 2016). Since excessive adipose tissue deposition is linked to obesity, adipose tissue-derived factors and some metabolic hormones are also crucial in the regulation of hormonal crosstalk in obese males. Obesity frequently results in leptin resistance, which inhibits GnRH and causes the release of LH and FSH (Ojeda *et al*., 2006).

The fundamental hormonal cause of male infertility linked with obesity may be due to impaired control of testicular activities by gonadotropin and GnRH. Along with contributing to male infertility, obese men experience physical issues including erectile dysfunction and elevated scrotal temperatures (Du Plessis *et al*., 2010).

3. RESEARCH METHODOLOGY

3.1 Study Area

The study was conducted at Tripoli Infertility Treatment Center in Tripoli. This center was chosen because it is the only governmental center in Tripoli that provides free services to patients, and also because a large number of patients come to it from all over Libya.

3.2 Target Population

The target population for this study included all patients who came for treatment during the period from April 2022 to July 2022 at Tripoli Infertility Treatment Center.

3.3 Sample size

The study included 100 men with age (20-45 years) with a duration of infertility of more than one year. The sample for this study was categorized into three groups. First, the normal weight group (BMI 18.5-24.9) consisted of 30 patients, the second group, the overweight group, (BMI 25-29.9) included 30 patients, and the third group, the obese group (BMI more than 30) included 40 patients.

3.4 Sampling Techniques

Kumar (1999) defines sampling as a process of selecting a number of individuals for a study in such a way that individuals represent the larger group from which they were selected. This study used stratified sampling and simple random sampling techniques.

3.4.1 Stratified Sampling

The population, from which the sample was taken, was not homogenous. there were patients of different weights and ages. Therefore, Patients were subdivided into smaller homogeneous groups in order of their weight (Best and Kahn, 2006).

3.4.2 Simple Random Sampling

Patients involved in the study were selected randomly from all patients attending the center. All individuals were chosen in such a way that each had an equal and independent chance of being selected (Krishnaswami, 2002).

3.5 Data Collection Methods

All subjects were exposed to medical history assessment and a physical examination by an andrologist to see if there is the possibility of the presence of a varicocele, or a hydrocele, the location and consistency of testis in scrotum, and size of the testis and epididymis were also recorded and each patient was given a preliminary andrological diagnosis.

Exclusion criteria included apparent genital infection, uncontrolled diabetes mellitus, uncontrolled hypertension, severe cerebrovascular or cardiovascular disease, Azoospermia patients, patients taking exogenous hormones (hypogonadism), orchidectomy for any indication, and patients with a current history of alcohol or drug abuse**.**

Data collection methods refer to many methods and procedures developed to aid in the acquisition of data (Best and Kahn, 2006). The data collected by measuring the patient's weight and height to determine BMI, in addition to measurement of waist circumference, beside to a fasting venous blood samples to investigate serum reproductive hormones for each patient, also a semen sample was taken to determine various semen parameters, regarding risk factors of infertility and to find out which patients meet the exclusion criteria; a questionnaire was used for this purpose.

Overall, the study employed five data collection methods: (1) patients' questionnaires, (2) blood samples; (3) semen samples (4) measurement of BMI; and (5) measurement of Waist circumference as shown in (table 2).

Table (2): Data Collection Methods for Each Research Theme

Research them	BMI	Hormones level	Semen analysis	Waist circumference	Questioner
Obese or not	√			√	
Risk factor					√
Fertility			√		
Hormonal disturbance		√			

3.5.1 Patients' Questionnaires

Questionnaires were prepared and administered to the patients after undergoing measurements of their weight and height to determine BMI and measurement of waist circumference. The questionnaires helped in gathering facts about risk factors of men infertility, and the questions were mainly close-ended and it has been taken into consideration that the question is easy for patients to score (Appendix A).

3.5.2 Measurement of BMI and WC

Determined of overweight and obesity was studied through measurement of weight in relation to height, a DTWS Dial Type Weighing Scale was used for measuring each patient's weight and height in the study sample, the weight of the patients was measured in kilograms and their heights was measured in centimeters without shoes, such data were later used in the calculation of BMI, which was calculated as a ratio of weight in kilograms and height square in meters (Sharkey, 1997), also, a tape measure was used to measure the waist circumference,

it is the distance around the abdomen at the mid-point between the anterior superior iliac spine and the lowest portion of the costal margin.

3.5.3 Investigate Reproductive hormones levels

Fasting venous blood samples (10mL) were withdrawn from each subject and centrifuged and serum of FSH, LH, PRL, E2, and total testosterone levels were estimated by the cobas e 411 analyzers, a fully automated analyzer that uses a patented ElectroChemiLuminescence (ECL) technology for immunoassay analysis, the steps are detailed in (Appendix B).

3.5.4 Semen analysis

Semen samples were donated once in a special laboratory room by masturbation into disposable sterile plastic containers. The collected samples were evaluated according to the WHO Guidelines (2010) (Appendix C), semen was analyzed manually by reading the sample under a microscope. The semen analysis was done according to WHO guidelines (Appendix D).

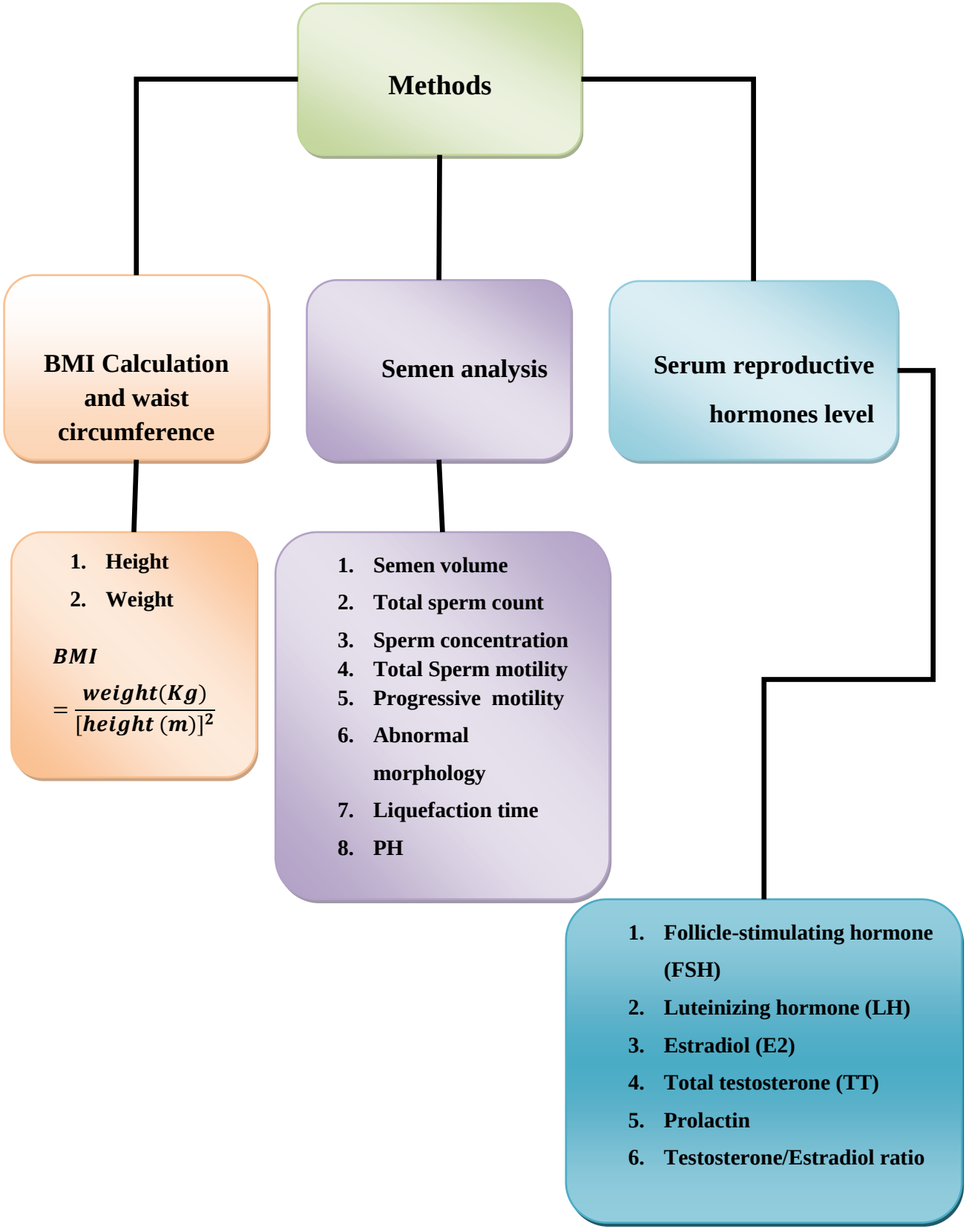

Figure (2): Illustration describing the methodology used in the study.

3.6 Data Analysis Procedures

3.6.1 Patients' Questionnaires

All data are collected and entered into SPSS for descriptive statistical analysis to know the prevalence of different characteristics and possible risk factors of infertility in all participants.

3.6.2 Analysis of BMI and WC measurements

3.6.2.1 Analysis of BMI measurements

Weight and height measurements were subjected to calculations of BMI to determine their implication to the patient's weight status. The following formula was used in BMI calculation: BMI = weight in kg/height in m2. Results were used to classify weight status as "normal weight, overweight, obesity" as shown in (Table 3).

Table (3): BMI and Body Weight Status by Implications.

BMI	The implication of the weight status
18.5-24.9	Normal weight
25-29.9	Overweight
>30	Obese

(WHO, 2000)

3.6.2.2 Waist circumference measurements Analysis

It is measured around the abdomen at the mid-point between the anterior superior iliac spine and the lowest portion of the costal margin. Measurement of waist circumference is necessary for the determination of the distribution of fat. as shown in (Table 4).

Table (4): Classification of waist circumference.

Waist circumference	The implication of the weight status
<94	Normal fat distribution
94-101.9	Moderate central fat accumulation
>102	High central fat accumulation

(Padaruth *et al*., 2019).

3.6.3 Analysis results of reproductive hormones

After the results of all hormone investigations were collected, they compared with normal levels of hormones in healthy adult men. (Table 5)

Table (5): Normal levels of reproductive hormones and TSH in adult men.

The Hormone	The normal range in adult male
Follicle Stimulating Hormone (FSH)	1.5-12.4 mIU/mL
Luteinizing Hormone (LH)	1.7-8.6 mIU/Ml
Prolactin	4.6-21.4 ng/mL
Total Testosterone	2.8-8.0 ng/mL
Estradiol	7.6-42.6 pg/mL
Thyroid Stimulating Hormone (TSH)	0.4-4.5 mU/L

(Gardner & Shoback, 2012)

3.6.4 Analysis of Semen parameters

The data of semen analysis shows a various semen parameter that include the following; (1) Semen volume, (2) sperm count, (3) sperm concentration, (4) sperm motility, (5) sperm morphology**,** (6) liquefaction, (7) sperm vitality, and (8) pH, and the results were compared with the lower reference limit for semen characteristics according to WHO as shown in (table 6).

Table (6): Lower reference limits for semen characteristics.

Parameter	Lower reference limit
Semen volume (ml)	1.5 (1.4–1.7)
Total sperm number (10^6 per ejaculate)	39 (33–46)
Sperm concentration (10^6per ml)	15 (12–16)
Total motility (PR + NP, %)	40 (38–42)
Progressive motility (PR, %)	32 (31–34)
Vitality (live spermatozoa, %)	58 (55–63)
Sperm morphology (normal forms, %)	4 (3.0–4.0)

PR progressive motility, NP nonprogressive motility

(WHO, 2010).

Statistical analysis

Statistical analysis was done by SPSS version 28 (IBM Co., Armonk, NY, USA). Numerical variables were presented using mean and standard deviation (SD) and were analyzed by ANOVA (F) test with post hoc test (Tukey). Categorical variables were presented using frequency and percentage (%) and analyzed using the Chi-square test. Spearman's rank correlation coefficient was used to estimate the degree of correlation between two non-parametric numerical variables. A two-tailed P value < 0.05 was considered statistically significant.

4. RESULTS

The study participants were categorized and further analyzed according to BMI into three groups as follows:

- Normal weight: Included 30 participants with BMI ranging between 18.0 and 24.9 kg/m^2.
- Overweight: Included 30 participants with BMI ranging between 25 and 29.9 kg/m^2.
- Obese: Included 40 participants with a BMI of 30.0 kg/m^2 or more (Table 7).

Table (7): body mass index Classification of the study group.

Nutritional status	BMI	Number of participants
Normal weight	18.0-24.9 kg/m^2	30
Overweight	25-29.9 kg/m^2	30
Obese	>30 kg/m^2	40

4.1 Baseline characteristics of all study participants

As demonstrated in Table 8, this study included 100 men suffering from infertility for more than a year and their ages ranged between 25 and 45 years with a mean of 38.67±4.87 years. BMI ranged from 18.3 to 44.9 kg/m^2 with a mean of 28.65 ± 5.44 kg/m^2, out of the 100 participants, 24% had children with their current wives, regarding the prevalence of different characteristics and possible risk factors, the most common was smoking in 39% of participants followed by varicocele surgery which was made up approximately one-third of participants (34%) and participants with diabetes mellites made up 16% of total participants, only 4% had chronic diseases and 3% were previously subjected to hernia repair, the less common risk factors were hypertension or hyperlipidemia, undescended testicular disease, testicular injury, and alcohol drinking in 2%, 2%, 1%, and 1% respectively.

Table (8): Baseline characteristics of all study participants.

		Total Participants (n=100)
Age (years)	Mean ± SD	38.67 ± 4.87
	Range	25 – 45
BMI (kg/m^2)	Mean ± SD	28.65 ± 5.44
	Range	18.3 – 44.9
WC (cm)	Mean ± SD	97.59 ± 14.6
	Range	65 – 133
Lifestyle and risk factors		
Having children		24 (24%)
DM		16 (16%)
Hypertension or hyperlipidemia		2 (2%)

Continue to table (8): Baseline characteristics of all study participants.

	Total Participants (n=100)
Previous hernia repair	3 (3%)
Undescended testicular disease	2 (2%)
Testicular torsion	0 (0%)
Testicular or penile injury	1 (1%)
STDs	0 (0%)
Varicocele surgery	34 (34%)
Testosterone or cortisone or any building supplements take	0 (0%)
Mumps after puberty	0 (0%)
Smoking	39 (39%)
Alcohol drinking	1 (1%)
Exposure to radiation	0 (0%)
Exposure to toxins or chemicals	0 (0%)

Data are presented as frequency (%) unless otherwise mentioned, WC: Waist circumference, DM: Diabetes mellitus, STDs: Sexually Transmitted Diseases

4.2 Hormonal and semen analysis characteristics

Hormonal and semen analysis of all the studied participants was shown in table 9, regarding FSH, it ranged from 0.99 to 27.21 IU/L with a mean of 5.79 ± 3.97 IU/L, LH ranged from 1.88 to 14.29 IU/L with a mean of 6.65 ± 2.46 IU/L, in term of estradiol, it ranged from 2.68 to 96.08 pg/mL with a mean of 32.18 ± 11.91 pg/mL, total testosterone ranged from 0.93 to 14.7 (nmol/L) with a mean of 4.61 ± 1.96 (nmol/L), regarding the total testosterone/estradiol ratio, it ranged from 0.08 - 8.31 with a mean of 1.61 ± 1.22, pH ranged from 7 to 9 with a mean of 8.16 ± 0.36 and prolactin had a range from 2.93 to 65.32 with a mean of 13.97 ± 9.15 (ng/mL), the samples were liquefied for a period ranging from 10 to 120 min with a mean time of 37.81 ± 16.26 min, the semen volume ranged from 0.5 to 7 mL with a mean of 2.53 ± 1.29 mL, and regards sperm concentration, it ranged from 0.01 to 200 million sperms/mL with a mean of 34.95 ± 31.42 million/mL, and total sperm count ranged from 0.01 to 660 million/mL with a mean of 89.53 ± 104.02 million/mL, sperm analysis showed that the average percentage of sperms that was abnormal morphology (large head or double tail) was 89 ± 12 % [from 25% of the sperm to the whole sperm (100%)], the progressive motility of participants' sperms ranged from 0 to 40% with a mean of 13 ± 10% and total motility ranged from 0 to 80% with a mean of 50 ± 20%.

Table (9): Semen analysis characteristics of all study participants.

Sex hormone and semen parameters	Mean ± SD Min-max
FSH (IU/L)	5.79 ± 3.97
	0.99 - 27.21
LH (IU/L)	6.65 ± 2.46
	1.88 - 14.29
Estradiol (pg/mL)	32.18 ± 11.91
	2.68 - 96.08
Total testosterone (nmol/L)	4.61 ± 1.96
	0.93 - 14.7
Prolactin (ng/mL)	13.97 ± 9.15
	2.93 - 65.32
Testosterone/ estradiol ratio	1.61 ± 1.22
	0.08 - 8.31
pH	8.16 ± 0.36
	7 – 9
Liquefaction time (min)	37.81 ± 16.26
	10 – 120
Semen volume (mL)	2.53 ± 1.29
	0.5 – 7
Sperm concentration (million/mL)	34.95 ± 31.42
	0.01 – 200
Total sperm count. (million/mL)	89.53 ± 104.02
	0.01 – 660
Abnormal morphology (%)	89 ± 12
	25 – 100
Progressive motility (%)	13 ± 10
	0 – 40
Total motility (%)	50 ± 20
	0 – 80

Data are presented as mean ± SD and range, FSH: Follicle-Stimulating Hormone, LH: Luteinizing hormone

4.3 Association between BMI and serum hormone profile

The only sex hormone associated with BMI was prolactin (P value= 0.011) being significantly lower in overweight and obese participants when compared to those with normal weight (P value=0.031, 0.016 respectively) while both groups with overweight and obesity had no statistically significant difference in prolactin level, there was no statistically significant difference between the remaining sex hormones represented by (FSH, LH, estradiol, total testosterone) and BMI as shown in Table 10 and Figure 3 in appendix G.

Table (10): Association between BMI and serum hormone profile.

Sex hormone		Normal weight (n=30)	Overweight (n=30)	Obese (n=40)	P value
FSH (IU/L)	mean± SD	5.65 ± 4.63	5.34 ± 2.6	6.24 ± 4.32	0.629
	range	2.1 - 27.21	1.75 - 12.49	0.99 - 20.4	
LH (IU/L)	mean± SD	7.34 ± 2.49	6.24 ± 2.09	6.43 ± 2.63	0.174
	range	3.74 - 14.29	2.55 - 11.17	1.88 - 13.91	
Estradiol (pg/mL)	mean± SD	30.68 ± 10.68	36.39 ± 13.59	30.14 ± 10.89	0.066
	range	5 - 49.58	21.32 - 96.08	2.68 - 61.79	
Total testosterone (nmol/L)	mean± SD	5.08 ± 1.67	4.82 ± 1.98	4.09 ± 2.07	0.086
	range	2.02 - 8.35	0.93 - 8.12	2.07 - 14.7	
Prolactin (ng/mL)	mean± SD	18.12 ± 12.2[a]	12.27 ± 7.46 [b]	12.13 ± 6.44 [b-c]	0.011
	range	4.75 - 65.32	5.65 - 44.84	2.93 - 34.2	
Testosterone/ estradiol ratio	mean± SD	1.9 ± 1.46	1.35 ± 0.6	1.59 ± 1.33	0.22
	range	0.51 - 8.31	0.08 - 2.79	0.33 - 7.29	

Data are presented as mean ± SD and range, FSH: Follicle-Stimulating Hormone, LH: Luteinizing hormone, Statistical significance at $p<0.05$, Different lower-case letters indicate significant difference

4.4 Association between BMI and semen analysis parameters

There was no statistically significant difference between BMI and semen analysis represented by (pH, Liquefaction time, semen volume, sperm concentration, and total sperm count), results are presented in Table 11.

Table (11): Association between BMI and semen parameters.

Semen parameters		Normal weight (n=30)	Overweight (n=30)	Obese (n=40)	P value
pH	mean± SD	8.25 ± 0.37	8.15 ± 0.33	8.1 ± 0.38	0.227
	range	7.5 - 9	7.5 - 9	7 - 9	
Liquefaction time (min)	mean± SD	32.63 ± 21.53	42.17 ± 12.3	38.43 ± 13.35	0.071
	range	10 - 120	20 - 60	15 - 65	
Semen volume (mL)	mean± SD	2.3 ± 1.11	2.29 ± 1.03	2.87 ± 1.51	0.089

Continue to table (11): Association between BMI and semen parameters.

Semen parameters		Normal weight (n=30)	Overweight (n=30)	Obese (n=40)	P value
Sperm concentration (million/mL)	mean± SD	33.58 ± 25.46	34.11 ± 25.51	36.6 ± 39.19	0.911
	range	1 - 100	0.2 - 100	0.01 - 200	
Total sperm count (million/mL)	mean± SD	103.3 ± 118.44	73.71 ± 60.07	91.07 ± 118.38	0.546
	range	1.6 - 554	0.76 - 210	0.01 - 660	

Statistical significance at p<0.05, Different lower-case letters indicate a significant difference

Table 12 shows a significant relationship between the sperm total motility and BMI of the studied participants (P= 0.01) as obese participants had significantly higher sperm motility as compared to the overweight ones (P= 0.015) but had no different motility to that of normal weight ones. Overweight participants had significantly lower sperm motility compared to the normal ones (P= 0.03). In terms of sperm morphology and progressive motility, they were not associated with BMI. As shown in Figure 4 appendix G.

Table (12): Association between BMI and sperm quality manifested by (morphology and motility).

Semen parameters		Normal weight (n=30)	Overweight (n=30)	Obese (n=40)	P value
Abnormal morphology (%)	Mean ± SD	93 ± 6	89 ± 9	86 ± 15	0.059
	Range	77 - 100	70 – 99	25 - 100	
Progressive motility (%)	Mean ± SD	13 ± 9	11 ± 9	15 ± 12	0.322
	Range	0 - 35	0 – 40	0 – 40	
Total motility (%)	Mean ± SD	53 ± 16[a]	40 ± 22[b]	54 ± 20[ac]	0.010
	Range	3 - 75	0 - 75	0 - 80	

Statistical significance at p<0.05, Different lower-case letters indicate a significant difference.

4.5 Correlation between BMI, hormonal profile, and semen parameters.

The Spearman's Rank Correlation confirmed that LH (r= -0.212, P= 0.034), Total testosterone (r= -0.298, P= 0.003), prolactin (r= -0.201, P= 0.045), T/E2 ratio (r= -0.198, P= 0.049) and sperm Abnormal morphology (r= -0.203, P= 0.043) had a weak yet significant negative correlation with BMI (they worsened as BMI increased). Results are presented in Table 13 and Figures 5-6-7 and Figure 8 appendix G.

Table (13): Correlation between BMI, hormonal profile, and full semen parameters in all study participants.

Sex hormones and semen parameters	BMI (kg/m^2)	
	r_s	P
FSH (IU/L)	0.002	0.98
LH (IU/L)	-0.212	0.034
Estradiol (pg/mL)	-0.038	0.707
Total testosterone (nmol/L)	-0.298	0.003
Prolactin (ng/mL)	-0.201	0.045
T/E2 ratio	-0.198	0.049
Ph	-0.058	0.57
Liquefaction time (min)	0.149	0.14
Semen volume (mL)	0.166	0.099
Sperm concentration (million/mL)	-0.036	0.724
Total sperm count (million/mL)	-0.052	0.604
Abnormal morphology	-0.203	0.043
Progressive motility (%)	0.013	0.901
Total motility (%)	0.073	0.469

Statistical significance at $p<0.05$, r_s: Spearman's correlation coefficient, BMI: Body mass index, FSH: Follicle-Stimulating Hormone, LH: Luteinizing hormone.

4.6 Correlation between sex hormones and semen analysis.

4.6.1: Correlation between sex hormones (FSH, LH, estradiol) and semen analysis.

Table 14 demonstrated a significant negative correlation between FSH and sperm concentration (r= -0.234, P= 0.019). A similar significant negative correlation was confirmed between estradiol and both progressive (r= -0.247, P= 0.013), and total motility (r= -0.259, P= 0.009). As demonstrated in figures 9 and 10 appendix G.

Table (14): Correlation between sex hormones (FSH, LH, estradiol) and semen parameters in all study participants.

semen parameters	FSH (IU/L)		LH (IU/L)		Estradiol (pg/mL)	
	r_s	P	r_s	P	r_s	P
pH	0.08	0.428	0.081	0.421	0.086	0.392
Liquefaction time (min)	0.078	0.438	0.113	0.261	0.104	0.303
Semen volume (mL)	0.119	0.239	-0.036	0.722	-0.114	0.26
Sperm concentration (million/mL)	-0.234	0.019	-0.144	0.152	-0.107	0.29
Total sperm count (million/mL)	-0.196	0.051	-0.115	0.255	-0.157	0.119
Abnormal morphology (%)	-0.048	0.634	0.09	0.376	0.041	0.687
Progressive motility (%)	0.063	0.536	0.116	0.252	-0.247	0.013
Total motility (%)	0.058	0.567	0.085	0.399	-0.259	0.009

Statistical significance at $p<0.05$, r_s: Spearman's correlation coefficient, FSH: Follicle-Stimulating Hormone, LH: Luteinizing hormone

4.6.2 Correlation between sex hormones (testosterone, prolactin, T/E2) and semen analysis

Table 15 shows that sex hormones (testosterone, prolactin, T/E2) did not correlate with different semen parameters represented by (pH, Liquefaction time, semen volume, sperm concentration, total sperm count, sperm morphology, and motility).

Table (15): Correlation between sex hormones (testosterone, prolactin, T/E2) and semen parameters in all study participants.

semen parameters	Total testosterone (nmol/L)		Prolactin (ng/mL)		T/E2 ratio	
	r_s	P	r_s	P	r_s	P
pH	0.039	0.703	-0.103	0.308	-0.06	0.556
Liquefaction time (min)	-0.021	0.837	-0.125	0.216	-0.094	0.351
Semen volume (mL)	0.042	0.679	-0.048	0.634	0.094	0.352
Sperm concentration (million/mL)	-0.011	0.917	-0.183	0.069	0.055	0.584
Total sperm count (million/mL)	0.013	0.9	-0.161	0.11	0.094	0.35
Abnormal morphology (%)	0.07	0.49	0.192	0.056	0.044	0.661
Progressive motility (%)	-0.032	0.751	-0.062	0.541	0.105	0.298
Total motility (%)	-0.047	0.644	-0.029	0.775	0.062	0.541

Statistical significance at $p<0.05$, r_s: Spearman's correlation coefficient

4.7 Association between waist circumference (WC) and serum sex hormones and semen parameters.

The study participants were categorized according to waist circumference into three groups as follows:

- **≤94 cm:** Included 46 participants with waist circumferences ranging between 65 and 94 cm.
- **≥95 – 101 cm:** Included 14 participants with waist circumferences ranging between 95 and 101 cm.
- **≥102 cm:** Included 40 participants with waist circumferences ranging between 102 and 133 cm. (Table 16).

Table (16): waist circumference classifications of the study group

	waist circumference	The number of participants.
Normal fat distribution	< 94 cm	46
Moderate central fat accumulation	94–101.9 cm	14
High central fat accumulation	> 102 cm	60

4.7.1 Association between WC and serum hormone profile.

Table 17 shows no statistically significant relationship between sex hormones represented by (FSH, LH, estradiol, testosterone, prolactin, and T/E2 ratio) and waist circumference.

Table (17): Association between WC and serum hormone profile.

Sex hormone		≤94 cm (n=46)	≥95 – 101.9 cm (n=14)	≥102 cm (n=60)	P value
FSH (IU/L)	mean± SD	5.79 ± 4.04	5.25 ± 2.04	5.99 ± 4.43	0.84
	range	2.1 - 27.21	2.14 - 9.11	0.99 - 20.4	
LH (IU/L)	mean± SD	6.98 ± 2.35	6.82 ± 2.18	6.2 ± 2.65	0.323
	range	2.55 - 14.29	3.49 - 11.17	1.88 - 13.91	
Estradiol (pg/mL)	mean± SD	31.57 ± 9.33	28.32 ± 10.51	34.23 ± 14.59	0.252
	range	5 - 49.58	2.68 - 43.24	9.69 - 96.08	
Total testosterone (nmol/L)	mean± SD	4.88 ± 1.64	4.65 ± 2.03	4.28 ± 2.25	0.367
	range	1.44 - 8.35	1.16 - 7.6	0.93 - 14.7	
Prolactin (ng/mL)	mean± SD	15.99 ± 10.63	10.06 ± 3.03	13.01 ± 8.23	0.072
	range	4.75 - 65.32	6.1 - 18.34	2.93 - 44.84	
Testosterone/ estradiol ratio	mean± SD	1.68 ± 1.23	2.01 ± 1.66	1.39 ± 0.98	0.22
	range	0.51 - 8.31	0.25 - 7.29	0.08 - 5.2	

Data are presented as mean ± SD and range, WC: Waist circumference, FSH: Follicle-Stimulating Hormone, LH: Luteinizing hormone, with Statistical significance at $p<0.05$.

4.7.2.1 Association between WC and semen parameters.

There was no statistically significant relation between waist circumference and semen analysis represented by (pH, Liquefaction time, semen volume, sperm concentration, and total sperm count) as shown in Table 18.

Table (18): Association between WC and semen parameters.

semen parameters		≤94 cm (n=46)	≥95 – 101.9 cm (n=14)	≥102 cm (n=60)	P value
pH	mean± SD	8.22 ± 0.36	8.07 ± 0.33	8.13 ± 0.37	0.308
	range	7.5 - 9	7.5 - 8.5	7 – 9	
Liquefaction time (min)	mean± SD	35.96 ± 19.26	42.5 ± 14.38	38.3 ± 12.72	0.411
		10 - 120	20 – 65	15 – 60	

Continue to Table (18): Association between WC and semen parameters.

semen parameters		≤94 cm (n=46)	≥95 – 101.9 cm (n=14)	≥102 cm (n=60)	P value
Semen volume (mL)	mean± SD	2.37 ± 1.09	2.58 ± 1.46	2.7 ± 1.44	0.493
	range	0.5 - 5	0.6 - 5.6	0.8 – 7	
Sperm concentration (million/mL)	mean± SD	32.77 ± 24.98	28.8 ± 22.64	39.6 ± 39.67	0.446
	range	1 - 100	0.2 – 70	0.01 – 200	
Total sperm count. (million/mL)	mean± SD	92.69 ± 101.79	60.87 ± 55.21	95.94 ± 118.94	0.538
	range	1.6 - 554	0.76 – 210	0.01 – 660	

Statistical significance at p<0.05, WC: Waist circumference

4.7.2.2 Association between WC and sperm quality manifested by (morphology and motility).

Table 19 shows a statistically significant association between waist circumference and sperm Abnormal morphology, and total motility (P= 0.045, 0.021 respectively). Participants with WC ≥102 cm had a significantly higher percentage of abnormal sperm morphology as compared to those with lower WC (≤94 cm) (P= 0.034), in terms of total motility, it was significantly higher in Participants with WC ≥102 cm when compared to those with WC ≥95 – 101 cm as (P= 0.016). There was no relation between waist circumference and progressive sperm motility. **(**Figure 11 and Figure 12 appendix G**).**

Table (19): Association between WC and sperm quality manifested by (morphology and motility).

semen parameters		≤94 cm (n=46)	≥95 – 101.9 cm (n=14)	≥102 cm (n=60)	P value
Abnormal morphology (%)	mean± SD	92 ± 7 [a]	89 ± 10 [ab]	86 ± 15 [bc]	0.045
	range	70 – 100	72 - 99	25 – 100	
Progressive motility (%)	mean± SD	013 ± 9	11 ± 9	15 ± 12	0.339
	range	0 – 40	0 - 30	0 – 40	
Total motility (%)	mean± SD	51 ± 18 [a]	36 ± 25 [ab]	53 ± 20 [ac]	0.021
	range	0 - 75	0 – 70	0 – 80	

Statistical significance at p<0.05, Different lower-case letters indicate significant difference, WC: Waist circumference

4.8 Correlation between WC, hormonal profile, and full semen analysis in all study participants.

The Spearman's Rank Correlation confirmed that LH (r= -0.221, P= 0.027), testosterone (r= -0.224, P= 0.025), prolactin (r= -0.231, P= 0.021) and T/E2 ratio (r= -0.198, P= 0.048) had a weak yet significant negative correlation with waist circumference as the higher WC became, the worse they got. **(**Table 20, Figures 13, 14**,** and 15 appendix G).

Table (20): **Correlation between WC, hormonal profile, and full semen parameters in all study participants**

Sex hormone	WC (cm)	
	r_s	P
FSH (IU/L)	-0.046	0.651
LH (IU/L)	-0.221	0.027
Estradiol (pg/mL)	0.045	0.653
Total testosterone (nmol/L)	-0.224	0.025
Prolactin (ng/mL)	-0.231	0.021
T/E2 ratio	-0.198	0.048
pH	-0.076	0.452
Liquefaction time (min)	0.179	0.075
Semen volume (mL)	0.095	0.345
Sperm concentration (million/mL)	0.021	0.837
Total sperm count (million/mL)	0.002	0.987
Abnormal morphology (%)	-0.19	0.058
Progressive motility (%)	0.068	0.501
Total motility (%)	0.097	0.335

Statistical significance at $p<0.05$, r_s: Spearman's correlation coefficient, WC: Waist circumference, FSH: Follicle-Stimulating Hormone, LH: Luteinizing hormone.

5. DISCUSSION

Several studies have examined the impact of obesity on male infertility with different numbers of patient groups. According to the new information, this impact is made up of a variety of variables and pathophysiological processes (Sermondade *et al*., 2013; Eisenberg *et al*., 2014). The generally accepted mechanism is described as the aromatization of testosterone to estradiol by peripheral adipose tissue and the blocking of negative feedback that lowers high levels of estradiol in the hypothalamus-pituitary gonadal axis (Giagulli *et al*., 1994; Kley *et al*., 1980; Vermeulen *et al*., 1993). As a result, lower sperm parameters and subfertility may arise from reduced testosterone and increased estrogen. Thereby, obesity may lead to hypogonadotropic hypogonadism and hyperestrogenism. Obesity can affect sperm quality, and sperm mitochondrial function negatively increased sperm DNA damage, and increased seminal oxidative stress (Kahn *et al*., 2017). Because there isn't enough research examining male infertility in obese individuals and because obesity can be accompanied by comorbid disorders that might influence fertility, there are differing views in the literature about the relationship between BMI and semen characteristics. the findings from several research that have looked at these aspects have been contradictory.

In this study, we investigated the correlation among BMI, WC, and semen-analysis parameters as well as serum concentrations of sex hormones. We observed that adiposity is related to some sperm parameters and serum concentration of some sex hormones when assessed by BMI as well as WC. Correspondingly, this was generally consistent with some previous studies that reported the negative association between obesity and total sperm counts or concentration, sperm motility, and normal sperm morphology.

A meta-analysis published by MacDonald *et al*. (2010) found no conclusive evidence between semen parameters with obesity. In this meta-analysis, there was convincing evidence that TT, sex hormone-binding globulin (SHBG), and free testosterone were negatively correlated with increased BMI. The biggest limitation was that 26 out of 31 research weren't suitable for the meta-analysis. The common limitation among the studies included in the meta-analysis was the use of self-reported weight and height measurements and the absence of uniform inclusion standards for recruited individuals. In 3200 subjects selected from the general community, Wu *et al*., (2008) found no link between LH and BMI and significant negative correlations between testosterone, free testosterone, and SHBG with BMI. In addition, Aggerholm *et al*., (2008) found no correlation between E2, LH, or FSH in 1989 males selected from the general community but detected a significant negative association

between testosterone and SHBG. In multi-institutional cohort research, Bieniek *et al*., (2016) found that there was a strong association between increasing BMI and sperm concentration and motility. In 1989 males selected from the general community, Aggerholm *et al*., (2008) found no link between E2, LH, or FSH but detected a significant negative relationship between testosterone and SHBG. Instead of a fertility clinic, men were selected from the general community, and a wide age range and significant proportions of overweight and obese men were included in the sample. As a result, the research sample was probably representative of men in most developed countries. This study's main limitation was that height and weight were self-reported. According to the Qin *et al*., (2007) study, there is a link between BMI and sperm concentration or total sperm count. The BMI distribution of the research population, with only 1.7% of the population representing obese men, may help to explain these contradictory findings. This study also used strict exclusion criteria, which may have created considerable bias. Regular drinkers, heavy smokers, and males with chronic conditions were all excluded from the study. Overall, however, the findings of this study were mixed and continue to support the conclusion that there is no proof of a relationship between BMI and semen parameters. According to Hofny *et al*., 2009 research, sperm concentration, and motility are negatively correlated with BMI and positively correlated with abnormal sperm morphology.

In our study, we did not observe a relationship between semen parameters (pH, Liquefaction time, ejaculate volume, sperm concentration, sperm morphology, and total sperm count) and the different levels of BMI. Only sperm total motility (progressive motility + non-progressive motility) was significantly lower in overweight men compared to normal-weight men. However, sperm morphology negatively correlated with increasing BMI. Also, there was an inverse association of BMI with serum levels of prolactin, which was significantly lower in overweight and obese participants when compared to those with normal weight, whereas, when we compare the serum prolactin level between the other both groups (overweight and obesity) we found that no statistically significant difference between the two groups. Also, we found that there was no statistically significant difference between the remaining sex hormones (FSH, LH, estradiol, and testosterone) and BMI.

Spermatogenesis is affected by the local hormonal balance of testicular testosterone and estradiol ratio. Because normal spermatogenesis is significantly impacted by the loss of this equilibrium, infertility may result. Therefore, assessing the change in the T/E2 ratio may provide helpful information (Parekattil and Agarwal 2012). In research by Keskin *et al*., 2017 with 454 patients, it was shown that there was no significant correlation between BMI

increase and semen parameters or T/E2 ratio, although there was a negative correlation between BMI and total testosterone and PRL. In research published by Oztekin *et al*., (2020), it was discovered that an increase in adipose tissue was shown to be negatively linked with T levels and the T/E2 ratio but not significantly correlated with changes in the semen parameters. In our study, we observed that increased BMI was negatively correlated with serum prolactin, total testosterone levels, and T/E2 ratios.

Generally speaking, much research in the literature has revealed a marginally significant connection between semen parameters and levels of reproductive hormones and increased BMI, the large sample size is probably responsible for these results. In a study by Zhao *et al*., (2020), the LH, FSH, and TT levels were all discovered to be inversely associated with sperm motility (all P for trend 0.05) after adjusting for age, body mass index, current smoking, and alcohol consumption. However, in mutual adjustment analysis, only LH remained an inverse association with sperm motility after adjusting for FSH and TT levels (P for trend = 0.04). Lower sperm progressive motility was similarly associated with higher LH concentrations (P for trend = 0.04). Furthermore, normal sperm morphology was inversely correlated with both LH and FSH levels. Kumanov *et al*., (2006) Observed that serum concentrations of LH and FSH were inversely correlated with sperm count, motility, and morphology, while testosterone was not correlated. Another study by Meeker *et al*. (2007) found that testosterone levels were considerably positively connected with motility whereas LH and FSH levels were significantly negatively correlated with sperm concentration, motility, and morphology. In contrast, two minor investigations (Uhler *et al*., 2003; Subhan *et al*., 1995) found that only FSH levels, but not LH or testosterone levels, have a negative connection with semen parameters.

In our study, we found a significant negative correlation between FSH and sperm concentration. And a similar significant negative correlation was confirmed between estradiol and both progressive and total motility, other sex hormones (testosterone, prolactin) and T/E2 did not correlate with different semen parameters (pH, Liquefaction time, semen volume, sperm concentration, total sperm count, sperm morphology and motility).

Although there is some evidence to support the concept that obesity has an impact on reproductive potential, several studies have found no connection between BMI and sperm parameters. However, self-reported BMI measurements were included in more than half of the studies. Moreover, BMI was the only metric used to measure adiposity. Notably, rather than BMI, the distribution of body fat as determined by WC may be a more reliable indicator

of the adverse metabolic effects of excessive body size. Eisenberg *et al*., (2014). A few research have investigated how WC affects the profile of the semen. Infertile males were shown to have a negative correlation between sperm counts and WC in research by Fejes *et al*., (2005) and Hammiche *et al*., (2012). According to Maghsoumi *et al*., 2020, infertile men with normal WC had better sperm parameter quality than infertile men who were overweight or obese; the existence of a similar relationship in men who are not infertile is still up for debate. depending on Eisenberg *et al*., (2014) the results indicate that ejaculate volume decreases linearly as waist circumference and BMI increase, and it seems that the magnitude of the association is similar. They also discovered that lower levels of concentration and sperm count were associated with both BMI and waist circumference and that the median sperm count was significantly associated with waist circumference but not with BMI.

In this study, we observe that there was no statistically significant relation between waist circumference and semen analysis represented by (pH, Liquefaction time, semen volume, sperm concentration, and total sperm count). Also, we found that there is a statistically significant association between waist circumference and sperm morphology, and total motility. Participants with WC ≥102 cm had a significantly higher percentage of abnormal sperm morphology as compared to those with lower WC (≤94 cm). In terms of total motility, it was significantly higher in Participants with WC ≥102 cm when compared to those with WC ≥95 – 101 cm. And there was no relation between waist circumference and progressive sperm motility also there is no statistically significant relation between sex hormones represented by (FSH, LH, estradiol, testosterone, prolactin, and T/E2 ratio) and waist circumference.

Numerous factors contribute to the study's strengths, including the relatively high sample size, the inclusion of different sex hormones, the correction for potential confounding, and the mutual adjustment technique used to identify independent effects. However, because our data were cross-sectional, it was difficult to conclude the causes. Furthermore, there was only one assessment of circulating hormones that might not accurately reflect long-term levels. To support our findings, prospective trials including ongoing circulating hormone monitoring are required. Finally, one semen sample may not accurately represent a man's long-term values since semen properties vary over time among people. One semen sample, however, may be sufficient to detect average variations in semen quality between people, according to other research that found no significant changes between the first semen sample and the remaining repetitions.

6. SUMMARY, CONCLUSION, AND RECOMMENDATIONS

6.1 Summary

In summary, in this study, we evaluated the relationship of semen parameters and reproductive hormones with BMI and WC in a large patient group after excluding possible risk factors that may affect infertility, and we found that there was no statistically significant difference between BMI and semen analysis represented by (pH, Liquefaction time, semen volume, sperm concentration, and total sperm count). Only the sperm total motility had a significant relation with BMI of all studied participants, the obese participants had significantly higher sperm motility as compared to the overweight ones but had no different motility to that of normal weight ones, Overweight participants had significantly lower sperm motility compared to the normal ones. sperm morphology and progressive motility were not associated with BMI.

The only sex hormone associated with BMI was prolactin, being significantly lower in overweight and obese participants when compared to those with normal weight, while both groups with overweight and obesity had no statistically significant difference in prolactin. There was no statistically significant difference between the remaining sex hormones (FSH, LH, estradiol, and testosterone) and BMI. However, there is a significant negative correlation between FSH and sperm concentration. A similar significant negative correlation was confirmed between estradiol and both progressive and total motility. Other sex hormones (testosterone, prolactin, T/E2) did not correlate with different semen parameters represented.

The Spearman's Rank Correlation confirmed that LH, testosterone, prolactin, T/E2 ratio, and sperm morphology had a weak yet significant negative correlation with BMI (they worsened as BMI increased).

There is a statistically significant association between WC and sperm morphology and total motility. Participants with WC ≥102 cm had a significantly higher percentage of sperm abnormal morphology as compared to those with lower WC (≤94 cm). In terms of total motility, it was significantly higher in Participants with WC ≥102 cm when compared to those with WC ≥95 – 101 cm. There was no statistically significant relation between WC and other semen parameters. There is no statistically significant relation between sex hormones and WC.

The Spearman's Rank Correlation confirmed that LH, testosterone, prolactin, and T/E2 ratio had a weak yet significant negative correlation with WC, the higher WC became, the worse they got.

6.2 Conclusion

In conclusion, no relationship was observed between BMI and semen parameters upon the overall group comparison, and there was no statistically significant difference between sex hormones and BMI, therefore, increasing BMI not affected the men's fertility.

Regarding the affecting of WC on men's fertility, there was a statistically significant association between WC and sperm morphology, the patients with WC more than 102cm have a significantly higher percentage of abnormal sperm morphology compared to patients with WC less than 94cm. So increasing WC may be affected men's fertility.

6.3 Recommendations

A more advanced method to study the impact of overweight and obesity on men’s fertility. Such a method may include conducting a longitudinal study that will help to get systematic progress of overweight and obese men.

Another study can be done to investigate the impact of decreased body weight in the central region (waist area) on semen parameters.

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8. Appendix

Appendix A

Patients' Questionnaire

Name of the patient _____

How long have you been trying to conceive with your current partner?

_____

Have you ever had children with your current wife? Yes or NO _____

Do you have diabetes? Yes or NO _____

Do you suffer from high blood pressure or high cholesterol? Yes or NO _____If the answer is yes, which one _____

In addition to high blood pressure and diabetes, do you have any other chronic diseases? _____If the answer is yes, what is it? _____

Have you ever had a hernia repair before? Yes or NO _____

Have you ever had undescended testicle disease (not descending into the scrotum) Yes or NO_____

Have you ever had a torsion of the testicle? Yes or NO _____

have you ever had an injury to the testicles or penis and required hospitalization Yes or NO _____

Have you ever had an STDs? Yes or NO _____if the answer is yes, which one? _____

Have you ever had varicocele surgery? Yes or NO _____

Do you take testosterone or cortisone or any building supplements? Yes or NO _____

Did you have mumps after puberty? Yes or NO _____

Do you smoke? Yes or NO _____ If the answer is yes how many pack sper day _____

Do you drink alcohol? Yes or NO _____ _____If the answer is yes, what is the average that you drink per week? _____

What are your jobs? _____

Are your exposed to any chemicals or toxins in your work? Yes or NO _____

Have you ever been exposed to a large amount of radiation or been exposed to radiation for a long time? _____

Appendix B

Steps of Reproductive Hormones investigations

Reproductive hormones were estimated by the cobas e 411 analyzers, a fully automated analyzer that uses a patented ElectroChemiLuminescence (ECL) technology for immunoassay analysis, non-fasting blood samples were taken from the cubital vein in a period between 8 and 10 am. The blood sample waited to be clotted (it took between 15 to 30 minutes). The next step is the Isolation of serum from blood samples, it's done by centrifugation of the sample at 4000g for 10min. After Preparing the cobas e 411 analyzers by placing the test solutions. About 200 µ of serum was taken and put in the test cup. then Placed in cobas e 411 analyzers and analyzed for luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), total testosterone, and prolactin and it took approximately 50 minutes to get the results.

Appendix C

Semen sample collection

The sample should be collected in a private room near the laboratory, to limit the exposure of the semen to fluctuations in temperature and to control the time between collection and analysis.

The sample should be collected after a minimum of 2 days and a maximum of 7 days of sexual abstinence. If additional samples are required, the number of days of sexual abstinence should be as constant as possible at each visit.

The man should be given clear written and spoken instructions concerning the collection of the semen sample. These should emphasize that the semen sample needs to be complete and that the man should report any loss of any fraction of the sample.

The following information should be recorded on the report form: the man's name, birth date, and personal code number, the period of abstinence, the date and time of collection, the completeness of the sample, any difficulties in producing the sample, and the interval between collection and the start of the semen analysis.

The sample should be obtained by masturbation and ejaculated into a clean, wide-mouthed container made of glass or plastic, from a batch that has been confirmed to be non-toxic for spermatozoa.

The specimen container should be kept at ambient temperature, between 20 °C and 37 °C, to avoid large changes in temperature that may affect the spermatozoa after they are ejaculated into it. It must be labeled with the man’s name and identification number, and the date and time of collection.

The specimen container is placed on the bench or in an incubator (37 °C) while the semen liquefies.

Note in the report if the sample is incomplete, especially if the first, sperm-rich fraction may be missing. If the sample is incomplete, a second sample should be collected, again after an abstinence period of 2–7 days.

Appendix D

Semen analysis

The semen analysis report shows the various semen parameters which include the following; liquefaction, semen viscosity, semen volume, sperm count, sperm concentration, sperm motility, sperm morphology, PH, and if there are any inflammatory cells.

Liquefaction

Within a few minutes at room temperature, the semen usually begins to liquefy (become thinner), at which time a heterogeneous mixture of lumps will be seen in the fluid. As liquefaction continues, the semen becomes more homogeneous and quite watery, The complete sample usually liquefies within 15 minutes at room temperature, although rarely it may take up to 60 minutes or more.

Semen viscosity

After liquefaction, the viscosity of the sample was estimated by gently aspirating it into a wide-bore (approximately 1.5 mm diameter) plastic disposable pipette, allowing the semen to drop by gravity and observing the length of any thread. A normal sample leaves the pipette in small discrete drops. If viscosity is abnormal, the drop will form a thread more than 2 cm long.

Semen volume

The volume of the sample directly took by reading the graduation that is present on the plastic container in which the sample is found.

Semen pH

After Mixing the semen sample well, a drop of semen was spread evenly onto the pH paper. Waited for the color of the impregnated zone to become uniform (<30 seconds). And then Compared the color with the calibration strip to read the pH.

For normal samples, pH paper in the range of 6.0 to 10.0 should be used.

Initial microscopic investigation

By using a phase-contrast microscope at a total magnification of ×100 the sample was read and the following data were determined:

The total number of sperm, sperm concentration, sperm motility, sperm morphology, and if there are any cells other than spermatozoa (pus cells, bacteria, and candida).

Appendix E

Definition of Key Terms and Concepts

Being overweight is a possession of extra weight that is un-proportional to height and age. It is the body weight falling above the range associated with minimum mortality (Sharkey, 1997).

Obesity refers to much higher body fat percentages than that considered normal for age and sex. It can also be termed as the condition of having an excess of non-essential body fat, Body Mass Index (BMI) above 30 (Sharkey, 1997).

Body mass index (BMI) refers to a measure of relative body weight that takes height into account and is correlated with direct measures of body fat (Sharkey, 1997).

Hypertension refers to sustained abnormally high blood pressure (Insel and Roth, 2002).

A stroke is an impeded blood supply to the brain-destroying brain cells (Johnson, 2002).

Diabetes mellitus is a disease that causes a building of glucose in the bloodstream. It is associated with kidney failure, nerve damage, blood circulation problems, and blindness (Ekblom, 2005).

Sleep apnea is the most common sleep-related breathing disorder. It causes you to repeatedly stop and start breathing while you sleep (Jameson *et al*., 2018).

Varicocele is an enlargement of the veins within the loose bag of skin that holds the testicles (scrotum). These veins transport oxygen-depleted blood from the testicles. A varicocele occurs when blood pools in the veins rather than circulating efficiently out of the scrotum. (Partin *et al*., 2020).

Hypogonadism is a condition in which the male testes or the female ovaries produce little or no sex hormones (Melmed *et al*., 2019).

Hypogonadotropic hypogonadism is a form of hypogonadism that is due to a problem with the pituitary gland or hypothalamus (Melmed *et al*., 2019).

Gonadal dysgenesis is a disorder of sex development resulting from a mutation or deletion of a gene upstream from SOX9 (a transcription factor essential in Sertoli cell development) in the presence of a Y chromosome (or that portion of the Y chromosome including the TSPY locus) (Cheng *et al.*,2020).

Cryptorchidism an undescended testicle (testis) is when it fails to drop into the normal place in the scrotum (Partin *et al*., 2020).

DNA fragmentation index (DFI) is a percentage of the spermatozoon with high single- or double-strand breaks in nuclear DNA, in a semen sample (Panner *et al*., 2020).

Appendix F

Delimitations and Limitations of the Study

This study may lack relevance to represent some country patterns because there are many social classes and geographical locations that were not represented. However, for a centralized education system and life patterns of the Libyans, results may be feasible for generalization. On the other hand, the study faced impediments caused by the highly limited studies on overweight and obesity among infertile men in Libya and developing countries. Many of the studies on overweight and obesity were for women. This led to a shortage of information sources, whether books or journals.

Appendix G

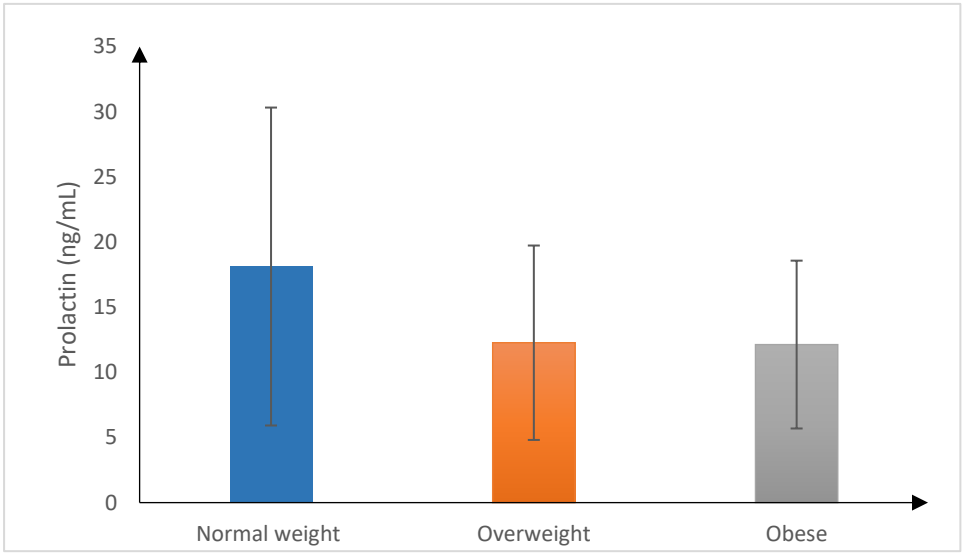

Figure (3): Association between BMI and prolactin among the study participants.

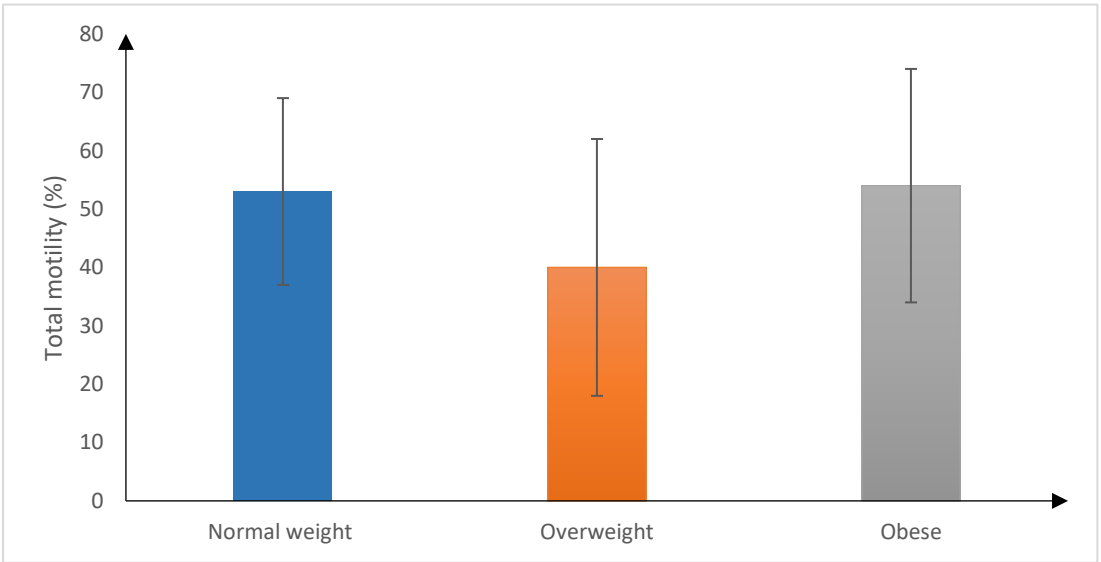

Figure (4): Association between BMI and total sperm motility among the study participants.

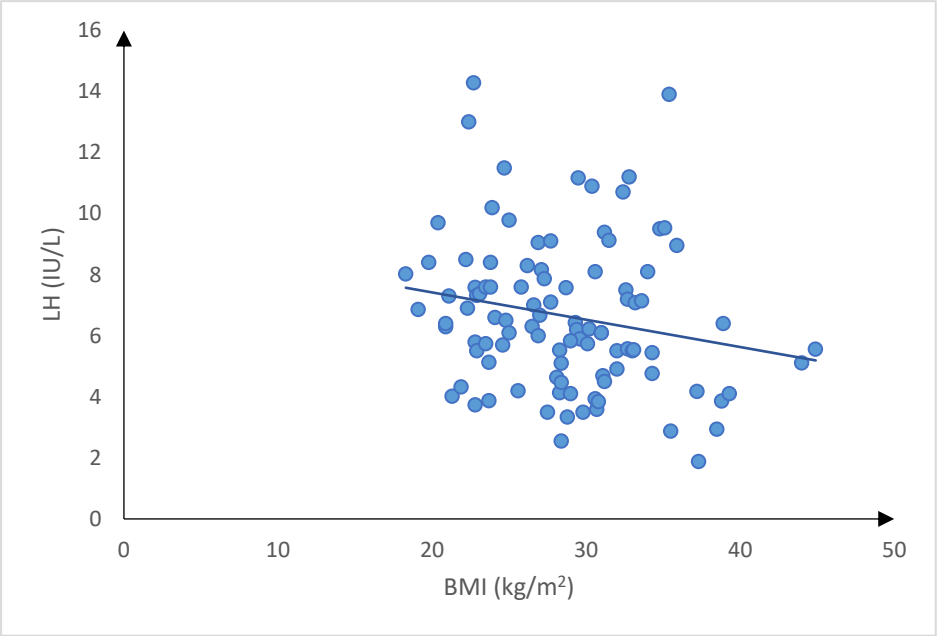

Figure (5): Correlation between BMI and LH among the study participants.

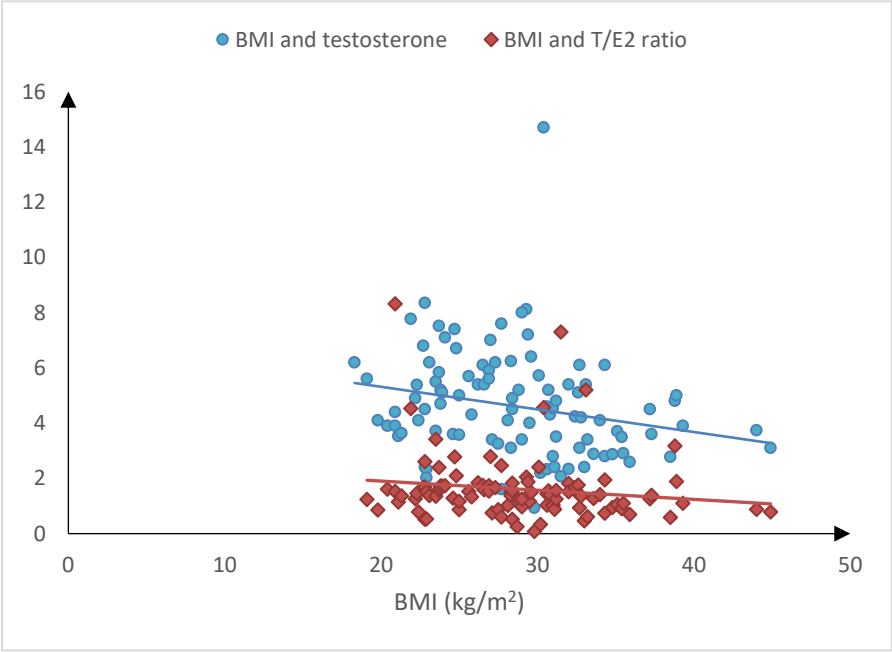

Figure (6): Correlation between BMI and testosterone, T/E2 among the study participants.

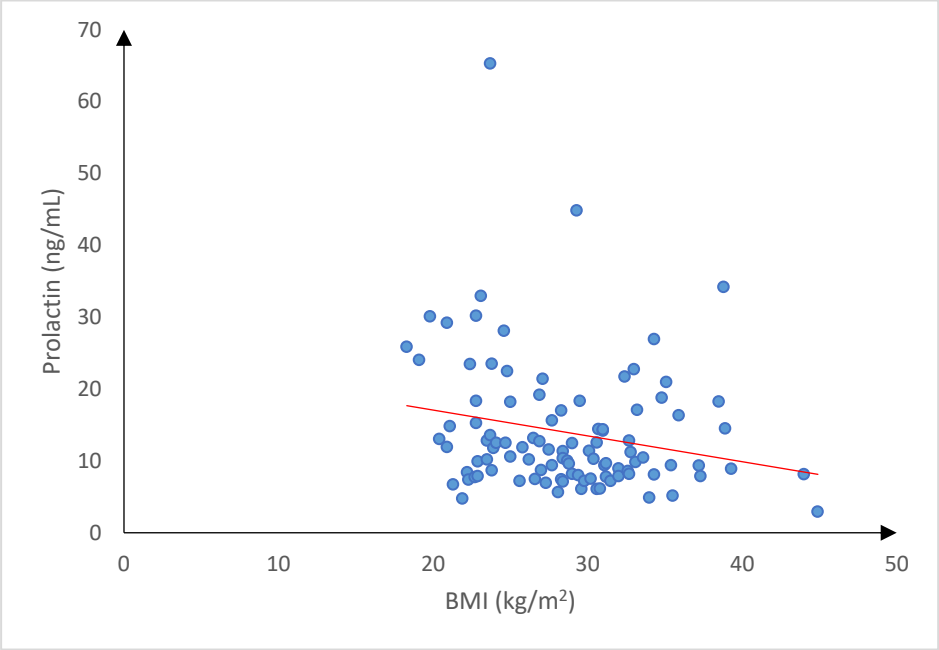

Figure (7): Correlation between BMI and prolactin among the study participants

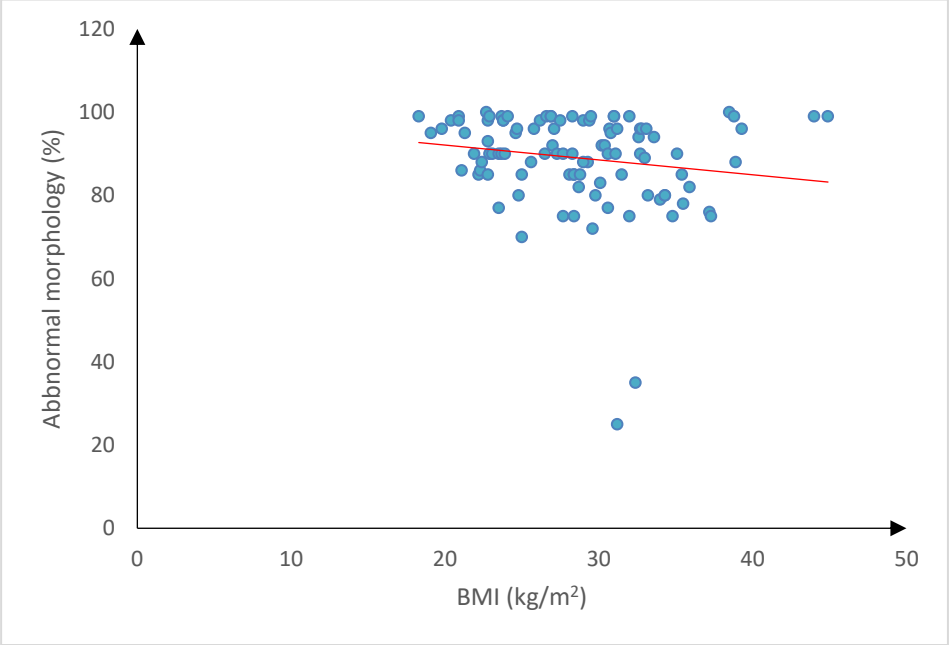

Figure (8): Correlation between BMI and abnormal sperm morphology among the study participants.

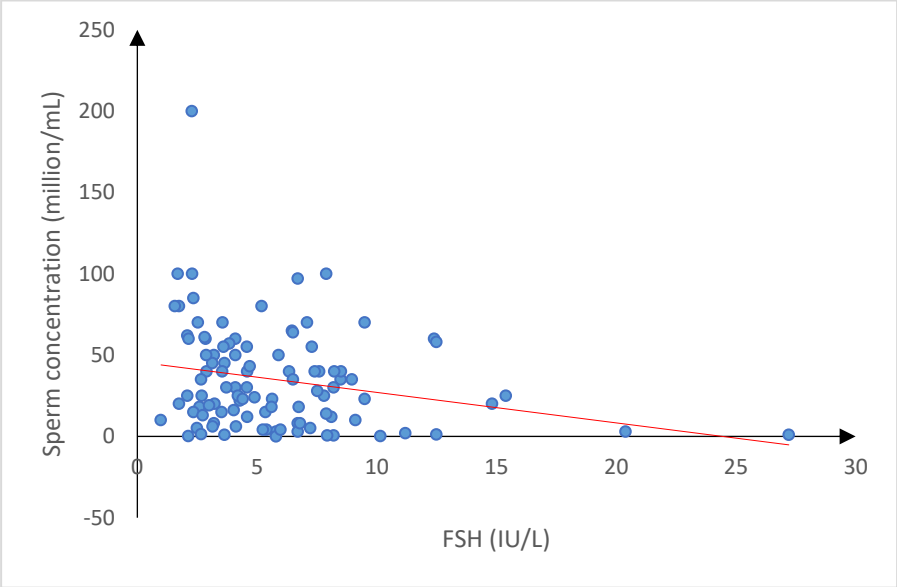

Figure (9): Correlation between FSH and sperm concentration among the study participants.

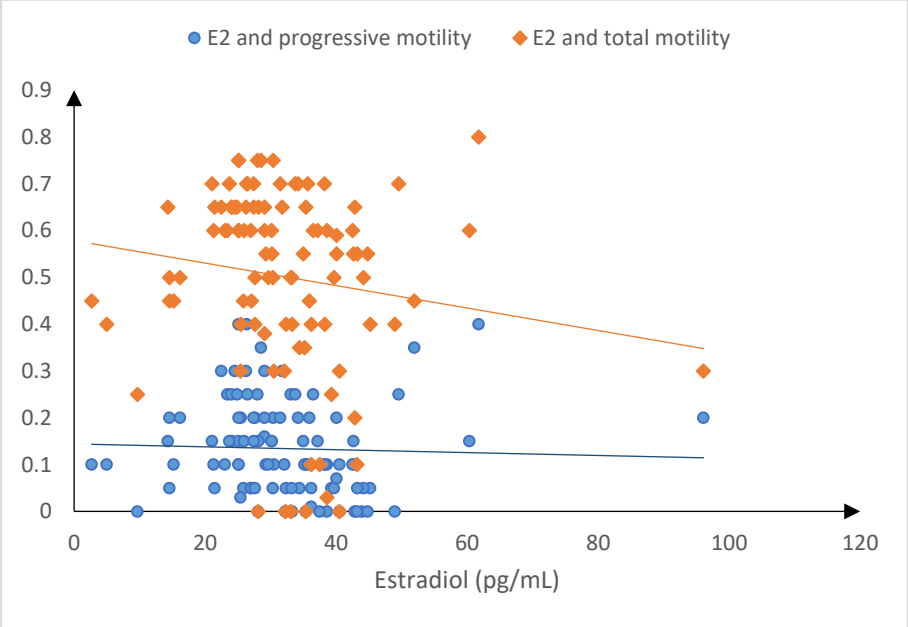

Figure (10): Correlation between estradiol and sperm motility among the study participants.

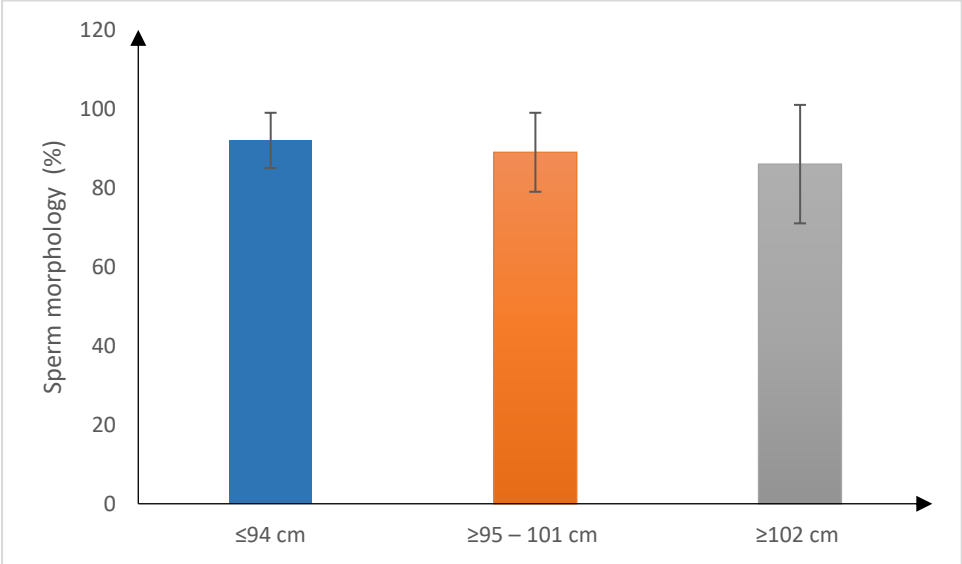

Figure (11): Association between WC and sperm morphology among the study participants.

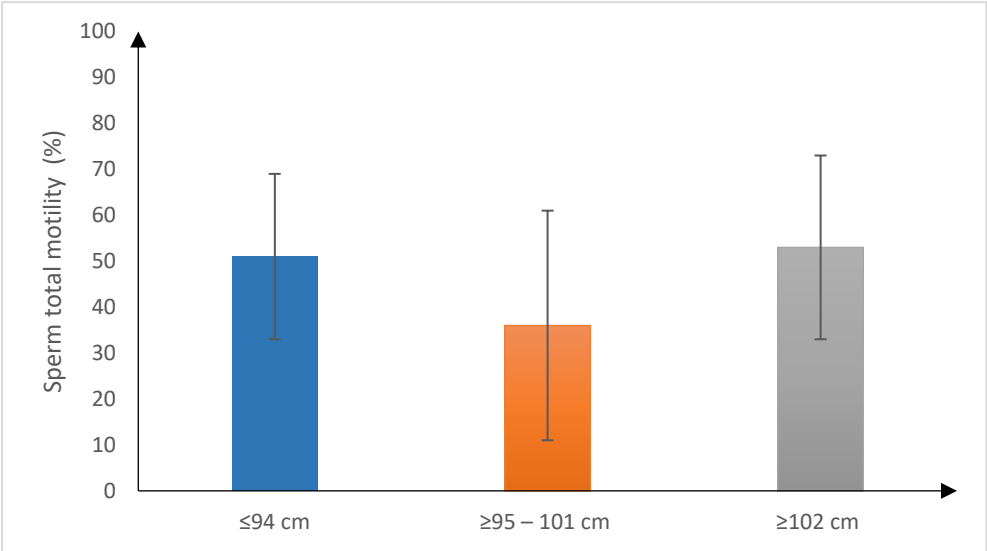

Figure (12): Association between WC and sperm total motility among the study participants.

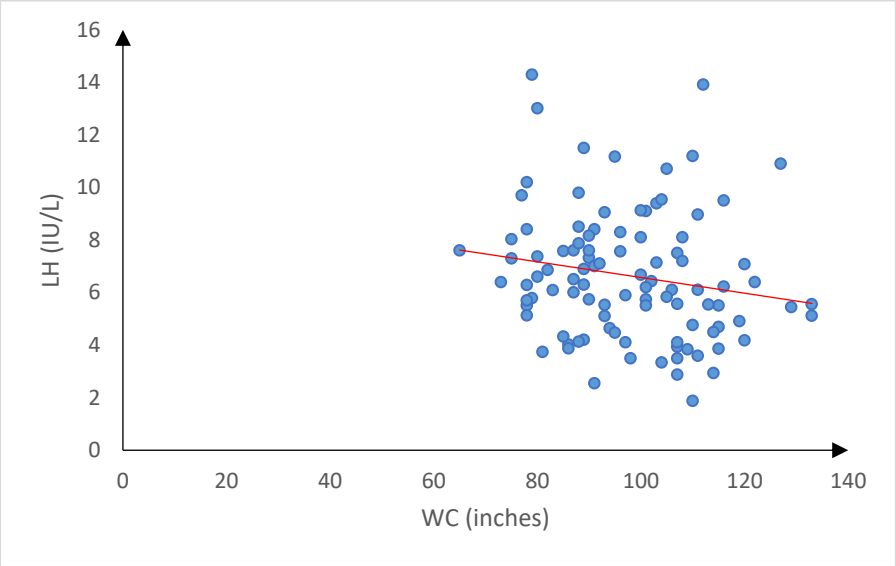

Figure (13): Correlation between WC and LH among the study participants.

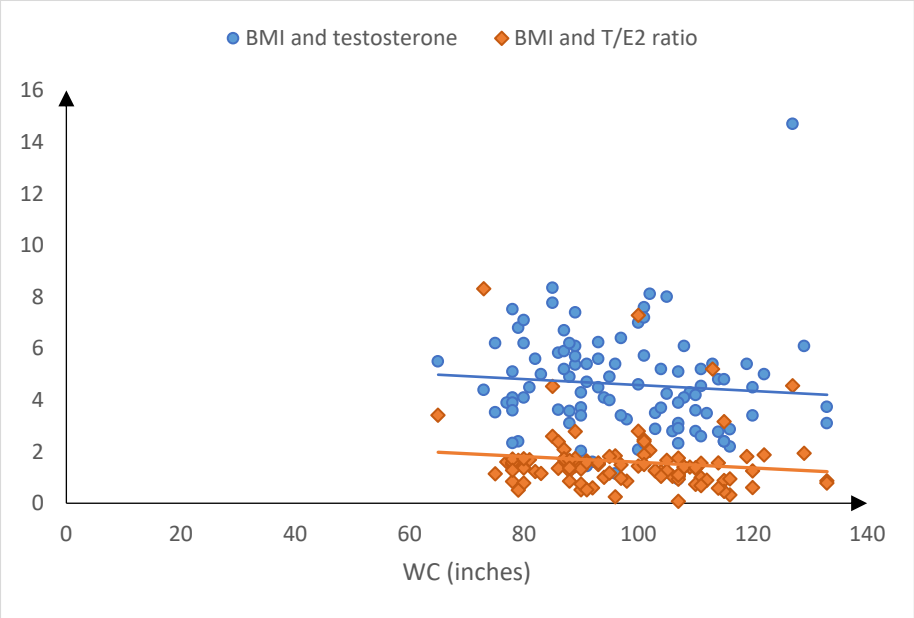

Figure (14): Correlation between WC and testosterone, T/E2 among the study participants.

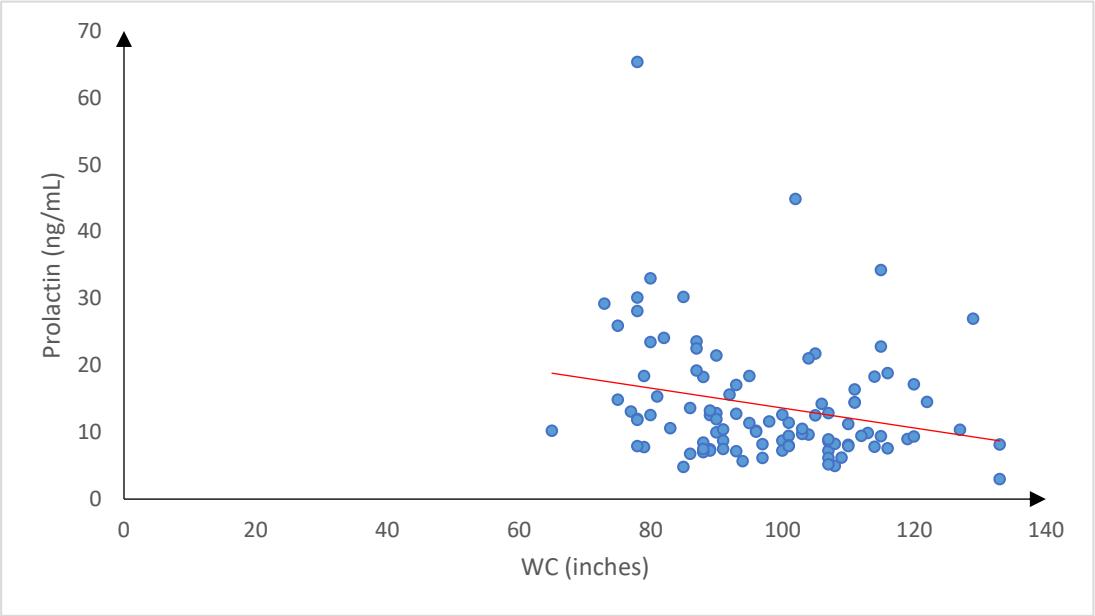

Figure (15): Correlation between WC and prolactin among the study participants.