Risk of obesity on male fertility in Gaza Governorate, Gaza Strip

خطورة السمنة على الخصوبة عند الرجال في قطاع غزة

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بسم الله الرحمن الرحيم

{ قل هل تستوى الذين يعلمون والذين لا يعلمون }

(9) الزمر...
Declaration

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Dedication

This work is dedicated to:

The dearest people to my heart, my mother and father, who have always supporting me

and my family members

The dearest to me; my wife, children : Zyaid, Maram,
Abdula and Ahmad
brothers and sisters

Dedication is almost expressed to the Palestinian people who have suffered and will be struggled with the persistence to have a free Palestine.
And all researchers who are working to improve the quality of life

Tarik Z. El-Refi
Acknowledgment

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At the end, I am very grateful to those who participated and helped me to complete this study.
Risk of obesity on male fertility in Gaza Governorate, Gaza Strip

Abstract

Obesity is a multifactorial disease characterized by an excess of adipose tissue. By 2015, approximately 700 million adults will be obese worldwide. Obesity affects endocrine and exocrine functions of male reproductive system. However, the underlying mechanisms of obesity-induced male infertility have not been fully understood.

Objective: To assess the risk of obesity on male fertility in Gaza Governorate, Gaza Strip.

Materials and Methods: This case-control study comprised 80 obese men (mean BMI=33.4±2.5 Kg/m²) and 80 healthy normal weight men (mean BMI=22.8±1.5 Kg/m²). Questionnaire interview was applied. Cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), total testosterone, follicular stimulation hormone (FSH), luteinizing hormone (LH), sperm concentration, sperm motility and sperm morphology were determined. Data were analyzed using SPSS version 18.0.

Results: Unemployment and low family income were more frequent among cases compared to controls (P=0.019 and P<0.001, respectively). Serum cholesterol, triglycerides and LDL-C were found to be higher in cases (212.6±42.4, 230.9±30.7 and 121.7±32.8 mg/dl) compared to controls (127.9±38.4, 113.8±35.6 and 93.9±22.9 mg/dl) with % differences of 49.8, 67.9 and 25.8 and P<0.001, respectively. Serum testosterone was significantly decreased in cases compared to controls (3.6±1.5 vs. 6.3±0.8 ng/ml, P<0.001), with percentage difference of 54.5%. On the other hand, serum LH and FSH were significantly increased in cases (9.7±5.3 and 12.3±6.4 mIU/ml vs. 4.7±2.6 and 4.6±1.3 mIU/ml, P=0.001, respectively), showing percentage differences of 69.4% and 91.1% respectively. There were significant decrease in semen volume, count and total count in cases (2.2±0.9 ml, 7.8±5.2 million/mL and
17.6±21.6 million) compared to controls (3.1±0.6 ml, 43.7±16.0 million/mL and 135.3±55.8 million). This decrease was significant (P<0.001), with percentage differences of 34.0, 139.5 and 15.4, respectively). Sperm total motility, fast progressive and slow progressive grades of motility were significantly decreased in cases (28.7±14.8, 0.23±1.27 and 18.7±11.6 vs. 57.7±11.2, 18.9±5.4 and 28.7±7.5 with % difference = 67.0, 195.3 and 42.1, P<0.001, respectively). On the other hand, the immotile sperms were significantly higher in cases than in controls (71.3±14.8 vs. 42.4±11.2, % differences=50.9 and P<0.001). The normal forms of sperms were lower in cases (25.9±4.7 vs. 39.0±4.6, % difference=40.5, P<0.001). Body mass index was positively correlated with cholesterol, triglyceride, LDL, LH, FSH, sperm immotility and abnormal sperm (r=0.737, r=0.836, r=0.493, r=0.445, r=0.492, r=0.738 and r=0.759, respectively and P<0.001). on the other hand, total testosterone, semen volume, sperm count, total sperm count, total motility, fast progressive and slow progressive grades of motility, and normal sperm were inversely correlated with BMI (r=-0.755, P<0.001; r=-0.465, P<0.001; r=-0.774, P<0.001; r=0.742, P<0.001; r=-0.738, P<0.001; r=-0.856, P<0.001; r=-0.494, P<0.001 and r=-0.759, P<0.001, respectively).

**Conclusions:** Obesity has a negative impact on male reproductive hormones as well as on semen quantity and quality.

**Keywords:** Obesity, male reproductive hormones, semen parameters, Gaza strip.
خطورة السمنة على الخصوبة عند الرجال في قطاع غزة

ملخص الدراسة

مقدمة:

السمنة هي مرض متعدد العوامل التي تتميز بوجود فائض من الأنسجة الدهنية. وبحلول عام 2015، حوالي 700 مليون من البالغين سوف يصابون بالسمنة في العالم. السمنة تؤثر على الغدد الصماء ووظائف إفرازات الجهاز التناسلي الذكرى. ومع ذلك، لم تتضح تماما الألياف الكامنة وراء السمنة التي تسهمها العقم عند الذكور.

الهدف:

تقييم مخاطر السمنة على خصوبة الرجال في قطاع غزة.

الطرق والأدوات:

الدراسة نوع من دراسة الحالات والشواهد. وتشتمل على 80 رجلاً يعانون من السمنة المفرطة (المجموعة المرضية) (متوسط مؤشر كلفة الجسم = 43.4 ± 33.4 كجم/م2) و 80 رجلاً من الأصحاء ذوي الوزن الطبيعي (المجموعة الضابطة) (متوسط مؤشر كلفة الجسم = 22.8 ± 15.1 كجم/م2). تمت المقابلة وتعيين الاستبيان. ومن ثم تم تحديد الكولسترول والدهون الثلاثية والكولسترول عالي الكثافة (HDL-C) والكولسترول منخفض الكثافة (LDL-C) والمستيروفون وهرمون (FSH) وهرمون (LH) وعدد الحيوانات المنوية وحركتها وأشكالها. وكذلك تم تحليل البيانات والنتائج التي تم الحصول عليها باستخدام البرنامج الإحصائي SPSS.

النتائج:

كانت البطالة وانخفاض دخل الأسرة أكثر تواتراً بين المجموعة المرضية مقارنة بالمجموعة الضابطة ذات دلالة إحصائية (P<0.001 وP<0.019) على التوالي. ولقد وجد أن الكولسترول والدهون الثلاثية والكولسترول منخفض الكثافة (LDL-C) مرتفعاً في الحالات المرضية (دلالة إحصائية) (212.6 ± 42.4 و 230 ± 30.7) والكولسترول عالي الكثافة (HDL-C) المنخفض (38.4 ± 35.6 و 93.9 ± 113.4) بالمقارنة مع المجموعة الضابطة (127.9 ± 32.8 و 121.7 ± 22.9) على التوالي. ونسبة الفرق = 49.8% و 67.9% و 52.8% و 25.8% على التوالي وmg/dl. ولقد كان انخفاض هرمون الفستيروفون في الحالات بالمقارنة مع الضوابط ذات دلالة إحصائية (P<0.001 وP<0.019) ونسبة الاختلاف = 15.1 ± 3.6 و 10.9% ± 6.3 ng/ml في الحالات قد أعطى دلالة إحصائية (P<0.001 وP<0.019) ونسبة الفرق = 9.7% و 5.3% على التوالي. وتباين نسبة 69.4% و 91.1% على التوالي. كان هناك انخفاض كبير بحجم الحيوانات المنوية وتركيزها (million 21.6 ± 17.6 و 5.2 ± 7.8، ml 0.9 ± 2.2 و 0.6 ± 3.4 وmillion/mL 16.0 ± 43.7 و 50.6 ± 135.3). وكان هذا الانخفاض ذات دلالة إحصائية (P<0.001) مع وجود نسبة اختلافات (34.0% و 139.5% و 15.4% على التوالي). وانخفضت بشكل ملحوظ حركة الحيوانات المنوية السريعة والبطيئة في الحالات (P<0.001 وP<0.001 وP<0.001 وP<0.001) ونسبة الفرق = 28.7 ± 5.4% و 11.6 ± 2.2 ميلاً و 18.7 ± 1.23 ميلاً و 57.7 ± 4.5 ميلاً و 28.7 ± 14.8 لفicianships. VI
ومن جانب آخر، كانت الحيوانات المنوية الغير متحركة أعلى بكثير في الحالات مقارنة بالمجموعة الضابطة (71.3 ± 14.8 مقابل 42.4 ± 11.2، ونسبة الاختلاف بينهم 0.9 % وذات دلالة (P<0.001). وكانت أشكال الحيوانات المنوية الطبيعية أقل في الحالات (25.9 ± 4.7 مقابل 4.6 ± 4.6 % ونسبة الاختلاف = 40.5 %، وذات دلالة إحصائية (P<0.001). وجدت العلاقة إيجابية بين مؤشر كتلة الجسم والكولسترول والدهون الثلاثية والكولسترول منخفض الكثافة (LDL-C) وهرمون (LH) وهرمون (FSH) وحركة الاحياء المنوية والحيوانات المنوية الغير طبيعية (r = 0.493 و r = 0.759 و P<0.001). ونسبة الاختلاف = 40.5 %، وذات دلالة إحصائية (P<0.001).

ومن ناحية أخرى، كانت العلاقة عكسية بين هرمون التستوستيرون وحجم السائل المنوي وتركيز الحيوانات المنوية والعدد الكلي للحيوانات المنوية ونسبة الحيوانات المت약كة، وسرعة الحركة وبطيئة الحركة، والحيوانات المنوية الطبيعية مع مؤشر كتلة الجسم (BMI) (r = 0.755 و P<0.001) و (r = 0.742 و P<0.001) و (r = 0.738 و P<0.001) و (r = 0.736 و P<0.001) و (r = 0.738 و P<0.001) و (r = 0.738 و P<0.001) و (r = 0.738 و P<0.001) و (r = 0.738 و P<0.001) و (r = 0.738 و P<0.001) و (r = 0.738 و P<0.001) و (r = 0.738 و P<0.001).

التعليق:

السمنة لها تأثير سلبي على الهرمونات التناسالية الذكرية وكذلك على كمية ونوعية السائل المنوي.

الكلمات المفتاحية:

السمنة والهرمونات الجنسية الذكرية، معايير السائل المنوي، قطاع غزة.
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Chapter 1

Introduction

1.1 Overview

Obesity is a complex, multifactorial disease that develops from the interaction between genotype and the environment. It is characterized by an excess of adipose tissue (Kopelman, 2000 and Kantachuvessiri, 2005). The most commonly used measurement for determining obesity is the body mass index (BMI), which is calculated as the weight (kg)/height (m$^2$) (World Health Organization, WHO, 2000). The WHO estimates that at least 400 million adults (9.8%) are obese (WHO, 2009). Severe obesity in the United States, Australia, and Canada is increasing faster than the overall rate of obesity (Tjepkema, 2005; Sturm, 2007 and Howard et al., 2008). In Arab countries including Palestine the prevalence of obesity also recorded high rates (Musaiger et al., 2000 and Abdul-Rahim et al., 2004).

A combination of excessive food energy intake and a lack of physical activity are thought to explain most cases of obesity (Lau et al., 2007). Other cases are due primarily to genetics, medical reasons, or psychiatric illness (Bleich et al., 2008). In addition, increased reliance on cars, and mechanized manufacturing contributed to obesity (Nestle and Jacobson, 2000 and James, 2008). Obesity can lead to serious medical, social, physical and psychological problems (Mota and Zanesco, 2007). However, pathogenesis of obesity is complicated and not well known. The mortality rises exponentially with increasing body weight (Bovbjerg, 2008).

Fertility is the natural capability of giving life. As a measure, "fertility rate" is the number of children born per couple, person or population. The testes are the main male reproductive organ and are very important for the normal functioning of the male reproductive system. The regulation of the testis function is mediated primarily by two pituitary hormones under control of gonadotropin-releasing hormone (GnRH) from hypothalamus: Luteinizing
hormone (LH) and follicular stimulating hormone (FSH). LH stimulates leydig cells to produce testosterone and FSH acts on Sertoli cells to stimulate spermatogenesis (Zirkin and Chen, 2000; Weinbauer et al., 2001 and Haider, 2004).

Over the last decade, obesity is linked to human fertility. The effect of obesity on male reproduction has been less well studied than those on female reproduction, but there is a growing body of evidence suggesting that obesity has a negative impact on male reproduction (Filer, 2009). The underlying mechanisms of obesity-induced male infertility have not been fully understood. However, obesity effects manifest in both the endocrine and exocrine functioning of the male gonads in terms of impaired hormonal profile and abnormal semen quality (Hammoud et al., 2008; and Du Plessis et al., 2010).

Although obesity is prevalent in Gaza Strip, the available studies are limited and focused on determination of leptin, Insulin and thyroid stimulating hormones in obesity (Zabut et al., 2007 and AL-jedi, 2011). No previous study investigated the impact of obesity on male fertility. Therefore, the present work is the first study to assess the risk of obesity on male fertility in terms of hormonal profile and semen quality in Gaza Governorate, Gaza Strip.

1.2 General objective
To understand the risk of obesity on male fertility in Gaza Governorate, Gaza Strip.

1.3 Specific objectives
1. To measure total testosterone, luteinizing hormone (LH) and follicular stimulation hormone (FSH) levels in the study population
2. To determine semen parameter including semen volume, sperm concentration, sperm motility and sperm morphology of the study population.
3. To estimate lipid profile including cholesterol, triglyceride, high density lipoprotein (HDL) and low density lipoprotein (LDL) of the study population
4. To verify relationships of obesity with hormonal and semen parameters.

1.4 Significance
1. Obesity is prevalent globally as well as in Gaza Strip.
2. No previous study was carried out on the impact of obesity on male fertility in Gaza Strip.
3. Understanding the status of sex hormones and semen quality in obese males may enable us to put new strategy to improve male fertility.
4. Understanding the association of body mass index and lipid profile, sex hormones and semen quality in obese men
Chapter 2

Literature Review

2.1 Definition of obesity

Obesity is a condition in which excess body fat has accumulated to an extent that health may be negatively affected. Obesity is the consequence of an overall positive energy balance maintained over time, that is, the metabolizable energy intake exceeds the energy expenditure for basal metabolic requirements, thermoregulation, physical activity, and growth (Rosenbaum et al., 1997 and National Institutes of Health, 2004).

2.2 Assessment and classification of obesity

The initial step in evaluation of obesity is calculation of BMI. To measure BMI, one begins by weighing the patient in light clothes and no shoes. Height is measured without shoes. BMI is calculated by dividing weight (in kilograms), by square height (in meters). BMI has replaced percentage ideal body weight as a criterion for assessing obesity for several reasons. BMI correlates significantly with body fat, morbidity and mortality, and it can be calculated quickly and easily. Furthermore, recommendations for treatment of obesity are based on BMI. A BMI of 25 kg/m² is the generally accepted threshold for identifying a patient at higher risk for obesity-related diseases, most notably type 2 diabetes, hypertension, and cardiovascular disease (Lyznicki et al., 2001).

Medical risk rises progressively with increasing degrees of obesity beginning with overweight, defined by BMI between 25.0 and 29.9 kg/m², through class I obesity (BMI, 30.0 to 34.9 kg/m²), class II obesity (BMI, 35.0 to 39.9 kg/m²), and class III or morbid obesity (BMI>40 kg/m²) (Hirsch et al., 2001). This classification system of obesity by BMI was developed by the World Health Organization Obesity Task Force (Table 2.1) and has been adopted by researchers on the identification, evaluation and treatment of overweight and obesity in adults (WHO, 2000).
Table 2.1. Different classes of body mass index (BMI), *(WHO, 2000)*.

<table>
<thead>
<tr>
<th>BMI groups</th>
<th>BMI (kg/m²)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight</td>
<td>&lt;18.5</td>
</tr>
<tr>
<td>Normal</td>
<td>18.5-24.9</td>
</tr>
<tr>
<td>Overweight</td>
<td>25.0-29.9</td>
</tr>
<tr>
<td>Obese I</td>
<td>30.0-34.9</td>
</tr>
<tr>
<td>Obese II</td>
<td>35.0-39.9</td>
</tr>
<tr>
<td>Extreme obesity</td>
<td>≥ 40</td>
</tr>
</tbody>
</table>

*(kg/m²): Kilogram / meter²*

2.3 Prevalence of obesity

The prevalence of obesity has reached alarming levels, affecting virtually both developed and developing countries. From 1976-1980 to 2005-2006, the prevalence of obesity increased from 13.4 to 35.1% in US adults age 20 to 74 *(National Center for Health Statistics, 2008)*. In European countries the prevalence of obesity has increased by about 10-40% in the majority of countries in the last decade. In Saudi Arabia, the prevalence of obesity rates at 23.6% in women and 14.2% in men, compared to 28.4% overweight females and 30.7% overweight males *(Othaimen et al., 2007)*. Obesity among Kuwaitis was found to be higher than reported elsewhere in the world (70.2% grade 1) and (36.4% grade 2) *(Al-Isa, 1995)*. In Northern Jordan, the prevalence of obesity was 28.1% for men and 53.1% for women *(Khader et al., 2008)*. The prevalence of obesity in Syria was higher in women (46.3%) than men (28.4%) *(Fouad et al., 2006)*. In urban Palestinian population the prevalence of obesity was high, 30% for men and 49% for women *(Abdul-Rahim et al., 2004)*. Finally, statisticians have predicted that, by 2015, approximately 2.3 billion adults will be overweight and 700 million will be obese *(WHO, 2009)*.
2.4 Risk factors of obesity

2.4.1 Diet

Consumption of an energy intake that is inappropriately large for a given energy expenditure leads to obesity. Overeating of certain specific dietary components may also lead to health risks. Obvious examples are saturated and trans-fatty acids. More recently attention has switched to high glycemic foods and to n-6 fatty acids (Prentice, 2001). In the last years, an increase occurred in the average amount of food energy consumed in US. For women, the average increase was 335 calories per day (1,542 calories in 1971 and 1,877 calories in 2004); while for men the average increase was 168 calories per day (2,450 calories in 1971 and 2,618 calories in 2004). Most of this extra food energy came from an increase in carbohydrate consumption rather than fat consumption (Wright et al., 2004). The primary sources of these extra carbohydrates are sweetened beverages, which now account for almost 25% of daily food energy in young adults in America (Caballero, 2007) and potato chips (Mozaffarian, 2011). Consumption of fast-food and sweetened drinks is believed to be contributing to the rising rates of obesity (Malik et al., 2006; Olsen and Heitmann, 2009 and Rosenheck, 2008). In Europe and UK, agricultural policy and techniques led to lower price of processed food compared to fruits and vegetables (Pollan and Michael, 2007). In the Arab World, the spread of the Western lifestyle, defined as "the intake of attractive energy dense food with undesirable composition, increased consumption of animal fats and sugars and reduced consumption of dietary fiber, along with a lack of sufficient physical activity", is one of the leading causes (Kelishadi and Roya, 2003).

2.4.2 Physical activity

One of the major contributing factor of obesity is lack of exercise or sedentary lifestyle. According to the center for disease control and prevention (CDC) being overweight and obese result from an energy imbalance caused by eating too many calories and not getting enough exercise (CDC, 2009). Worldwide
there has been a large shift towards less physically demanding work (Ness-Abramof, 2006 and WHO, 2009) and currently at least 60% of the world's population gets insufficient exercise (WHO, 2009). This is primarily due to increasing use of mechanized transportation and a greater prevalence of labor-saving technology in the home (Ness-Abramof, 2006 and WHO, 2009). In children, there appear to be declines in levels of physical activity due to less walking and physical education (Salmon and Timperio, 2007). World trends in active leisure time physical activity are less clear. The World Health Organization indicates people worldwide are taking up less active recreational pursuits, while a study from Finland (Borodulin et al., 2008) found an increase and a study from the United States found leisure-time physical activity has not changed significantly (Brownson et al., 2005). In both children and adults, there is an association between television viewing time and the risk of obesity (Vioque et al 2000 and Spence-Jones, 2003). A 2008 meta-analysis found 63 of 73 studies (86%) showed an increased rate of childhood obesity with increased media exposure, with rates increasing proportionally to time spent watching television (Emanuel, 2008).

2.4.3 Genetics

Like many other medical conditions, obesity is the result of interplay between genetic and environmental factors. Polymorphisms in various genes controlling appetite and metabolism predispose to obesity when sufficient calories are present. The percentage of obesity that can be attributed to genetics varies widely, depending on the population examined, from 6% to 85% (Yang et al., 2007). As of 2006, more than 41 sites on the human genome have been linked to the development of obesity when a favorable environment is present (Poirier et al., 2006). Some studies have focused upon inheritance patterns without focusing upon specific genes. One study found that 80% of the offspring of two obese parents were obese, in contrast to less than 10% of the offspring of two parents who were of normal weight (Kolata and Gina., 2007) A commonly quoted genetic explanation for the rapid rise in obesity is the thrifty gene hypothesis postulates that certain ethnic groups may be more
prone to obesity in a given homogeneous environment. In the past their ability to take advantage of rare periods of abundance by storing energy as fat would have been advantageous in an environment of varying food availability. Individuals with greater adipose reserves would be more likely to survive a famine. This tendency to store fat, however, seems be a disadvantage in modern societies with a stable food supply (Chakravarthy and Booth, 2004). Numerous studies provided strong evidence that genetics plays an important role in obesity (Garland et al., 2011).

2.4.4 Other causes

Medical illnesses that increase obesity risk include some congenital or acquired conditions: hypothyroidism, Cushing's syndrome, growth hormone deficiency, (Rosén et al., 1993) and the eating disorders: binge eating disorder and night eating syndrome. Certain medications may cause weight gain or changes in body composition; these include insulin, sulfonylureas, thiazolidinediones, atypical antipsychotics, antidepressants, steroids, certain anticonvulsants (phenytoin and valproate), pizotifen, and some forms of hormonal contraception (Haslam and James, 2005). Psychological factors may also contribute to the development of obesity (Aronne, 2001). An association between viruses and obesity has been found in humans and several different animal species. The amount that these associations may have contributed to the rising rate of obesity is yet to be determined (Falagas and Kompotis, 2006). There is an indication that gut flora in obese and lean individuals can affect the metabolic potential. This apparent alteration of the metabolic potential is believed to confer a greater capacity to harvest energy contributing to obesity. Whether these differences are the direct cause or the result of obesity has yet to be determined unequivocally (DiBaise et al., 2008).

2.5 Male fertility

Fertility is the natural capability of producing offspring's. As a measure, "fertility rate" is the number of children born per couple, person or population. The primary reproductive organs of the male are the testes, or male gonads, which
have both an exocrine (sperm producing) function and an endocrine (testosterone producing) function. The accessory reproductive structures are ducts or glands that aid in the delivery of sperm to the body exterior or to the female reproductive tract.

2.5.1 Structure of testis

The testis consists of convoluted seminiferous tubules embedded in a connective tissue matrix that called interstitium (Figure 2.1). The interstitium contains mixture of blood and lymph vessels, nerves, fibroblast cell, macrophages and Leydig cells. The epithelium of seminiferous tubules consists of continually dividing germ cells that produce sperm cells and supporting Sertoli cells. The sperm travel from the seminiferous tubules to the rete testis located in the mediastinum testis, to the efferent ducts, and then to the epididymis where newly-created sperm cells mature. The sperm move into the vas deferens, and are eventually expelled through the urethra and out of the urethral orifice through muscular contractions (Krohmer, 2004).

Figure 2.1. cross-section of the testis showing sperm producing tube (seminiferous tubule) and leydig cell (Ohl et al., 1996).
2.5.2 Sperm formation (spermatogenesis)
Spermatogenesis represents the entire process of germ cell development within the seminiferous epithelium of the adult testis. It can be divided into four phases and includes:

2.5.2.1 Proliferation and differentiation of spermatogonia
Spermatogonia are the diploid stem cells of spermatogenesis, and can be divided into type A and type B (Figure 2.2). Type A spermatogonia are further divided into A1-A4 spermatogonia (Steger et al., 1998). Type B spermatogonia are able to differentiate and enter the process of meiosis.

2.5.2.2 Spermatocytes/Meiosis
Primary and secondary Spermatocytes
Primary spermatocytes undergo the first meiotic division. The prophase of the first meiotic division takes about 1–3 weeks and is divided into several stages: the leptotene, zygotene, pachytene and diplotene stages. Secondary spermatocytes undergo the second meiotic division after a short interphase of about 6 h in the human without DNA synthesis. By this division, chromatids are finally separated leading to round spermatids with a haploid n (Kierszenbaum, 2002).

2.5.2.3 Spermatids/Spermiogenesis
The transformation of conventional round cell spermatids into spermatozoa with the capacity for motility and fertilization of an egg includes a complex sequence of events: 1) formation of the acrosome, 2) condensation of the nucleus, 3) development of the sperm tail, 4) reorganization of cellular organelles such as mitochondria and centrioles and 5) reduction of the cytoplasm (Bermudez et al., 1994 and Steger et al., 2003).
Figure 2.2 Sperm formation (spermatogenesis) (Steger et al., 1998).
2.5.2.4 Spermatozoon

The length of the human spermatozoon measures about 60 μm. The flat and oval head (diameter: 3 μm, length: 5 μm) consists of the acrosome and the extremely condensed nucleus (Figure 2.3). The acrosome covers the head surface, and contains numerous proteolytic enzymes. The release of these enzymes, the so-called acrosome reaction, enables the spermatozoon to penetrate the “corona radiata” of follicle cells and the zona pellucida of the egg. The flagellum measures about 55 μm in length. It possesses the central axoneme and is divided into (Henkel et al., 1994):

* The neck/connecting piece (1 μm) is the point of articulation between the sperm head and the flagellum.
* The mid-piece (6 μm) contains the mitochondria and the nine doublets of microtubules, which are associated with outer dense fibers. Outer dense fibers are believed to maintain the passive elastic structure for flagellar bending and also to protect it from shearing forces during epididymal transit and ejaculation (Baltz et al., 1990).
* The principal piece (45 μm) is a fibrous sheet.
* The end-piece (5 μm) only contains microtubules. Spermatozoa acquire motility during epididymal passage and their competence for fertilization during the passage of the female genital tract (capacitation).

Figure 2.3 Schematic drawing of the human spermatozoon (Henkel et al., 1994).
2.6 Semen parameters

2.6.1 Semen volume
Normal ejaculate volume is between 2 and 6 ml. 65% of the volume is from seminal vesicles, 30-35% is from the prostate and only 5% from the vasa. Low volume is associated with absence or decrease of seminal vesicle component of ejaculate or retrograde ejaculation (Irvine and Aituen 1994).

2.6.2 Sperm concentration
Sperm concentration refers to the number of sperm found in a measured quantity of semen. Normospermic specimen contains more than 20x10^6 sperms (World Health Organization, WHO, 1992).

2.6.3 Sperm motility
Sperm motility refers to the swimming capabilities of the sperm. If sperm cannot swim properly, they may have trouble reaching the egg and fertilizing it. Normally >50% of sperm in the specimen are motile (Irvine, 1995).

2.6.4 Sperm morphology
Sperm morphology refers to the shape of sperm. Oddly shaped sperm may not swim well enough to get to the egg and may not be able to fertilize an egg. Strict criteria for normal sperm morphology include (Bernstein et al., 1995):
- Sperm head: Smooth oval configuration. Length: 5-6 microns. Width: 2.5-3.5 microns.
- Acrosome comprises 40-70% of the anterior sperm head.
- Midpiece: Axially attached, 1.5 times the head length, ≤1μm in width.
- Tail: Straight, uniform, slightly thinner than the midpiece, uncoiled, ±45μm long.

2.7 Male fertility hormones

2.7.1 Testosterone
Testosterone is a male sex hormone, secreted by Leydig or interstitial cells of the testes in the male and by the follicular theca and interstitial cells of the
ovaries in the female. Testosterone secretion is regulated by negative feedback of testosterone on the release of LH from the pituitary gland. Testosterone is highly protein-bound. In males, 98% of the testosterone in circulation is bound; the value is slightly lower in females. The majority of the steroid is bound to a specific binding protein, sometimes referred to as sex hormone binding globulin (SHBG) or testosterone binding globulin (TeBG), and to serum albumin (Dunn et al., 1981).

2.7.2 Follicle stimulating hormone (FSH) and luteinizing hormone (LH)

Human FSH is a glycoprotein of approximately 30,000 daltons which, like LH, human chorionic gonadotropin (hCG) and thyroid stimulating hormone (TSH, thyrotropin), consists of two noncovalently associated subunits designated α and β (Pierce and Parsons, 1981). The α subunit of FSH contains 92 amino acids and is very similar to the α subunits of LH, hCG, and TSH. The β subunit of FSH is unique and confers its immunological and functional specificity. FSH and LH control growth and reproductive activities of the gonadal tissues (Catt and Pierce, 1978 and Daughaday, 1985). FSH promotes follicular development in the ovary and gametogenesis in the testis (Franchimont, 1973 and Catt and Pierce, 1978). The gonadotroph cells of the anterior pituitary secrete both FSH and LH in response to gonadotropin releasing hormone (LHRH or GnRH) from the medial basal hypothalamus (Bonnar et al., 1973). Both FSH and LH are secreted in a pulsatile manner, with rapid fluctuations over the normal range (Catt and Pierce, 1978 and Crowley et al., 1987 and Beastall et al., 1987). The pulsatility of FSH is less pronounced than that of LH. Release of both FSH and LH from the pituitary is under negative feedback control by the gonads (Bonnar et al., 1973).

In males, FSH, LH, and testosterone regulate spermatogenesis by the Sertoli cells in the seminiferous tubules of the testes. FSH is less sensitive to feedback inhibition by testosterone than is LH and is thought to be regulated independently by the inhibitory peptide inhibin produced by the Sertoli cells (Jeffcoate, 1975 and Griffin and Wilson, 1985). Because of the negative feedback mechanisms regulating gonadotropin release, elevated
concentrations of LH and FSH are indicative of gonadal failure when accompanied by low concentrations of the gonadal steroids. In males, these observations suggest primary testicular failure or anorchia. FSH may also be elevated in Klinefelter’s syndrome (seminiferous tubule dysgenesis) or as a consequence of Sertoli cell failure (Franchimont, 1973).

2.8 Interrelationships between the hypothalamus, pituitary and testis

The successful initiation of testicular function is dependent on the hypothalamic secretion of GnRH which in turn stimulates FSH and LH to act on the testis (Schanbacher, 1982). (Figuer 2.4)

![Figuer 2.4 The hypothalamic–pituitary–gonadal axis in men.](image-url)

(Dandona and Rosenberg, 2010).
These actions initiate spermatogenesis and testosterone production. The testis in turn, through the secretion of hormones produced in the Sertoli and Leydig cells, exerts a negative feedback control on the production of gonadotrophins (Griswold, 1998).

2.8.1 Control of LH secretion
There is a substantial body of evidence to indicate that the steroid hormones testosterone, estradiol and dihydrotestosterone inhibit LH secretion. From the studies by (Santen and Bardin, 1973), it is evident that testosterone acts at the hypothalamic level by decreasing GnRH pulse frequency without a change in pulse amplitude. However, the action of estradiol appears to be predominantly at the pituitary where it decreases LH pulse amplitude without changing pulse frequency. Further support for the action of testosterone at the hypothalamus emerged from the observation of a decrease in GnRH pulse frequency in portal blood (Kretser, 2002).

2.8.2 Control of FSH Secretion
There is a substantial body of evidence to indicate that testosterone and estradiol are capable of suppressing FSH in the male (Hayes et al., 2001). For many years, it was proposed that the action of the steroid hormones could account for the entire negative feedback exerted on FSH levels by the testis despite the existence of a hypothesis that a specific FSH feedback regulator named inhibin existed (Boepple et al., 2008).

2.9 Obesity and male fertility
Jarow et al. (1993) compared obese infertile with fertile non obese men to determine the independent and codependent effects of obesity and fertility status on the male hypothalamic-pituitary gonadal axis. The obese infertile group exhibited significant endocrinologic changes as compared with fertile non obese control group. Serum testosterone was significantly lower. The testosterone/estradiol ratio was significantly lower despite a lack of significant change in serum estradiol levels. Serum SHBG was significantly lower which correlated with elevated bioavailability of both testosterone and estradiol in the
obese infertile group. Serum luteinizing hormone levels were no different, suggesting that free testosterone levels were unchanged. Reduction of serum SHBG, total testosterone, and testosterone/estradiol ratio appear to be a marker of infertility among obese men.

Jensen et al. (1995) concluded that endurance training can modify significantly hormonal profiles and semen parameters in long-distance runners. In addition, Bray (1997) reported that in men, the primary effects of obesity are a weight related reduction in testosterone and, with massive overweight, a reduction in free testosterone.

Serum levels of total testosterone (TT), free testosterone (FT), luteinizing hormone (LH) and insulin in 37 obese men before and after weight loss compared with 20 normal weight men were verified (Lima et al., 2000). Moderately obese men (BMI=32.3±1.9 kg/m²) presented significantly decreased TT levels (390±120 ng/dl) as well as FT (16.0±4.8 pg/ml) as compared with normal controls. FT serum levels had a significant and negative correlation with BMI, whereas for TT concentrations this correlation was not significant. Serum LH concentrations (4.5±2.9 mIU/ml) were normal. Insulin levels were elevated in all patients (46.3±30.1 microU/ml). After weight loss there was a significant (P<0.01) increase in TT, FT and LH levels, whereas insulin concentrations significantly decreased. In massively obese men (BMI=43.0±6.7 kg/m2), TT (320±110 ng/dl), FT (11.0±2.1 pg/ml) and LH (3.1±1.3 mIU/ml) were decreased and significantly lower as compared with the previous group and normal controls. As expected, after weight loss TT, FT and LH levels increased significantly while insulin concentrations decreased.

Kaukua et al. (2003) studied the impact of a weight-loss program on sex hormones and sexual function among 38 middle-aged obese men (BMI≥35 kg/m²). The mean weight loss in the treatment group was 21 kg at the end of the 10 week very-low-energy diet (VLED). At the end of follow-up, the maintained weight loss was 17 kg of baseline weight. The control group was weight stable throughout the study. In the treatment group, increases in sex hormone-binding globulin (SHBG), testosterone, and high-density lipoprotein
cholesterol, as well as decreases in insulin and leptin, were maintained until the end of follow-up, although with VLED, the level of several hormones and metabolic variables improved transiently during the rapid weight loss. There were no significant changes in the questionnaire scores on sexual function in either group.

The relationship between BMI and semen quality among young 1,558 Danish men (mean age 19 years) from the general population was examined (Jensen et al. 2004). Serum testosterone, SHBG, and inhibin B all decreased with increasing BMI, whereas free androgen index and estradiol (E2), increased with increasing BMI. Serum FSH was higher among slim men. After control for confounders, men with a BMI<20 kg/m² had a reduction in sperm concentration and total sperm count of 28.1% (95% confidence interval [CI] 8.3%-47.9%) and 36.4% (95% CI 14.6%-58.3%), respectively, and men with a BMI>25 kg/m² had a reduction in sperm concentration and total sperm count of 21.6% (95% CI 4.0%-39.4%) and 23.9% (95% CI 4.7%-43.2%), respectively, compared to men with BMI between 20-25 kg/m². Percentages of normal spermatozoa were reduced, although not significantly, among men with high or low BMI. Semen volume and percentage of motile spermatozoa were not affected by BMI.

Kort et al., (2006) determined the relationship between BMI and semen parameters, including sperm chromatin integrity. Data on semen samples from 520 men who were grouped based upon calculated BMI values (normal, 20-24 kg/m²; overweight, 25-30 kg/m²; obese, >30 kg/m²) were analyzed. Linear regression revealed a significant (P<0.05) and negative relationship between BMI and the total number of normal motile sperm cells. ANOVA revealed a significant difference (P<0.05) in the total number of normal motile sperm cells among the different BMI groups. The number of normal motile sperm cells per BMI group was as follows: normal, 18.6x10⁶; overweight, 3.6x10⁶; and obese, 0.7x10⁶. All multiple pairwise comparisons were found to be significantly (P<0.05) different. The overall DNA fragmentation index (DFI) mean was 24.7±2.57. Linear regression revealed a significant (P<0.05) and positive
relation between BMI and DFI. Men presenting with a BMI greater than 25 kg/m² have fewer chromatin-intact normal-motile sperm cells per ejaculate.

A clinical study was carried out to examine the relationships between obesity, hormone profiles and semen analysis of 75 young Egyptian males (Abdullah and Bakry, 2008). Body mass index was calculated. Fasting blood samples were collected, serum levels of total testosterone, E2, FSH, LH and prolactin were measured. Semen quality measures for the first ejaculates were obtained at the start of the study. Testosterone (maximum ~ -70.4 %), LH (maximum ~ -53.0 %) and FSH (maximum ~ -44.9 %) all decreased significantly with increasing BMI, whereas prolactin (~ +233.6 %) and E2 (~ +773.1 %) increased significantly with increasing BMI. Men with high BMIs typically are found to have an abnormal semen analysis represented by decrease in sperm count (~ -30.4 %), decrease in sperm motility (~ -24.4%) as well as increase in the abnormal forms of spermatozoa (~ +182.1 %). These changes were statistically significant as compared with the normal subjects.

Hammoud et al. (2008) studied the effect of male obesity on sperm parameters and erectile dysfunction. The mean age of the study population was 32.8±0.3 years. Among the 526 patients, 10.2% (54 of 526) were excluded because of the presence of a male factor known to affect fertility. The incidence of oligozoospermia increased with increasing BMI: normal weight=5.32%, overweight=9.52%, and obese=15.62%. The prevalence of a low progressively motile sperm count was also greater with increasing BMI: normal weight=4.52%, overweight=8.93%, and obese=13.28%. The incidence of erectile dysfunction did not vary across BMI categories when corrected for potential contributing factors.

Stewart TM et al (2009) described clinical, semen and hormone characteristics obtained from 225 male partners of pregnant women in Australia, and tested the associations between these characteristics. Previously known associations between semen, hormone and clinical variables were confirmed as significant: sperm numbers (concentration and total sperm count) correlated positively with inhibin B and inversely with FSH and left
varicocele, while total testicular volume correlated positively with sperm numbers and inhibin B and inversely with FSH. However, only abstinence, total testicular volume, varicocele grade and obesity (BMI>30 kg/m²) were independently significantly related to total sperm count. Compared with those with BMI<30 (n=188), obese subjects (n=35) had significantly lower total sperm count (mean 324 vs 231 million, P=0.013) and inhibin B (187 vs 140 pg/ml, P<0.001) but not FSH (3.4 vs 4.0 IU/l, P=0.6).

The association between body weight and measures of male reproductive potential was examined in 483 male partners of subfertile couples (Chavarro et al., 2010). As expected, BMI was positively related to E2 levels and inversely related to total testosterone and SHBG levels. There was also a strong inverse relation between BMI and inhibin B levels and a lower testosterone:LH ratio among men with a BMI>or=35 kg/m². Body mass index was unrelated to sperm concentration, motility, or morphology. Ejaculate volume decreased steadily with increasing BMI levels. Further, men with BMI>or=35 kg/m² had a lower total sperm count than normal weight men (adjusted difference in the median [95% CI]=−86x10⁶ sperm [-134, -37]). Sperm with high DNA damage were significantly more numerous in obese men than in normal-weight men.

Hakonsen et al. (2011) assessed semen quality and reproductive hormones among 43 morbidly obese men (BMI ranging from 33-61 kg/m²) aged 20-59 years and studied if weight loss improved the reproductive indicators. At baseline, after adjustment for potential confounders, BMI was inversely associated with sperm concentration (P=0.02), total sperm count (P=0.02), sperm morphology (P=0.04), and motile sperm (P=0.005) as well as testosterone (P=0.04) and inhibin B (P=0.04) and positively associated to E2 (P<0.005). The median (range) percentage weight loss after the intervention was 15% (3.5-25.4). Weight loss was associated with an increase in total sperm count (P=0.02), semen volume (P=0.04), testosterone (P=0.02), SHBG (P=0.03) and anti-Müllerian hormone (P=0.02). The group with the largest weight loss had a statistically significant increase in total sperm count [193 millions (95% CI: 45; 341)] and normal sperm morphology [4% (95% CI: 1; 7)].
Serondade et al. (2012) conducted a systematic review of available literature to investigate the impact of BMI on sperm count in 9779 men according to the preferred reporting items for systematic reviews and meta-analyses statement. Overweight men were at significantly increased odds of presenting with oligozoospermia (OR, 1.11; 95% CI, 1.01-1.20) or azoospermia (OR, 1.39; 95% CI, 0.98-1.97) compared with normal-weight men. Likewise, obese men were at increased risk of oligozoospermia (OR, 1.42; 95% CI, 1.12-1.79) or azoospermia (OR, 1.81; 95% CI, 1.23-2.66) compared with normal-weight men. There was an inverse association between overweight or obesity and abnormal sperm count. In addition, Martini et al (2013) pointed out that the prevalence of obesity is increasing and consequently, the number of obese men with reduced fertility will also rise.
Chapter 3

Materials and Methods

3.1 Study design

Non experimental retrospective - case control study design

3.2 Study population

The target population included obese men (BMI≥30 Kg/m²) and healthy normal weight men (BMI=18.5-24.9 Kg/m²) from Gaza Governorate, Gaza Strip. Cases and controls were age matched.

3.3 Sampling and sample size

The case group was married obese men visiting obesity clinics in Gaza Governorate seeking for weight control. The control group included married normal weight men selected from the general population. The sample size calculations were based on the formula for case-control studies. EPI-INFO statistical package version 3.5.1 was used with 95% CI, 80% power and 50% proportion as conservative and OR > 2. The sample size in case of 1:1 ratio of obese non-obese (control) was found to be 71:71. For a no-response expectation, the sample size was increased to 80 obese men. The control group was consisted of 80 non-obese men.

3.4 Exclusion criteria

- Smoker men
- Men with disease known to affect fertility such as diabetes mellitus
- Men with testicular disease or testicular surgery
- Family history of infertility

### 3.5 Ethical Consideration

Consent form obtained from all participants to ensure their voluntary participation. All participants were informed about the purpose of the study. Confidentiality was maintained all the time during the study.

### 3.6 Data collection

#### 3.6.1 Questionnaire interview

A face to face interview was used for filling in the questionnaire which is designated for matching the study needs (Annex 1). The questionnaire was based on questionnaire problem weight clinics of Al bsama, and on that used in a similar study with some modifications (Jaber, 2011). During the study the interviewer explained to the patient confused questions that were not clear to them. Most questions were one of two types: the yes/no question, which offer a dichotomous choice; and the multiple choice question, which offers several fixed alternatives (Backstrom and Hursh-César, 1981). The questionnaire was validated by four experts in the fields of epidemiology and public health. The questionnaire was piloted with 10 patients, and modified as necessary to improve reliability. The questionnaire included questions on the personal data (age, have children and education) and socioeconomic data (occupation and family income/month).

#### 3.6.2 Body mass index

Body mass index was calculated as the ratio of body weight in Kg / height in square meter. The subjects were asked to remove shoes and heavy clothes before measurement of weight and height. The balance (Camry, Italy) was used for measuring weight in kilograms and stadiometer (Henglida, China) for measuring height in centimeters. People with BMI=18.5-24.9 were considered
to have normal weight and people with BMI ≥ 30.0 were classified obese (WHO, 2000).

3.6.3 Semen collection and analysis
Semen samples were collected from obese and non-obese men by masturbation in sterile polypropylene containers after sexual abstinence of 2-7 days. Semen volume was measured. Routine semen analysis was carried out by light microscopy. The concentration, motility and morphology of spermatozoa were assessed according to WHO criteria (WHO, 1992). The normal criteria of semen parameters are (WHO, 2010):

* Volume (over 2 cc is normal)
* Sperm count (over 20 million per cc or 40 million total is normal).
* Sperm morphology (percentage of sperm that have a normal shape)
* Sperm motility (percentage of sperm that can move forward normally)

Motility is graded from a to d, as follows:
* Grade a (fast progressive) sperms are those which swim forward fast in a straight line - like guided missiles.
* Grade b (slow progressive) sperms swim forward, but either in a curved or crooked line, or slowly (slow linear or non linear motility).
* Grade c (non progressive) sperms move their tails, but do not move forward (local motility only).
* Grade d (immotile ) sperms do not move at all.

3.6.4 Blood sampling and processing
Twelve hours overnight fasting venous blood sample (8 ml) was drawn by the researcher himself into vacutainer tubes from obese and non-obese men. Blood was left for a while without anticoagulant to allow blood to clot. Then, serum samples were obtained by centrifugation at room temperature at 3000 rpm for 10 minutes for biochemical analysis. Serum testosterone, FSH, LH and lipid profile (cholesterol, triglycerides, HDL-C and LDL-C) were determined.
3.7 Hormonal analysis

3.7.1 Determination of serum testosterone

Testosterone hormone level was determined according to the method of Tiez, (1986) using ELISA TECO kit for testosterone.

Principle

The testosterone ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. The microtiter wells are coated with an antibody directed towards an unique antigenic site on the testosterone molecule. Endogenous testosterone of a patient sample competes with a testosterone horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off. The amount of bound peroxidase conjugate is reverse proportional to the concentration of testosterone in the sample. After addition of the substrate solution, the intensity of colour developed is reverse proportional to the concentration of testosterone in the patient sample.

Kit components

1. Microtiter wells, 12x8 (break apart) Strips, 96 wells coated with mouse monoclonal anti-testosterone antibody
2. Standard (Standard 0-6), 7 vials, 1 ml, ready to use concentrations: 0 - 0.2 - 0.5 - 1.2 - 6 - 16 ng/ml Conversion: 1 ng/ml = 3.467 nmol/l
3. Enzyme conjugate, 1 vial, 25 ml, ready to use testosterone conjugated to horseradish peroxidase
4. Substrate solution, 1 vial, 25 ml
5. Stop Solution, 1 vial, 14 ml, ready to use contains 0.5M H2SO4 avoid contact with the stop solution. It may cause skin irritations and burns.
6. Wash Solution, 1 vial, 30 ml (40X concentrated)

Note: Additional standard 0 for sample dilution is available on request.

Assay procedure

All samples and reagents were allowed to reach at room temperature ~25°C).
Reagents mixed by gentle inversion before use. Standards, controls and samples assayed in duplicate.

1. Microtitration Strip was marked to be used.
2. Twenty-five µL of the standards, controls and samples were added into each appropriate well.
3. Two hundred µL of Conjugate Reagent were added into each well using a precision pipette.
4. The wells were mixed for 10 seconds.
5. The wells were incubated for 60 minutes at room temperature (~25°C).
6. Each well was aspirated and washed 3 times by added 400 µL of working Wash Solution.
7. Two hundred µL of substrate solution were added into each well using a precision pipette and gently mixed for 10 seconds.
8. The wells were incubated in the dark for 15 minutes at room temperature (~25°C).
9. One hundred µL of Stop Solution were added into each well using a precision pipette and mixed for 10-20 seconds.
10. The absorbances of the solution in each well were read at 450 nm.

**Calculation**

The absorbance for each standard, control, or samples were obtained, and then the standard curve prepared by plotted the absorbance readings for each of the standards along the Y-axis versus standard concentrations in ng/mL along the X-axis, the mean absorbance values for each sample were determined the corresponding concentration of testosterone in ng/mL from the standard curve (Figure 3.1).
Figure 3.1. Standard curve for testosterone hormone

Normal reference values of testosterone for adult male: 2.0-7.0 ng/mL.

3.7.2 Determination of serum luteinizing hormone

Luteinizing hormone level was determined according to Lenton et al., (1982) method using ELISA TECO kit for LH.

Principle

The essential reagents required for an immunoenzymometric assay include excess amount of antibodies (both enzyme conjugated and immobilized) with high affinity, high specificity and contain different epitopes with distinct recognition and native antigen. In this assay procedure, the immobilization takes place at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-LH antibody. Upon mixing, a reaction results between the native antigen contained in serum, the monoclonal biotinylated antibody and the enzymelabeled antibody, without competition or steric hindrance, to form a soluble sandwich complex. Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody.
After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen value, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

**Kit components**

One Strip holder containing 96 microtitration wells coated with streptavidin, six LH reference standards with concentrations of approximately (0, 5.0, 25, 50, 100 and 200 mIU/mL). Enzyme Conjugate, TMB Chromogen Solution, Stop Solution and Wash Solution concentrate.

**Assay Procedure**

All samples and reagents were allowed to reach at room temperature (~25°C). Reagents mixed by gentle inversion before use. Standards, controls and samples assayed in duplicate.

1. Microtitration Strip was marked to be used.
2. Fifty µL of the standards, controls and samples were added into each appropriate well.
3. One hundred µL of Conjugate Reagent were added into each well using a precision pipette and then mixed for 30 seconds.
4. The wells were incubated for 60 minutes at room temperature (~25°C).
5. Each well was aspirated and washed 3 times by added 300 µL of working ash Solution.
6. One hundred µL of TMB reagent were added into each well and gently mixed for 10 second.
7. The wells were incubated in the dark for 15 minutes at room temperature (~25°C) without shaking.
8. Fifty µL of Stop Solution were added into each well and gently mixed for 10-20 second.
9. The absorbance for each well was read at 450 nm.
Calculation

The absorbance for each standard, control, or samples were obtained, and then the stander curve prepared by plotted the absorbance readings for each of the standards along the Y-axis versus standard concentrations in mIU/mL along the X-axis, the mean absorbance values for each sample were determined the corresponding concentration of LH in mIU/mL from the standard curve (Figure 3.2).

![Figure 3.2. Luteinizing hormone standard curve.](image)

Normal reference values of LH for adult male: 2.0-13.0 mIU/mL.

3.7.3 Determination of serum follicle stimulating hormone

Follicle stimulating hormone level was determined according to Vitt et al., (1998) method using ELISA TECO kit for FSH.

Principle

The essential reagents required for an immunoenzymometric assay include excess amount of antibodies (both enzyme conjugated and immobilized) with
high affinity, high specificity and contain different epitopes with distinct recognition and native antigen. In this assay procedure, the immobilization takes place at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-FSH antibody. Upon mixing, a reaction results between the native antigen contained in serum, the monoclonal biotinylated antibody and the enzymelabeled antibody, without competition or steric hindrance, to form a soluble sandwich complex. Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen value, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

**Kit components**

One Strip holder containing 96 microtitration wells coated with streptavidin, six FSH reference standards with concentrations of approximately (0, 5.0, 10, 25, 50 and 100 mIU/mL). Enzyme Conjugate, TMB Chromogen Solution, Stop Solution and Wash Solution concentrate.

**Assay Procedure**

All samples and reagents were allowed to reach at room temperature (~25°C). Reagents mixed by gentle inversion before use. Standards, controls and samples assayed in duplicate.

1. Microtitration Strip was marked to be used.
2. Fifty µL of the standards, controls and samples were added into each appropriate well.
3. One hundred µL of Conjugate reagent were added into each well using a precision pipette and then mixed for 30 seconds.
4. The wells were incubated for 60 minutes at room temperature (~25°C).
5. Each well was aspirated and washed 3 times by added 300 µL of working wash solution.
6. One hundred µL of TMB reagent were added into each well and gently mixed for 10 second.
7. The wells were incubated in the dark for 15 minutes at room temperature (~25°C) without shaking.
8. Fifty µL of Stop Solution were added into each well and gently mixed for 10-20 second.
9. The absorbance for each well was read at 450 nm.

**Calculation**

The absorbance for each standard, control, or samples were obtained, and then the standard curve prepared by plotted the absorbance readings for each of the standards along the Y-axis versus standard concentrations in mIU/mL along the X-axis. The mean absorbance values for each sample were determined the corresponding concentration of FSH in mIU/mL from the standard curve (Figure 3.3).

![Figure 3.3. Follicle stimulating hormone standard curve.](image)

Normal reference value of FSH for adult male: 2.5-10.0 mIU/mL.
3.8 Determination of lipid profile

3.8.1 Determination of total cholesterol

Total Cholesterol was determined by enzymatic colorimetric method using Globe diagnostics kit, Italy.

Principle

The measurement is based on the following enzymatic reactions:

\[
\text{CHE} \quad \text{Cholesterol esters} + \text{H}_2\text{O} \rightarrow \text{Cholesterol} + \text{Fatty acids}
\]

\[
\text{CHOD} \quad \text{Cholesterol} + \text{O}_2 \rightarrow \text{Cholest}-4\text{-en}-3\text{-one} + \text{H}_2\text{O}_2
\]

\[
\text{POD} \quad 2\text{H}_2\text{O}_2 + \text{hydroxybenzoate} + \text{4-AmminoantiPyrine} \rightarrow \text{Red complex} + 4\text{H}_2
\]

The intensity of the red complex is proportional to the total cholesterol present in the sample.

Assay procedure

About 0.5 ml of serum was transferred to the Min-dray BS-120 chemistry autoanalyzer to perform the test according to these parameters:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent</td>
<td>300 (µL)*</td>
</tr>
<tr>
<td>Serum</td>
<td>3 (µL)</td>
</tr>
<tr>
<td>Incubation period</td>
<td>3(min)**</td>
</tr>
<tr>
<td>Reaction type</td>
<td>End point</td>
</tr>
<tr>
<td>Wavelength</td>
<td>510 (nm)***</td>
</tr>
</tbody>
</table>

* µL : microliter, **min :minute, ***nm : nanometer

Reference range

Cholesterol values according to a study on a population of adults are the following:

Recommended values < 200 mg/dl
Upper limit 200 - 239 mg/dl
High value ≥ 240 mg/dl

3.8.2 Determination of triglycerides

Triglycerides were determined by enzymatic colorimetric method using Globe diagnostics kit, Italy.

**Principle:**

Glycerol, released from triglycerides after hydrolysis with lipoprotein lipase is transformed by glycerokinase into glycerol-3-phosphate which is oxidized by glycerolphosphate oxidase into dihydroxyacetone phosphate and hydrogen peroxide. In presence of peroxidase, the hydrogen peroxide oxidizes the chromogen ESPT (4-aminophenazone/N-ethylmethylanilin-propan-sulphonate sodic) to form purple quinoneimine whose colour intensity, measured at 510 nm, is proportional to the concentration of triglycerides in the sample.

\[
\text{LPL} \\
\text{Triglycerides} \rightarrow \text{Glycerol} + \text{Fatty acids}
\]

\[
\text{GK} \\
\text{Glycerol} + \text{ATP} \rightarrow \text{Glycerol-3-phosphate} + \text{ADP}
\]

\[
\text{GPO} \\
\text{Glycerol-3-phosphate} + \text{O}_2 \rightarrow \text{Dihydroxyacetone phosphate} + \text{H}_2\text{O}_2
\]

\[
\text{POD} \\
2 \text{H}_2\text{O}_2 + \text{Amminoantipirine} + \text{ESPT} \rightarrow \text{Quinoneimine} + \text{HCl} + 4 \text{H}_2\text{O}
\]

**Assay procedure:**

About 0.5 ml of serum was transferred to the Mindray BS-120 chemistry autoanalyzer to perform the test according to these parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent</td>
<td>300 (µL)*</td>
</tr>
<tr>
<td>Serum</td>
<td>3 (µL)</td>
</tr>
<tr>
<td>Incubation period</td>
<td>3(min)</td>
</tr>
<tr>
<td>Reaction type</td>
<td>End point</td>
</tr>
<tr>
<td>Wavelength</td>
<td>510(nm)</td>
</tr>
</tbody>
</table>

* µL : microliter, **min :minute, ***nm : nanometer
Reference range:
  Recommended values < 200 mg/dL
  Upper limit 200-400 mg/dL
  High values > 400 mg/dL

3.8.3 Determination of high density lipoprotein

High density lipoprotein was determined by liquid HDL precipitant for the determination of HDL Cholesterol using Globe diagnostics kit, Italy.

Principle:
The VLDL and LDL-C from serum or plasma are precipitated by phosphotungstate in the presence of magnesium ions. After removed by centrifugation the clear supernatant is containing high density lipoproteins (HDL-C) and used for the determination of it.

Assay procedure:
1. pipette into conic test tubes:
   - 0.5 ml serum
   - 0.5 ml precipitating reagent
2. Mix gently by inversion, wait 5 minutes and centrifuge at (3000 rpm) for 20 minutes.
3. Recovery the supernatant for the HDL cholesterol determination as follows:
   - About 0.5 ml of supernatant was transferred to the Mindray BS-120 chemistry autoanalyzer to perform the test according to these parameters:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent</td>
<td>200 (µL)*</td>
</tr>
<tr>
<td>Serum</td>
<td>3 (µL)*</td>
</tr>
<tr>
<td>Incubation period</td>
<td>3 (min)**</td>
</tr>
<tr>
<td>Reaction type</td>
<td>End point</td>
</tr>
<tr>
<td>Wavelength</td>
<td>510 (nm)***</td>
</tr>
</tbody>
</table>

* µL: microliter, **min: minute, ***nm: nanometer
Reference value:
Based on the risk for heart diseases the sequent reference ranges are suggested:
- Low value < 40 mg/dl (high risk)
- Medium value 40 - 59 mg/dl (moderate risk)
- High value > 60 mg/dl (low risk)

3.8.4 Determination of Low density lipoprotein cholesterol

Determination of LDL- C was calculated from the primary measurements using the empirical equation.

\[ \text{LDL- C (mg/dl)} = \text{total cholesterol} - \text{triglyceride}/5 - \text{HDL- C} \]

3.9 Statistical analysis

Data were computer analyzed using SPSS/ PC (Statistical Package for the Social Science Inc. Chicago, Illinois USA, version 18.0) statistical package. Simple distribution of the study variables and the cross tabulation were applied. Chi-square \( (\chi^2) \) was used to identify the significance of the relations, associations, and interactions among nominal various variables. Yates's continuity correction test, of \( \chi^2 \)_{(corrected)} was used when not more than 20% of the cells had an expected frequency of less than five and when the expected numbers were small. The independent sample t-test procedure was used to compare means of quantitative variables by the separated cases into two qualitative groups such as the relationship between cases and controls hormones. Pearson's correlation test between BMI and other studied variables was applied.

Percentage difference equals the absolute value of the change in value, divided by the average of the 2 numbers, all multiplied by 100.

\[ \text{Percent difference} = \left( \frac{|(V1 - V2)|}{((V1 + V2)/2)} \right) \times 100. \]

The results were accepted as statistical significant when the P-value was less than 5% (P<0.05).
SPSS program version 18.0 was also used for correlation graph plotting between BMI and other studied variables.
Chapter 4

Results

4.1 Anthropometric measurements of the study population

The study population comprised 80 obese men (case group). A total of 80 healthy normal weight men were served as controls. Table 4.1 shows the anthropometric measurements of the study population. The mean weight of controls was 76.8±7.8 kg compared to 112.6±11.1 kg of cases. The weight difference was significant (P<0.001) with % difference=37.8% higher in cases. There was no significant difference in the mean height of cases compared to controls (1.82±0.08 vs. 1.83±0.06 m, % difference=0.5, P=0.815). Therefore, the body mass index (BMI) of cases was significantly higher than that of controls (33.4±2.5 vs. 22.8±1.5, % difference=37.7, P<0.001).

Table 4.1 Anthropometric measurements of the study population.

<table>
<thead>
<tr>
<th>Anthropometric measurement</th>
<th>Control (n=80) mean± SD</th>
<th>Case (n=80) mean± SD</th>
<th>% difference</th>
<th>t</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)*</td>
<td>76.8±7.8 (59.1-93.7)</td>
<td>112.6±11.1 (90.0-132.1)</td>
<td>37.8</td>
<td>16.595</td>
<td>0.000</td>
</tr>
<tr>
<td>Height (m)**</td>
<td>1.83±0.06 (1.68-1.94)</td>
<td>1.82±0.08 (1.67-1.96)</td>
<td>0.5</td>
<td>0.235</td>
<td>0.815</td>
</tr>
<tr>
<td>BMI***</td>
<td>22.8±1.5 (18.9-24.9)</td>
<td>33.4±2.5 (30.0-40.60)</td>
<td>37.7</td>
<td>22.443</td>
<td>0.000</td>
</tr>
</tbody>
</table>

*Kg: kilogram, ** m: meter, ***BMI: Body mass index: People with BMI=18.5-24.9 were considered to have normal weight and people with BMI ≥ 30.0 were classified obese (WHO, 2000). All values are expressed as mean ±SD. P>0.05: not significant, P<0.05: significant.

4.2 Personal data of the study population

Personal profile of the study population is illustrated in Table 4.2. Age classification showed that 16 (20.0%) controls and 16 (20.0%) cases were 20-25 years old. Age group 26-30 years comprised 20 (25.0%) controls and 20
(25.0%) cases. Controls and cases aged 31-35 were 18 (22.5%) and 16 (20.0%), and those aged 36-40 were 26 (32.5%) and 28 (35.0%) years. The difference between controls and cases in term of age distribution was not significant ($\chi^2=0.192$, $P=0.979$). The mean ages of controls and cases were 31.7±5.6 and 31.7±6.1 years, respectively with ranges of 20-40 years. The independent sample t-test also showed no significant difference between mean ages of controls and cases ($t=0.000$, $P=1.000$). Eighty (100%) controls and 74 (92.5%) cases have children compared to 0 (0.0%) controls and 6 (7.5%) cases have no children. The difference between the two groups was significant ($\chi^2_{corrected} = 4.329$, $P=0.037$). Analysis of the educational status of the study population showed that 18 (22.5%) controls and 22 (27.5%) cases had a university degree, 54 (67.5%) and 42 (52.5 %) finished secondary school, 8 (10.0%) and 10 (12.5%) had finished preparatory school, and 0 (0.0%), 6 (7.5%) had passed primary school. The difference between various education levels of controls and cases was not significance ($\chi^2_{corrected} = 5.708$, $P=0.127$).

Table 4.2 Personal data of the study population.

<table>
<thead>
<tr>
<th>Personal character</th>
<th>Controls (n=80)</th>
<th>Cases (n=80)</th>
<th>test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Age (Year)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-25</td>
<td>16</td>
<td>20.0</td>
<td>16</td>
<td>20.0</td>
</tr>
<tr>
<td>26-30</td>
<td>20</td>
<td>25.0</td>
<td>20</td>
<td>25.0</td>
</tr>
<tr>
<td>31-35</td>
<td>18</td>
<td>22.5</td>
<td>16</td>
<td>20.0</td>
</tr>
<tr>
<td>36-40</td>
<td>26</td>
<td>32.5</td>
<td>28</td>
<td>35.0</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>31.7±5.6</td>
<td></td>
<td>31.7±6.1</td>
<td></td>
</tr>
<tr>
<td>Have children</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>80</td>
<td>100</td>
<td>74</td>
<td>92.5</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>7.5</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>University or Diploma</td>
<td>18</td>
<td>22.5</td>
<td>22</td>
<td>27.5</td>
</tr>
<tr>
<td>Secondary school</td>
<td>54</td>
<td>67.5</td>
<td>42</td>
<td>52.5</td>
</tr>
<tr>
<td>Preparatory school</td>
<td>8</td>
<td>10.0</td>
<td>10</td>
<td>12.5</td>
</tr>
<tr>
<td>Primary school</td>
<td>0</td>
<td>0.0</td>
<td>6</td>
<td>7.5</td>
</tr>
</tbody>
</table>

*P-value of $\chi^2_{(corrected)}$ test.
P>0.05: not significant, P<0.05: significant.
4.3 Socioeconomic characters of the study population

Table 4.3 provides socioeconomic characters of the study population. The employed controls and cases were 34 (42.5%) and 20 (25.0%), whereas 46 (57.5%) controls and 60 (75.0%) cases were unemployed. The difference between the two groups was significant, with increasing unemployment among obese men ($\chi^2=5.479$, $P=0.019$). Regarding family income/month, significant difference was also recorded between controls and cases, with less income among obese men ($\chi^2=20.486$, $P<0.001$).

<table>
<thead>
<tr>
<th>Socioeconomic character</th>
<th>Controls (n=80)</th>
<th>Cases (n=80)</th>
<th>$\chi^2$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed</td>
<td>34</td>
<td>42.5</td>
<td>20</td>
<td>25.0</td>
</tr>
<tr>
<td>Unemployed</td>
<td>46</td>
<td>57.5</td>
<td>60</td>
<td>75.0</td>
</tr>
<tr>
<td>Family income/month (NIS)**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1000</td>
<td>8</td>
<td>10.0</td>
<td>30</td>
<td>37.5</td>
</tr>
<tr>
<td>1000-2000</td>
<td>36</td>
<td>45.0</td>
<td>34</td>
<td>42.5</td>
</tr>
<tr>
<td>&gt;2000</td>
<td>36</td>
<td>45.0</td>
<td>16</td>
<td>20.0</td>
</tr>
</tbody>
</table>

** NIS: new Israeli Shekels, $P<0.05$: significant.

4.4 Lipid profile of the study population

Lipid profile including cholesterol, triglycerides, (LDL-C) and (HDL-C) of the study population is illustrated in Table 4.4. The average levels of cholesterol, triglycerides and LDL-C were found to be higher in cases (212.6±42.4, 230.9±30.7 and 121.7±32.8 mg/dl) compared to controls (127.9±38.4, 113.8±35.6 and 93.9±22.9 mg/dl) with % differences of 49.8, 67.9 and 25.8 and $P=0.000$, respectively. In contrast, HDL-C was lower in cases than in controls (51.9±4.0 vs. 52.7±5.4 mg/dl, % difference=1.5). However, this change was not significant ($t=0.918$, $P=0.360$).
Table 4.4 Lipid profile of the study population.

<table>
<thead>
<tr>
<th>Lipid parameter</th>
<th>Control (n=80) mean±SD</th>
<th>Case (n=80) mean±SD</th>
<th>% difference</th>
<th>t</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/dl) (min-max)</td>
<td>127.9±38.4 (65-195)</td>
<td>212.6±42.4 (94-303)</td>
<td>49.8</td>
<td>9.32</td>
<td>0.000</td>
</tr>
<tr>
<td>Triglycerides (mg/dl) (min-max)</td>
<td>113.8±35.6 (53-184)</td>
<td>230.9±30.7 (144-292)</td>
<td>67.9</td>
<td>15.75</td>
<td>0.000</td>
</tr>
<tr>
<td>LDL-C (mg/dl) * (min-max)</td>
<td>93.9±22.9 (53-143)</td>
<td>121.7±32.8 (49-178)</td>
<td>25.8</td>
<td>4.390</td>
<td>0.000</td>
</tr>
<tr>
<td>HDL-C (mg/dl) ** (min-max)</td>
<td>52.7±5.4 (45-63)</td>
<td>51.9±4.0 (45-59)</td>
<td>1.5</td>
<td>0.918</td>
<td>0.360</td>
</tr>
</tbody>
</table>

*LDL-C: Low density lipoprotein cholesterol, **HDL-C: High density lipoprotein cholesterol. P>0.05: not significant, P<0.05: significant.

4.5 Hormonal levels of the study population

Table 4.5 illustrates hormonal levels of the study population including total testosterone, luteinizing hormone and follicle stimulating hormone. The mean level of testosterone was significantly decreased in cases compared to controls (3.6±1.5 vs. 6.3±0.8 ng/ml, P<0.001), with percentage difference of 54.5%. On the other hand, the mean levels of LH and FSH were significantly increased in cases compared to controls (9.7±5.3 and 12.3±6.4 mIU/ml vs. 4.7±2.6 and 4.6±1.3 mIU/ml, P<0.001, respectively), showing percentage differences of 69.4% and 91.1%, respectively.

Table 4.5 Hormonal levels of the study population.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Control (n=80) mean±SD</th>
<th>Case (n=80) mean±SD</th>
<th>% difference</th>
<th>t</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. testosterone* (ng/ml) (min-max)</td>
<td>6.3±0.8 (4.3-7.0)</td>
<td>3.6±1.5 (1.01-8.04)</td>
<td>54.5</td>
<td>10.190</td>
<td>0.000</td>
</tr>
<tr>
<td>LH** (mIU/ml) (min-max)</td>
<td>4.7±2.6 (2.0-13.0)</td>
<td>9.7±5.3 (1.20-26.80)</td>
<td>69.4</td>
<td>5.290</td>
<td>0.000</td>
</tr>
<tr>
<td>FSH*** (mIU/ml) (min-max)</td>
<td>4.6±1.3 (2.6-9.7)</td>
<td>12.3±6.4 (1.8-37.0)</td>
<td>91.1</td>
<td>7.117</td>
<td>0.000</td>
</tr>
</tbody>
</table>

*T. Testosterone: Total testosterone, **LH: Luteinizing hormone, ***FSH: Follicle stimulating hormone. P>0.05: not significant, P<0.05: significant.

40
4.6 Semen parameters of the study population

4.6.1 Sperm concentration of the study population

The sperm concentration of the study population including volume, count and total count is pointed out in Table 4.6. There were significant decrease in sperm volume, count and total count in cases (2.2±0.9 ml, 7.8±5.2 million/mL and 17.6±21.6 million) compared to controls (3.1±0.6 ml, 43.7±16.0 million/mL and 135.3±55.8 million). This decrease was significant (P<0.001), with percentage differences of 34.0, 139.5 and 15.4, respectively).

Table 4.6 Sperm concentration of the study population.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=80) mean±SD</th>
<th>Case (n=80) mean±SD</th>
<th>% difference</th>
<th>t</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VOLUME (mL)</td>
<td>3.1±0.6 (2.0-4.5)</td>
<td>2.2±0.9 (0.8-5.5)</td>
<td>34.0</td>
<td>5.353</td>
<td>0.000</td>
</tr>
<tr>
<td>Count (million/mL)</td>
<td>43.7±16.0 (23.0-83.0)</td>
<td>7.8±5.2 (1.5-25.0)</td>
<td>139.5</td>
<td>13.55</td>
<td>0.000</td>
</tr>
<tr>
<td>Total Count (million)</td>
<td>135.3±55.8 (9.0-294.0)</td>
<td>17.6±21.6 (3.2-137.5)</td>
<td>15.4</td>
<td>12.44</td>
<td>0.000</td>
</tr>
</tbody>
</table>

P>0.05: not significant, P<0.05: significant.

4.6.2 Sperm motility of the study population

Table 4.7 summarizes the sperm motility (total motility, fast progressive, slow progressive, non-progressive and immotile) of the study population. Sperm total motility was markedly decrease in cases compared to controls (28.7±14.8 vs. 57.7±11.2, % difference=67.0 and P<0.001). In the context of sperm motility, fast progressive and slow progressive grades were also significantly decreased in cases (0.23±1.27 and 18.7±11.6) compared to controls (18.9±5.4 and 28.7±7.5), showing percentage differences of 195.3 and 42.1 and P<0.001. However, the non-progressive grade of sperm motility exhibited no significant difference between cases and controls (9.8±4.7 vs. 10.1±4.5, % difference=2.8 and P=0.789). On the other hand, the immotile sperms were significantly
higher in cases than in controls (71.3±14.8 vs. 42.4±11.2, % differences=50.9 and P<0.001).

Table 4.7 Sperm motility of the study population.

<table>
<thead>
<tr>
<th>Motility (%)</th>
<th>Control (n=80) mean±SD</th>
<th>Case (n=80) mean±SD</th>
<th>% difference</th>
<th>t</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total motility (min-max)</td>
<td>57.7±11.2 (40-80)</td>
<td>28.7±14.8 (4-66)</td>
<td>67.0</td>
<td>9.861</td>
<td>0.000</td>
</tr>
<tr>
<td>Fast Progressive (min-max)</td>
<td>18.9±5.4 (10-40)</td>
<td>0.23±1.27 (0-8)</td>
<td>195.3</td>
<td>21.150</td>
<td>0.000</td>
</tr>
<tr>
<td>Slow progressive (min-max)</td>
<td>28.7±7.5 (15-40)</td>
<td>18.7±11.6 (0-50)</td>
<td>42.1</td>
<td>4.560</td>
<td>0.000</td>
</tr>
<tr>
<td>Non–progressive (min-max)</td>
<td>10.1±4.5 (2-22)</td>
<td>9.8±4.7 (2-28)</td>
<td>2.8</td>
<td>0.269</td>
<td>0.789</td>
</tr>
<tr>
<td>Immobile (min-max)</td>
<td>42.4±11.2 (20-60)</td>
<td>71.3±14.8 (34-96)</td>
<td>50.9</td>
<td>9.860</td>
<td>0.000</td>
</tr>
</tbody>
</table>

P>0.05: not significant, P<0.05: significant.

4.6.3 Sperm morphology of the study population

Table 4.8 presents the normal and abnormal sperm morphology of the study population. The normal forms of sperms were lower in cases in comparison to controls (25.9±4.7 vs. 39.0±4.6, % difference=40.5, P<0.001). In contrast, abnormal forms of sperms were higher in cases when compared to controls (74.1±4.7 vs. 61.0±4.6 % difference=19.5, P<0.001).

Table 4.8 Sperm morphology of the study population.

<table>
<thead>
<tr>
<th>sperm forms (%)</th>
<th>Control (n=80) mean±SD</th>
<th>Case (n=80) mean±SD</th>
<th>% difference</th>
<th>t</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal form (min-max)</td>
<td>39.0±4.6 (30-51)</td>
<td>25.9±4.7 (12-38)</td>
<td>40.5</td>
<td>12.68</td>
<td>0.000</td>
</tr>
<tr>
<td>Abnormal form (min-max)</td>
<td>61.0±4.6 (49-70)</td>
<td>74.1±4.7 (62-88)</td>
<td>19.5</td>
<td>12.63</td>
<td>0.000</td>
</tr>
</tbody>
</table>

P<0.05: significant.
4.7 Correlations of body mass index with the studied parameters of the study population

4.7.1 Correlations of body mass index with lipid profile of the study population

The correlation between BMI and lipid profile of the study population is demonstrated in Table 4.9. Pearson correlation test revealed positive significant correlation between BMI and cholesterol, triglyceride and LDL-C levels (r=0.737, P<0.001; r=0.836, P<0.001 and r=0.493, P<0.001, respectively, Figures 4.1, 4.2 and 4.3). However, negative not significant correlation was registered between BMI and HDL-C (r=-0.034, P=0.693)

Table 4.9 Body mass index (BMI) in relation to lipid profile of study population.

<table>
<thead>
<tr>
<th>Lipid Profile (mg/dl)</th>
<th>BMI (Kg/m²)</th>
<th>Pearson correlation (r)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>0.737</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.836</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>LDL-C*</td>
<td>0.493</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>HDL-C**</td>
<td>-0.034</td>
<td></td>
<td>0.693</td>
</tr>
</tbody>
</table>

*LDL-C: Low density lipoprotein cholesterol, **HDL-C: High density lipoprotein cholesterol. P>0.05: not significant, P<0.05: significant.
Figure 4.1 Correlation between BMI and cholesterol of the study population.

Figure 4.2 Correlation between BMI and triglyceride of the study population.
4.7.2 Correlations of body mass index with hormonal levels of the study population

Table 4.10 gives the relation between BMI and hormonal levels of the study population. BMI was negatively correlated with total testosterone levels ($r=-0.755$ and $P<0.001$, Figure 4.4). On the other hand, LH and FSH were positively correlated with BMI ($r=0.445$, $P<0.001$ and $r=0.492$, $P<0.001$ respectively, Figures 4.5 and 4.6).

Table 4.10 Body mass index (BMI) in relation to hormonal level of the study population.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>BMI (Kg/m2)</th>
<th>Pearson correlation (r)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Testosterone (ng/ml)</td>
<td>-0.755</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>LH* (mIU/ml)</td>
<td>0.445</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>FSH* (mIU/ml)</td>
<td>0.492</td>
<td></td>
<td>0.000</td>
</tr>
</tbody>
</table>

*LH: Luteinizing hormone, **FSH: Follicle stimulating hormone. P<0.05: significant.
Figure 4.4 Correlation between BMI and total testosterone of the study population.

Figure 4.5 Correlation between BMI and Luteinizing hormone of the study population.
4.7.3 Correlations of body mass index with sperm concentration of the study population

Body mass index in relation to sperm concentration (volume, count and total count) of the study population is indicated in Table 4.11 and Figures 4.7, 4.8 and 4.9. BMI showed negative significant correlations with sperm volume, count and total count ($r=-0.465$, $P<0.001$; $r=-0.774$, $P<0.001$ and $r=-0.742$, $P<0.001$).

Table 4.11 Body mass index (BMI) in relation to sperm concentration of the study population.

<table>
<thead>
<tr>
<th>sperm concentration</th>
<th>BMI</th>
<th>Pearson correlation (r)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (mL)</td>
<td>-0.465</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Count (million/mL)</td>
<td>-0.774</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Total count (million)</td>
<td>-0.742</td>
<td>0.000</td>
<td></td>
</tr>
</tbody>
</table>

P<0.05: significant.
Figure 4.7 Correlation between BMI and volume of the study population.

Figure 4.8 Correlation between BMI and count sperm of the study population.
Figure 4.9 Correlation between BMI and total count sperm of the study population.

4.7.4 Correlations of body mass index with sperm motility of the study population

Table 4.12 presents BMI in relation to sperm motility of the study population. There were negative significant correlations of BMI with total motility, fast progressive and slow progressive grades of motility (\(r=-0.738, P<0.001\); \(r=-0.856, P<0.001\) and \(r=-0.494, P<0.001\) Figures 4.10, 4.11 and 4.12). However non-progressive grad of sperm motility showed negative not significant correlation with BMI (\(r=-0.064\) and \(P=0.573\)). On the other hand, BMI showed positive significant correlation with sperm immotility (\(r=0.738\) and \(P<0.001\) Figure 4.13).
Table 4.12 Body mass index (BMI) in relation to sperm motility of all study population.

<table>
<thead>
<tr>
<th>Motility (%)</th>
<th>BMI (Kg/m²)</th>
<th>Pearson correlation (r)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total motility</td>
<td>-0.738</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Fast progressive</td>
<td>-0.856</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Slow progressive</td>
<td>-0.494</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Non-progressive</td>
<td>-0.064</td>
<td>0.573</td>
<td></td>
</tr>
<tr>
<td>Immotile</td>
<td>0.738</td>
<td>0.000</td>
<td></td>
</tr>
</tbody>
</table>

P<0.05: significant.

Figure 4.10 Correlation between BMI and total motility sperm of the study population.
Figure 4.11 Correlation between BMI and percentage of fast progressive motile sperm of the study population.

Figure 4.12 Correlation between BMI and percentage of slow progressive motile sperm of the study population.
4.7.5 Correlations of body mass index with sperm morphology of the study population

Correlation of BMI with sperm morphology of the study population is shown in Table 4.13 and Figures 4.14 and 4.15. Body mass index displayed negative significant correlation with normal form of sperm ($r=-0.759$ and $P<0.001$) and positive significant correlation with abnormal form of sperm ($r=0.759$ and $P<0.001$)

Table 4.13 Body mass index (BMI) in relation to sperm morphology of the study population.

<table>
<thead>
<tr>
<th>sperm morphology (%)</th>
<th>BMI (Kg/m²)</th>
<th>Pearson correlation (r)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal form</td>
<td>-0.759</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>Abnormal form</td>
<td>0.759</td>
<td></td>
<td>0.000</td>
</tr>
</tbody>
</table>

P<0.05: significant.
Figure 4.14 Correlation between BMI and normal form sperm of the study population.

Figure 4.15 Correlation between BMI and abnormal form sperm of the study population.
Chapter 5

Discussion

Obesity is a modern day pandemic with serious co-morbidities, both physical and psychological. By 2015, the foresight report estimates that 36% of males and 28% of females (aged between 21 and 60) will be obese worldwide. By 2025 it is estimated that 47% of men and 36% of women will be obese (National Health Service, 2011). Studies clearly showed that obese men have an increased chance of subfertility and subfecundity due to various mechanisms (physical, genetic, hormonal, adipokine, cytokine) that ultimately lead to erectile dysfunction and abnormal semen parameters (Cabler et al., 2010). Despite its high prevalence and the subsequent health problems, there are under-diagnosis and under-reporting of obesity in the Gaza Strip. Data on obesity were limited to annual reports emerged from the Palestinian Ministry of Health. Recently, few studies have been focused on determination of leptin, Insulin, thyroid stimulating hormone, ghrelin, and obestatin hormones in obesity (Zabut et al., 2009, AL-jedi, 2011, Al-smairi, 2012 and Omran, 2012). However, no previous study investigated the reproductive hormones and semen parameters in obese men. Therefore, the present work is the first to assess risk of obesity on male fertility in Gaza Strip.

5.1 Personal and socioeconomic data of the study population

The present case control study was carried out on 80 obese men with mean age=31.7±6.1 years and BMI=33.4±2.5 kg/m². The controls were 80 healthy normal weight men with mean age=31.7±5.6 years and BMI=22.8±1.5 kg/m². Personal data of the study population showed that obese men had less number of children than non-obese men. It is well document that obesity have negative impact on male fertility which may be reflected in the lower number of children reported in the present study (Jokela et al., 2008; Phillips and Tanphaichitr, 2010 and Martini et al., 2012).
Socioeconomic data revealed that about half of controls and three quarters of obese men were unemployed, reflecting the unemployment crisis in Gaza strip. Unemployment crisis in Gaza strip was reported to exceed 46% as a result of continuous siege on economic conditions in Gaza strip (Giacaman et al 2009 and International Labour Organization, 2013). In addition, obesity was more frequent in unemployed men. This finding is in agreement with that obtained by Tunceli et al. (2006) who found that obesity is associated with reduced employment. In other words, Lindeboom et al. (2010) reported a significant negative association between obesity and employment. Regarding family income/month, obesity was more prevalent in families with less income. This result is supported by the above finding that obesity is more frequent in unemployed men. Similar results were obtained by Filzgerald et al. (2006); Kuntz and Lampert (2010) and Markwick et al. (2013). The inability of low income families to afford healthy food may be a major contributor to the increase prevalence of obesity (Akil and Ahmad, 2011).

5.2 Lipid profile of the study population

The mean levels of cholesterol, triglycerides and LDL-C were significantly increased in cases compared to controls. This finding is in accordance with that addressed by Bhatti et al. (2001); Thorpe et al. (2004) and Fox, (2011). The lipid disturbance observed in the current study could be attributed to the development of insulin resistance in obese men. Obesity results in an increased flux of free fatty acids into the circulation and uptake by the myocyte or hepatocyte. Activated fatty acids are “metabolized” primarily via one of two pathways, oxidation or storage. When fatty acid flux exceeds the ability of these pathways to dispose of activated fatty acids, intermediaries of fatty acid metabolism accumulate. In turn, these fatty acid intermediates can negatively regulate insulin action (Schenk et al., 2008). Reaven et al. (2004) and Al-Jedi (2011) found that insulin resistance was significantly higher in obesity. Hyperinsulinemia is known to enhance hepatic very low density lipoprotein synthesis and thus may directly contribute to the increased plasma triglyceride and LDL-C cholesterol levels (Hwang et al., 2006).
5.3 Hormonal levels of the study population

The serum total testosterone was significantly decreased in obese men compared to controls, whereas serum LH and FSH were significantly increased in obese men. These findings are in accord with that obtained by Kaukua et al. (2003); Mohr et al. (2006); Hammoud et al. (2008); Shayeb et al. (2009); Hofny et al. (2010); Wang et al. (2011) and Kelly and Jones (2013). In the hypogonadism study in males, obese men were more than twice (odds ratio=2.38) as likely as nonobese men to have low testosterone levels (Mulligan et al., 2006). Indeed, both weight loss and exercise increase total testosterone (Kaukua et al., 2003 and Corona et al., 2011).

Obesity, particularly the accumulation of excess visceral fat, appears to activate a self-perpetuating cycle of insulin resistance, systemic inflammation, and other metabolic derangements, including decreased testosterone (Grossmann, 2011). The negative significant correlation between BMI and testosterone observed in the present study do confirm the previous studies (MacDonald et al., 2010; Lucca et al., 2012 and Buvat et al., 2013) and the negative impact of obesity on fertility. Low testosterone levels promote insulin resistance in men (Pitteloud et al., 2005). In such conditions, there is an excess of serum fatty acids which converted into phospholipids and cholesterol in liver. These two substances along with excess triglycerides formed at the same time in liver may be discharged into blood in the form of lipoproteins (Jaworski et al., 2007 and Schenk et al., 2008). This may be the cause stands behind the elevation of serum lipid profile observed in the present study.

Increase levels of LH and FSH recorded in obese men in the present study is more likely to be due to negative feedback control excreted by low testosterone level on hypothalamic-pituitary axis. This negative feedback mechanism is well documented in the literature (Hayes et al., 2001; Phillips and Tanphaichitr, 2010; Guyton and Hall, 2011 and Martini et al., 2013). In addition, the positive significant correlation of BMI with FSH and LH noted in the current study is in agreement with that reported by Hofny et al. (2010). Correlation of
BMI with total testosterone, FSH and LH with BMI may explain the role of such tri-hormonal interplay in subfertility in obese men.

Finally, Phillips and Tanphaichitr, (2010) proposed three main biological mechanisms link obesity to impaired male reproductive function: 1) hypogonadism: hypogonadotropic hypogonadism caused by negative feedback by estrogens or insulin/leptin resistance and hypergonadotropic hypogonadism caused by direct actions of leptin on the testis, 2) testicular heat-stress-/hypoxia-induced apoptosis and 3) endocrine disruption by obesogens: environmental chemicals that may be stored in adipose tissue and have the potential to modulate both estrogenic and adipogenic pathways.

5.4 Semen parameters of the study population

The present data revealed that obesity had a negative impact on both semen quality and quantity. Similar result were addressed (Jensen et al., 2004; kort et al., 2006; Stewart et al., 2009 and Hakonsen et al., 2011)

5.4.1 Sperm concentration of the study population

Semen volume, sperm count and total count were significantly decreased in obese men compared to normal weight men. Hypospermia, oligospermia and decreased sperm total count observed in the present study are in agreement with that reported in obese men by Stewart et al. (2009); Hofny et al. (2010) and Sermondade et al. (2012). In addition, Jensen et al. (2004) reported a 21.6 and 23.9% significant reduction in sperm concentration and total sperm count, respectively, in men with BMI >25 compared with those classified as normal. In this context, the present data showed negative significant correlations of BMI with semen volume, sperm count and total count. Similar associations were previously documented (Hammoud et al., 2008; Martini et al 2010 and Braga et al., 2012). Low sperm concentration noted in this study may be attributed to many factors including: 1) Reduced spermatogenesis as a result of low testosterone level recorded in the present study (Meeker et al., 2007 and Martini et al., 2013), 2) Increase of oxidative stress which is
associated with increase insulin resistance and dyslipidemia observed in the present study (Agarwal et al., 2006 and Aitken et al., 2012), 3) Environmental toxins accumulate in fatty tissues around the scrotum and testis may have a direct localized effect on spermatogenesis (Oliva et al., 2001) and 4) Sedentary lifestyle which is often associated with Obesity, which may lead to increased local testicular temperature and result in low sperm count (Paul et al., 2009).

5.4.2 Sperm motility of the study population

Sperm motility of the study population showed that the mean total motility, progressive and slow progressive grades of motility were significantly decreased in cases compared to controls. In contrast, immotile sperm was significantly increased in cases. Higher incidence of asthenozoospermia was observed in obese men (Hammoud et al., 2008 and Du Plessis et al., 2010). In addition, in nine morbidly obese patients Martini et al. (2010) found a significant increase in the percentage of non-motile spermatozoa (45.7±5.5, n=9 in men with BMI≥40 vs 33.3±1.5, n=146 in men with 30≤BMI<40, P<0.024). When sperm motility was related to BMI, total motility, progressive and slow progressive degrees of motility were found to be negatively correlated with BMI, whereas sperm immotility was positively correlated with BMI. Similar associations were recorded previously (Kort et al., 2006; Qin et al., 2007; Hammoud et al., 2008; Martini et al., 2010; Braga et al., 2012 and Sermondade et al., 2012).

Asthenozoospermia observed in the present study may be explained on the bases that: 1) Low testosterone level recorded in this study decrease the activity of seminal neutral $\alpha$-glucosidase (NAG) which modulates the maturation process of spermatozoa during their passage through the epididymis; motility acquisition is one of its characteristics (Mahmoud et al., 1998 and Chauvin and Griswold, 2004). Interestingly, there is a significant inverse association between BMI and seminal NAG levels (Martini et al., 2010), 2) increase adipokines secreted by the adipose tissue reduce human
sperm motility in a time and dose-dependent manner, promoting an increased production of nitric oxide up to pathological levels (Lampiao and Plessis, 2008 and Martini et al., 2012) and 3) increase reactive oxygen species may responsible for alteration in sperm motility (Agarwal et al., 2006).

5.4.3 Sperm morphology of the study population

Microscopic observation revealed higher number of abnormal sperms in cases compared to controls. This teratozoospermia was positively correlated with BMI. These findings are in accord with that found by others who reported abnormal sperm morphology in obese men (Hofny et al., 2010 and Hakonsen et al., 2011). Teratozoospermia observed in obese men may be due to: 1) Increase sperm DNA fragmentation. Kort et al. (2006), found that an increase in the BMI above 25 kg/m² causes an increase in sperm DNA fragmentation and a decrease in the number of normal chromatin-intact sperm cells per ejaculate, relative to the degree of obesity and 2) increase reactive oxygen species that may be responsible for alteration in sperm morphology (Agarwal et al., 2006).
6.1 Conclusions

* The BMI of cases and controls was 33.4±2.5 and 22.8±1.5 kg/m\(^2\), respectively.
* The number of cases who reported not to have children were more than controls.
* Unemployed and low family income was more frequent among cases compared to controls.
* The mean levels of cholesterol, triglycerides and low density lipoprotein cholesterol were significantly increased in obese men compared to controls.
* Serum testosterone was significantly lower in cases compared to controls.
* Serum LH and FSH were significantly increased in cases compared to controls.
* The sperm concentration including volume, count and total count was significantly decreased in cases compared to controls.
* In cases total motility, fast progressive and slow progressive grades of motility were significantly decreased compared to controls.
* Immotile sperm was significantly increased in cases compared to controls.
* Normal sperm form was significantly decreased in cases compared to controls.
* Body mass index was positively correlation with cholesterol triglyceride and LDL-C.
* There was an inverse correlation between BMI and testosterone whereas LH and FSH were positively correlated with BMI.
* Sperm volume, count, total count, total motility, progressive and slow progressive grades of motility were negatively correlation with BMI whereas sperm immotility and abnormal form sperm were positively correlation with BMI.
6.2 Recommendations

* Launching of health education programs on obesity particularly among unemployed individuals and families with low income are highly recommended
* Follow up a healthy diet in term of reducing obesity
* Estimation of lipid profile is recommended as part of the laboratory investigations in obese men so as to promote management strategies to prevent deleterious effect of obesity on male fertility.
* Further research is needed to clarify the role of insulin resistance in male fertility
Chapter 7

References


Jaber E. 2011. Leptin Status and Some Biochemical Parameters in Germ Cell Aplasia Among Infertile Men in Gaza Strip Faculty of Medicine The Islamic University of Gaza.


Appendices

Annex 1

Questionnaire

no. ( )

This questionnaire is designed to study risk of obesity on male fertility in Gaza Governorate, Gaza Strip.

I am researcher / Tarik Z. El-Refi (Master student / The Islamic University) will be very grateful if you help me in completing this study which focuses on the risk of obesity on male fertility in Gaza Strip.

Name: …………………..

Address ………………………………………………………………

Tel. ……………………………….. Mobile: ………………………………..

Weight ……..….kg          Height……….m          BMI………….

Personal data:

1. Age :-----------------

2. Do you have children ?  □ Yes  □ No

   No. of children ---------

3. Education
   □ University or Diploma □ Secondary □ Preparatory
   □ Primary □ illiterate

Socioeconomic characters:

4. Occupation: □ Employed  □ Unemployed

   If employed, Type of job ---------------------------------------------

5. family income/month
   □  <1000              □ 1000 – 2000 □  > 2000

I agree to complete this questionnaire concerning my health

Signature …………………….. Date ……………………..

Thank you for confiding us

Researcher / Tarik Z. El-Refi
استبيان

رقم ( )

أخي الكريم / أبنا الباحث طارق الريحاني(طالب ماجستير / الجامعة الإسلامية ) أرجو مساعدتي في إتمام هذه
الدراسة والتي صممت لدراسة خطورة السمنة على الخصوبة عند الرجال في قطاع غزة، وذلك من خلال تعقب
هذا الاستبيان وتحرير لك نلبسة سكان مني وعينة دم لإجراء بعض الفحوصات والتجارب عليها.

الاسم: .................................................................

العنوان: .................................................................

رقم الهاتف: .................................................................

الوزن: ................................................................. كجم

الطول: ................................................................. متر

(BMI) ................................................................. كجم / م²

البيانات الشخصية:

1. العمر:

2. هل عدكم أطفال □ نعم □ لا

عدد الأطفال: .................................................................

3. مستوى التعليم □ تعليم ثانوي □ إعدادي □ دبلوم / جامعة □ من أيام

البيانات الاجتماعية والاقتصادية:

4. الوظيفة □ موظف □ غير موظف

إذا كنت موظف ، نوع الوظيفة .................................................................

5. دخل العائلة الشهري □ أقل من 1000 شيقل □ 1000 - 2000 شيقل □ أكثر من 2000 شيقل

أنموافق على تعبئة هذا الاستبيان الذي يتعلق بصحتي.

التوقع / .................................................................

التوقيع / .................................................................

شكراً لتفتحمنا

الباحث / طارق زياد الريحاني