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Risk of cigarette smoking on male fertility in Gaza Governorate, Gaza Strip

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Declaration

I hereby declare that this submission is my own work and, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree of the university or other institute, except where due acknowledgement has been made in the text.

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Abstract

Background: Epidemiological studies have shown an association between smoking and increasing risk of male infertility.

Aim: To assess the risk of cigarette smoking on male fertility in Gaza Governorate, Gaza Strip.

Place of study: Specialized laboratories in Gaza Governorate, Gaza Strip.

Time of study: From October, 2010 to June, 2011

Subjects and Methods: The present case control study included 54 smokers (cases) and 54 non smokers (controls) matched with age. Data were collected by questionnaire interview, semen and hormonal analysis. Data were analyzed using SPSS version 18.

Results: The mean ages of controls and cases were 33.5±7.6 and 32.7±6.3 years, respectively. The percentage of cases who had children was lower than controls (37.0% v 81.5%, P=0.000). The percentages of cases who had testes surgery, previous semen and hormone analysis were higher than controls (33.3% v 11.1%; 88.9% v 29.9% and 83.3% v 9.3%, P=0.005, P=0.000 and P=0.000, respectively). Passive smoking was significantly associated with male fertility (P=0.000). The majority of cases were cigarette smokers and around half of them smoked more than 20 cigarettes/day. In addition, more than half of the cases smoked for 5-15 years. The longer duration of smoking, the less number of cases having children (P=0.026). Smoking had negative impact on sexual desire and erection in about quarter of cases. The mean sample volume, total count and active sperm were significantly decreased in cases compared to controls (2.7±1.3, 16.7±16.1 and 22.0±16.3 v 4.1±1.9, 52.6±14.6 and 43.3±11.2, with P=0.004, P=0.000 and P=0.000, respectively), whereas the mean abnormalities and dead sperms were markedly increased (23.0±9.9 and 57.9±16.2 v 10.9±6.0 and 35.8±10.2, respectively, P=0.000). When semen parameters were related to the number of smoked cigarettes/day and duration of smoking, there was a significant decrease in the number of active sperms whereas dead sperms were

significantly increased with increasing the number of smoked cigarettes/day and smoking duration. In addition, total sperm count was significantly decreased only with increasing smoking duration. The mean levels of testosterone, luteinizing hormone (LH), follicular stimulating hormone (FSH) and prolactin in cases showed no significant decrease compared to controls. Also, the number of smoked cigarettes/day and duration of smoking had no significant effect on hormonal profile.

Conclusions: Cigarette smoking affects fertility by its main negative impact on semen parameters rather than hormonal profile at least in our patients.

Key words: Cigarette smoking, Risk evaluation, Male fertility, Gaza Strip.

خطر التدخين على خصوبة الذكور في محافظة غزة، قطاع غزة

الملخص

أظهرت الدراسات الوبائية وجود علاقة بين التدخين وزيادة خطر العقم عند الذكور.

الهدف من الدراسة

تهدف الدراسة إلى تقيم خطر التدخين على خصوبة الرجال في محافظة غزة، قطاع غزة.

مكان الدراسة

المختبرات الخاصة في محافظة غزة، قطاع غزة.

وقت الدراسة

من أكتوبر2010 حتى يونيو2011

الأشخاص والطرق المستخدمة بالدراسة

شملت الدراسة مقارنة إحصائية بين 54 مدخن (حالة) و 54 من غير المدخنين (ضوابط) متطابقين بالعمر. وقد تم جمع البيانات عن طريق استبيانات من خلال مقابلة شخصية، تحليل للحيوانات المنوية والهرمونات وقد تم تحليل البيانات باستخدام SPSS نسخة 18.

النتائج

متوسط عمر العينة الضابطة والعينة التجريبية (33.5±7.6 و32.7±6.3) سنة على التوالي، وقد كانت نسبة المدخنين المنجبين أقل من نسبة العينة الضابطة (37% مقابل 81%) ذات دلالة إحصائية. نسبة من قام بعمل جراحة للخصية وفحص سابق للحيوانات المنوية والهرمونات في المدخنين أكبر منها في العينة الضابطة (33.3%مقابل 11.1%، 88.9% مقابل 29.9% و 83.3% مقابل 9.3%) على التوالي وكلها ذات دلالة إحصائية.

التدخين السلبي كان مرتبطا مع خصوبة الرجال بدلالة إحصائية واضحة. غالبية المدخنين كانوا يدخنون سجائر وحوالي النصف منهم يدخنون أكثر من 20 سيجارة يوميا لمدة تتراوح من5-15 سنة. تم ملاحظة أنه بزيادة مدة التدخين أدى ذلك إلى قلة عدد الأولاد والقدرة على الإنجاب لدى المدخنين بدلالة إحصائية. من خلال الدراسة تبين لنا التدخين له تأثير سلبي على الرغبة الجنسية والانتصاب مع ربع المدخنين تقريبا. متوسط حجم عينة الحيوانات المنوية، العدد الكلي والنشيط منها أقل في المدخنين عنه في غير المدخنين (2.7±1.3، 16.7±16.1و22±16.3 مقابل 4.1±1.9، 52.6±14.6 و43.3±11.2بدلالة إحصائية على التوالي). بينما متوسط الحيوانات المنوية المشوهة والميتة في المدخنين كان بزيادة ذات دلالة إحصائية عنه في الغير

مدخنين (23±9.9 و 57.9±16.2مقابل 10.9±6 و 35.8±10.2 على التوالي). عند عمل مقارنة بين معلمات الحيوانات المنوية مع عدد السجائر يوميا ومدة التدخين تم ملاحظة أن هناك نقص بدلالة إحصائية في عدد الحيوانات المنوية النشطة، بينما الميت منها كان بزيادة ذات دلالة إحصائية مع زيادة عدد السجائر ومدة التدخين. بالإضافة إلى أن العدد الكلي للحيوانات المنوية كان بنقص ذو دلالة إحصائية مع زيادة مدة التدخين فقط.

متوسط هرمونات التستيستيرون، LH، FSH والبرولاكتين لم تتأثر مقارنة مع الغير مدخنين. أيضا عدد السجائر يوميا ومدة التدخين لم تؤثر سلبا على الهرمونات.

تخلص الدراسة إلى

تدخين السجائر يؤثر على الخصوبة عن طريق تأثيره السلبي على معلمات الحيوانات المنوية أكثر منه على الهرمونات على الأقل من خلال الدراسة على مرضانا.

الكلمات المفتاحية

تدخين السجائر، تقيم خطورة، خصوبة الرجال، قطاع غزة.

Dedication

This work is dedicated to:
The dearest persons to my heart my father and my mother
The dearest to me; my wife, children : Shahed, Mohammed and Raghad
brothers and sisters

Dedication is almost expressed to the Palestinian people who
have suffered and will be struggling with the persistence to
have a free Palestine.

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At the end, I am very grateful to those who participated and helped me to complete this study.

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Abbreviations

GnRH	Gonadotropin-releasing hormone
LH	Luteinizing hormone
FSH	Follicular stimulating hormone
TSH	Thyroid stimulating hormone
WHO	World Health Organization
DNA	Deoxyribonucleic acid
SHBG	Sex hormone binding globulin
TeBG	Testosterone binding globulin
hCG	Human chorionic gonadotropin
hPRL	Human prolactin
TRH	Thyrotropin-releasing hormone
E2	Estradiol
T	Testosterone
DHEA	Dehydroepiandrosterone
DHT	Dihydrotestosterone
ED	Erectile dysfunction
ELISA	Enzyme-linked immunosorbent assay

Chapter 1

Introduction

1.1 Overview

Fertility is the natural capability to produce offspring. As a measure, "fertility rate" is the number of children born per couple, person or population. The testes are the main male reproductive organs 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 and Haider, 2004).

Human fertility depends on factors of nutrition, sexual behavior, culture, instinct, endocrinology, timing, economics, way of life, and emotions. There are also some other factors that have relative effect on male fertility like cigarette smoking (Kidd et al., 2001). Fertility does not decline in men as sharply as it does in women. There have been examples of males being fertile at 94 years old (Sherman, 1991).

According to the World Health Organization (2002), approximately one-third of the world’s male adult population (above 15 years of age) smokes. The combustion of tobacco yields about 4000 chemical compounds, some of which are deadly toxic. Given that cigarette smoke contains more than 30 compounds known to be mutagens, or carcinogens such as" radioactive polonium, benzo(a)pyrine, dimethylbenz(a)anthracene, dimethylnitrosamine, naphthalene, and methylnapthalene" which have a direct deleterious effects on human embryos and female and male germ cells are probable (Zenzes, 2000).

Other chemical compounds in tobacco including nicotine and its metabolite cotinine which may lead to poor semen function and resultant infertility (Arabi and Shareghi, 2005). Cigarette smoke contains also carbon monoxide which may affect male reproduction via direct effect on the testicular function and spermatogenesis; this mechanism may involve the hormonal control of spermatogenesis or via direct effect upon the germ cells and Sertoli cells of the seminiferous epithehum. Such alterations may lead to infertility and/or production of mutated spermatozoa which may subsequently cause an adverse pregnancy outcome if the mutated spermatozoa were to fertilize an egg (Hanrahan et al., 1996). Difficulties in getting pregnant may be from the effects of the nasty chemicals in cigarette smoke that affect the man's sperm and libido (Quitters Guide, 2008).

The rate of smoking in the Palestinian territories is among the highest in the world compared to the other population. The prevalence of smoking in Gaza Strip was estimated to be 13.7% (The Palestinian Central Bureau of Statistics, 2007). Sear smokers annually in the West Bank and Gaza are approximately $ 450 million. The number of deaths caused by smoking each year in the West Bank and Gaza Strip, at least 4 thousand deaths (Statistics Of Smoking in Gaza Strip, Tahady.Com, 2006).

The subject of possible detrimental effects of cigarette smoking on reproductive performance and specifically on semen parameters in the male is of great interest (Wong et al., 2000; Ramlau-Hansen et al., 2007 and Coelho et al., 2009). But the data available are somewhat inconclusive. Despite the increased number of smokers in the Gaza Strip, particularly in young adulthood there was no study link between smoking and fertility in men. This will be the first study to investigate risk evaluation of cigarette smoking on male fertility in Gaza Governorate, Gaza Strip

1.2 General objective

The general objective of the present study is to assess the risk of cigarette smoking on male fertility in Gaza Governorate, Gaza Strip.

1.3 Specific objectives

1. To assess the impact of smoking on semen parameters including volume, total count, abnormalities and motility.
2. To estimate the effect of smoking on testosterone, FSH and LH and prolactin hormones.
3. To find out possible relations between number of cigarette/day in relation to semen parameters and hormonal profile of smokers.
4. To investigate possible relations between duration of smoking in relation to semen parameters and hormonal profile of smokers.

1.4 Significance

1. Smoking is a global problem and there are deficiency in health education programs to determine risk of smoking on fertility.
2. The rate of smoking in the Palestinian territories is among the highest in the world compared to the other population. The prevalence of smoking in Gaza Strip was estimated to be 13.7%.
3. Cigarette smoking has become a serious health and social problem today in Gaza Strip.
4. No published research was done on the effect of smoking on male fertility in Gaza Strip.

Chapter 2

Literature review

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.

2.1 Structure of testis

The testis consists of convoluted seminoferous 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).

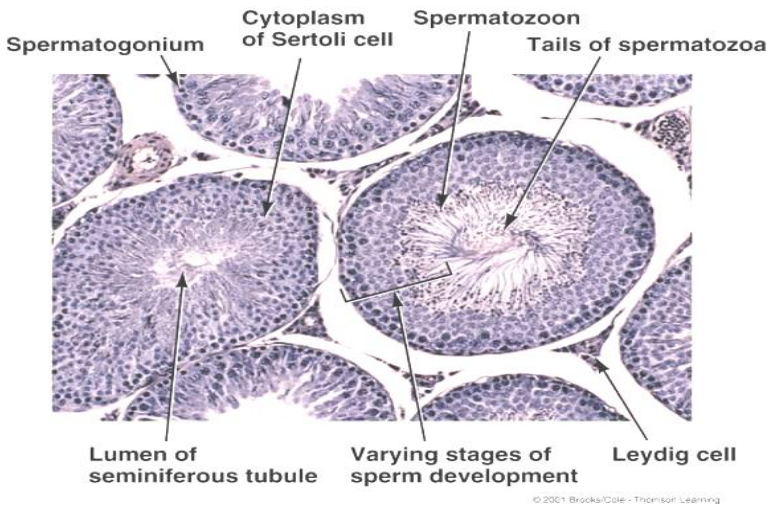

Figure 2.1. cross-section of the testis showing sperm producing tube (seminiferous tubule) and leydig cell (www.octc.kctcs.edu/GCaplan/anat2/notes/male.jpg)

2.2 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:

2.2.1 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.

2.2.2 Spermatocytes/Meiosis

2.2.2.1 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).

2.2.3 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).

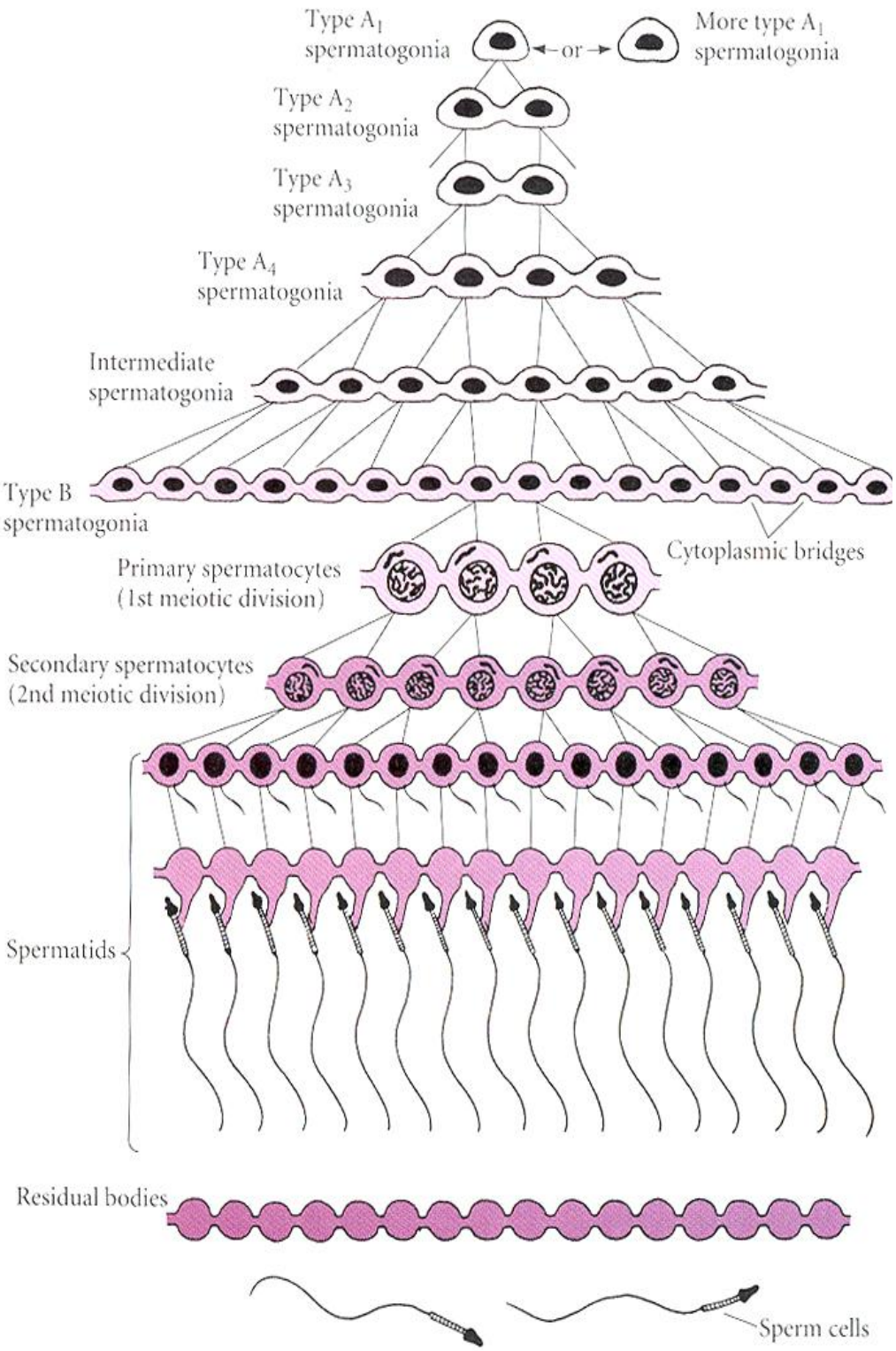

Figure 2.2 Sperm formation (spermatogenesis)
(http://i.quizlet.net/i/PQtxZrsghxHmImLojule_g_m.jpg)

2.2.4 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).

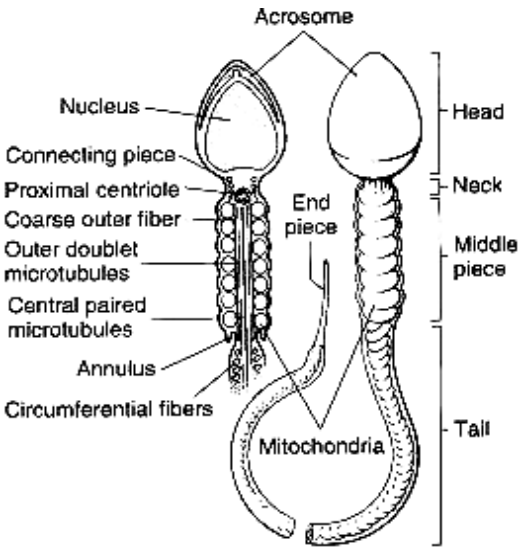

Figure 2.3 Schematic drawing of the human spermatozoon
(www.genericlook.com/anatomy/Spermatozoa/)

2.3 Semen parameters

2.3.1 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 et al., 1994).

2.3.2 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).

2.3.3 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).

2.3.4 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.

2.4 Male fertility hormones

2.4.1 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 luteinizing hormone (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).

2.4.2 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), 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, 1973). Both FSH and LH are secreted in a pulsatile manner, with rapid fluctuations over the normal range (Catt, 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, 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. FSH may also be elevated in Klinefelter's syndrome (seminiferous tubule dysgenesis) or as a consequence of Sertoli cell failure (Franchimont, 1973).

2.4.3 Prolactin hormone

Human prolactin (hPRL) is a single chain polypeptide of 199 amino acids and a molecular weight of approximately 23,000 daltons. Its existence as a distinct chemical entity, separate from growth hormone, was established through a series of studies between 1965 and 1971 (Frantz, et al.,1972 and Niall, 1972). Prolactin is produced by the anterior pituitary and its secretion is regulated physiologically by inhibitory (Talwalker, et al., 1963) and releasing (Bowers, et al., 1971) factors of the hypothalamus. Prolactin appears in the blood promptly after administration of thyrotropin-releasing hormone (TRH) (Friesen, et al., 1972).

The major physiologic action of prolactin is the initiation and maintenance of lactation in women. Hyperprolactinemia has been established as a common cause of infertility and gonadal disorders in men and women. Prolactin has been shown to inhibit the secretion of ovarian steroids (Demura, et al., 1982 and Kauppila, et al., 1982) and to interfere with follicle maturation and the secretion of LH and FSH (Andersen, et al., 1982) in the human female. Measurement of elevated serum prolactin levels may provide the first quantitative evidence of pituitary dysfunction (Franks, et al., 1977). Quantitation of prolactin levels is also of interest in the evaluation and management of patients with amenorrhea and galactorrhea (Frantz, 1978).

2.5 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). 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).

2.5.1 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).

2.5.2 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).

2.6 Smoking and male fertility

Attia et al. (1989) compared serum levels of estradiol (E2), prolactin (PRL), and total testosterone (T) in 50 heavy smokers (median 23.5 cigarettes/day) and 35 men who never smoked. The median age was 25.4 years among smokers and 27.4 years among nonsmokers. The differences between these 2 groups of men in terms of E2 and PRL levels were significant. Smokers showed elevated E2 levels (median, 59.8±1.83 pcg/ml) compared with nonsmokers (median, 48.6±0.9 pcg/ml) (P=0.001). The median serum PRL level was 10.11±0.55 ng/ml in smokers compared with 7.88±0.54 ng/ml in nonsmokers (P=0.001). The median level of serum T did not differ significantly between smokers (4.53±0.17ng/ml) and nonsmokers (4.55±0.24 ng/ml).

Field et al. (1994) examined the relationship of cigarette smoking to serum dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), androstenedione, cortisol, testosterone, albumin-bound testosterone, free testosterone, dihydrotestosterone (DHT), and sex hormone-binding globulin (SHBG) in 1241 randomly sampled middle-aged U.S. men. Compared with nonsmokers and independent of relative weight and age, cigarette smokers had increased serum levels of DHEA (18% higher, P=0.0002), DHEAS (13% higher, P=0.0007), cortisol (5% higher, P=0.01), androstenedione (33% higher, P=0.0001), testosterone (9% higher, P=0.009), DHT (14% higher, P=0.004), and SHBG (8% higher, P=0.004).

A cross-sectional study was conducted to determine the semen parameters of proven fertile males in Singapore and compare it with the World Health Organization (WHO) recommended normal values and to examine some factors that may affect spermatogenesis (Chia et al., 1998). A total of 243 men, whose wives were pregnant at the time of collection of semen, provided a semen sample each after sexual abstinence for 3 days. A questionnaire was used to elicit occupational exposure, alcoholic consumption, smoking history and past significant medical history. Most subjects had normal sperm volume (56.4%), concentration (79.8%), motility (69.5%) and viability (53.5%) based on WHO criteria. However, fertile men had a low mean percentage of normal sperm

morphology (20.0%), although they were normally distributed. Cigarette smoking was associated with significantly lower semen volumes even after adjusting for alcohol consumption. The WHO criterion for normal sperm morphology is too stringent, and should be adopted with caution.

Wong et al. (2000) evaluated the impact of cigarette smoking on male factor subfertility and the semen parameters of sperm count, motility, and morphology by questionnaire and determination of the cotinine concentrations in blood and seminal plasma of fertile and subfertile males. A total of 107 fertile and 103 subfertile males from Netherlands provided a standardized blood and semen specimen and completed a self-administered questionnaire about their smoking habits. A higher frequency of cigarette smoking was observed in subfertile males than in fertile males, with an odds ratio of 1.7 (95% confidence interval, 0.9-3.2). The self-reported number of cigarettes smoked per day correlated with the cotinine concentrations in blood and seminal plasma for both groups. A small but statistically significant correlation was found between cotinine concentrations in seminal plasma and the percentage of abnormal sperm morphology, but not for other semen parameters.

The effect of smoking on the semen quality in infertile men in China was studied (Zhang et al., 2000). Adult non-drinker males attending the infertility clinic, including 110 non-smokers and 191 smokers, were recruited for the study. Sixty-one fertile, non-smoker and non-drinker males, who had one or more children, served as the controls. The semen volume, acidity, sperm density, viability and forward progression, as well as the seminal plasma contents of Zn, Cu and SOD were much lower in the medium, heavy and long-term smokers than in the non-smokers ($P<0.01$). The sperm density, viability and forward progression, and the seminal plasma Zn, Cu and SOD levels were negatively correlated with the amount and duration of cigarette smoking ($P<0.01$).

English et al. (2001) determined whether smoking affects the level of bioavailable testosterone. A case-control study of 25 healthy male smokers and 25 healthy never-smokers, matched by age and body mass index was carried

out. Early morning levels of total, free and bioavailable testosterone, 17beta-oestradiol, sex hormone binding globulin (SHBG) and cotinine were determined and compared between the two groups. Levels of total (18.5±4.6 nM versus 15.1±4.9 nM, P=0.01) and free testosterone (462±91 pM versus 402±93 pM, P=0.03) were found to be higher in smokers compared with non-smokers respectively, as was SHBG (34.1±12.8 versus 28.1±9.0 nM, P=0.06). There were no significant differences in the levels of bioavailable testosterone (3.78±1.59 versus 3.51±1.26 nM, P=0.49) or 17beta-oestradiol (44.5±11.4 versus 42.3±11.5 pM, P=0.50) between smokers and non-smokers respectively. These data suggest that cigarette smoking has no significant effect on the biologically active fraction of testosterone, but may influence the levels of total and free testosterone through changes in the levels of SHBG.

The role of smoking on the risk of erectile dysfunction (ED) using data from a cross-sectional study on prevalence and risk factors for ED in 2010 Italian men aged more than 18 years were analyzed (Mirone et al., 2002). In comparison with never smokers, current smokers had an odds ratio (OR) of ED of 1.7 (95% confidence interval (CI), 1.2–2.4) and ex-smokers of 1.6 (95% CI, 1.1–2.3). The association between smoking and ED risk was present in subjects without a history of any cardiovascular disease, cardiopathy, hypertension, diabetes and neuropathy, but not in those with a history of these conditions. For example, the ORs of ED in smokers, in comparison with never smokers, were respectively 2.4, 2.0 and 1.7 in men with no history of any cardiovascular disease, diabetes and neuropathy, but respectively 1.0, 1.0 and 1.2 in those with a history of the conditions.

In a cohort study Künzle et al. (2003) investigated the effect of cigarette smoking on main sperm variables in 655 smokers and 1,131 nonsmokers attending the andrology laboratory in the context of infertility investigation in the couple in Switzerland. Cigarette smoking was associated with a significant decrease in sperm density (−15.3%), total sperm count (−17.5%), total number of motile sperm (−16.6%), and citrate concentration (−22.4%). The percentage of normal forms was significantly reduced in smokers, and sperm vitality,

ejaculate volume, and fructose concentration were slightly but non significantly affected.

In a prospective study Taszarek-H et al. (2005) investigated different semen parameters of infertile smokers with a history of cigarette smoking in Poland. Twenty seven infertile men who smoked cigarettes, 79 infertile men who were nonsmokers and 82 healthy nonsmoking donors were evaluated. Smokers had significantly less spermatozoa with motility grade B (9.37% versus 11.9%, $P<0.05$), decreased results of hypoosmotic swelling test (46.39% versus 52.11%, $P<0.05$) than nonsmokers infertile men. The concentration of leukocytes in semen was significantly higher in smokers (0.26 mln/ml versus 0.11 mln/ml, $P>0.05$), which can also suggests oxidative imbalance in the ejaculates of these men. No differences were found in the assessment of sperm concentration, motility grade A, C, D, percentage normal forms and different sperm defects, induced acrosomal reaction and acidic aniline blue staining test between these two groups ($P>0.05$). Our data demonstrate that cigarette smoking alters semen quality which could worsen fertilizing capability in infertile men.

Hosseinzadeh et al. (2007) assessed the effect of cigarette smoking on sperm parameters both before and after swim-up. Semen sample provided from fertile smoker (n=25), fertile nonsmoker (n=21), infertile smoker (n=23) and infertile nonsmoker men (n=32). Semen analysis was performed manually according to the World Health Organization Standards Guidelines. Results showed that sperm parameters quality in smoker men was approximately lower than nonsmoker men and that cigarette smoking has dose dependent effect on sperm parameters, but this effect was not significant. Therefore, it appears that cigarette smoking is associated with reduced sperm quality and the risk of idiopathic male infertility in smoker men.

The association between current smoking and semen characteristics and hormonal levels in a large group of healthy men was studied (Ramlau-Hansen et al., 2007). From 1987 to 2004, seven separate occupational or environmental

semen quality studies were co-ordinated. A total of 2562 men participated, each providing semen and blood sample and answering a questionnaire about lifestyle and factors related to health. Appropriate semen and smoking data were available for 2542 men. Adjusting for study, age and other covariates, an inverse dose–response relation between smoking and semen volume, total sperm count and percentage motile sperm was observed. Heavy smokers had a 19% lower sperm concentration than non-smokers. A positive dose–response relationship between smoking and testosterone, LH and the LH/free testosterone ratios was found.

In a retrospective comparative study Coelho et al. (2009) analyzed the effects of cigarette consumption on semen parameters of 327 men in Portugal. The semen parameters were first compared between smokers and nonsmokers and then a heavy smokers/light smokers analysis took place. A total of 135 (41%) were smokers and 55 (40.7%) were heavy smokers. The demographic characteristics were similar between groups. The tobacco use was associated with an increased incidence of oligo/zoosperm, without difference in the other semen parameters. It was also noted a negative correlation between heavy smoking habits and semen volume abnormalities.

Collodel et al. (2009) investigated whether smoking cigarettes increases the effect of varicocele on sperm morphology. The semen quality of 2 groups of patients with varicocele were compared, those who smoked (n=121) and those who did not (n=158). The semen parameters were evaluated, and sperm morphology was assessed using transmission electron microscopy and quantitatively elaborated. In the smoker and nonsmoker varicocele-associated cases, sperm motility and the results from transmission electron microscopy analysis were significantly impaired compared with controls. However, a non significant difference was detected when the semen parameters were compared. Subsequently, the patients were divided into 4 groups: mild (≥1 but ≤10 cigarettes/d), moderate (>10 but <20 cigarettes/d), and heavy (≥20 cigarettes/d) smokers and a group of randomly chosen nonsmoker patients with varicocele. The sperm motility, sperm concentration, and fertility index

decreased and the percentage of sperm pathologic features increased as the number of cigarettes smoked daily increased.

The effect of smoking cigarette or shisha tobacco on semen parameters in 100 men with subfertility was investigated (Hussein et al., 2011). There were 50 smokers with mean age of 37.1±8.3 years and 50 non-smokers with a mean age of 39.5±9.0 years. When compared with non-smokers, the smokers had a significantly higher mean percentage of abnormal sperm forms (92.3% vs 89.4%) ($P<0.01$), a significantly lower mean sperm count (20.6 M/mL vs 44.9 M/mL), a significantly lower mean semen volume (2.01 mL vs 2.52 mL), and a significantly lower mean percentage of sperm motility (25.7 vs 37.9) (all with $P<0.01$). There were no statistically significant differences in semen parameters between men smoking cigarettes or shisha. There were statistically significant negative correlations between smoking index and the percentage of sperm motility ($r=-0.38$; $P=0.006$) and between smoking duration and the percentage of sperm motility ($r=-0.32$; $P=0.026$). There was also a marginally significant positive correlation between smoking index and the percentage of abnormal sperm forms ($r=0.28$; $P=0.046$). Correlations with other semen parameters were not significant.

Chapter 3

Materials and Methods

3.1 Study design

The present study is a case control design.

3.2 Target population

The target population is smokers and non smokers men aged 20-50 years old from Gaza Governorate, Gaza Strip.

3.3. Sampling and sample size

Samples were collected from married men who visiting central laboratories such as Shaker and Balsam laboratories in Gaza Governorate and seeking for semen analysis. Samples were defined as non probability accidental samples. Men were divided into smoker and nonsmoker groups. 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 smoker non smoker (control) was found to be 51:51. For a no-response expectation, the sample size was increased to 54 smokers and was recruited from Gaza central laboratories. The controls were consisted of 54 nonsmokers. Case and control men were matched for age.

3.4 Exclusion criteria

Men with known causes of infertility, using contraceptive methods, newly married men and men with chronic diseases or use chronic medications.

3.5 Limitation of the study

Place of the study was changed from Nuseirat area to Gaza governorate due to deficiency of samples.

3.6 Ethical considerations

The necessary approval to conduct this study was obtained from Helsinki committee in Gaza Strip (Annex 1). Helsinki committee is an authorized professional body for giving permission to researchers to conduct their studies with ethical concern in the area. The participants were given a full explanation about the purpose of the study and assurance about the confidentiality of the information and that the participation is optional.

3.7 Data collection

3.7.1 Questionnaire interview

A face to face interview was used for filling in the questionnaire which is designated for matching the study need. The questionnaire (Annex 2) was based on Centers for Disease control and Prevention smoking questionnaire with some modification (Centers for Disease control and Prevention, CDC, 2009). During the study the interviewer explained to the patients any of the confused questions that may be not clear to them. Most questions were the yes/no questions, which offer a dichotomous choice (Backstrom and Hursh-César, 1981). The questionnaire was validated by four experts in the fields of epidemiology, public health, and environment. The questionnaire was piloted with 10 patients, and with few modification as necessary to improve reliability. The questionnaire included questions on the personal data (age, having children, education and employment), medical history (Testes trauma and surgery, previous semen and hormone analyses) and smoking data (number of cigarette/day, smoking duration, impact of smoking on sexual desire and erection).

3.7.2 Semen collection and analysis

Semen samples were collected from smokers and non smokers by masturbation in sterile polypropylene containers after sexual abstinence of 3–5 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).

3.7.3 Blood sampling and processing

Venous blood sample (5 ml) was drawn by a well trained medical technologist into vacutainer tubes from smokers and nonsmokers. Blood left for a while without anticoagulant to allow blood to clot. Then serum samples were obtained by centrifugation at room temperature at 3000 rpm/10 minutes for hormonal analysis.

3.7.4 Hormonal analysis

3.7.4.1 Testosterone hormone assay

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

Principle of the assay

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 H_2SO_4.
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 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 minute at room temperature (~25°C).
6. Each well was aspirated and washed 3 times by adding 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 minute 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 of results

The absorbance for each standard, control, or samples were obtained, and then the stander curve prepared by plotting 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).

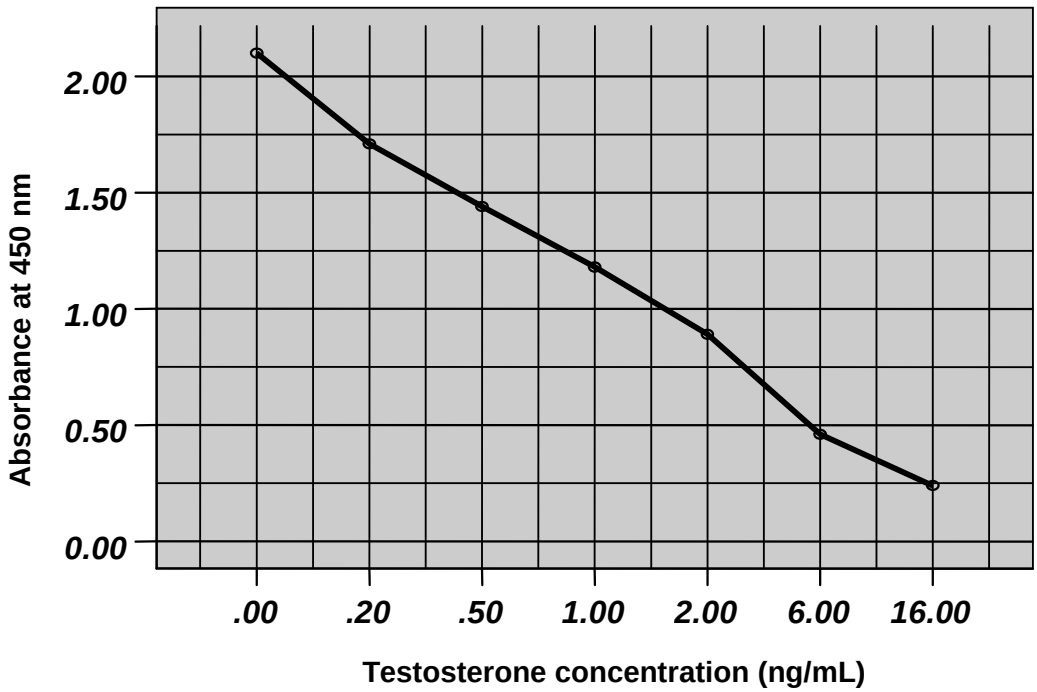

Figure 3.1. Standard curve for testosterone hormone

Normal reference values of testosterone for adult male

The normal range between 2.0-7.0 ng/mL.

3.7.4.2 Luteinizing hormone (LH) assay

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

Principle of the assay

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 enzyme-labeled 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 stripholder 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 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 seconds.
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 of results

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).

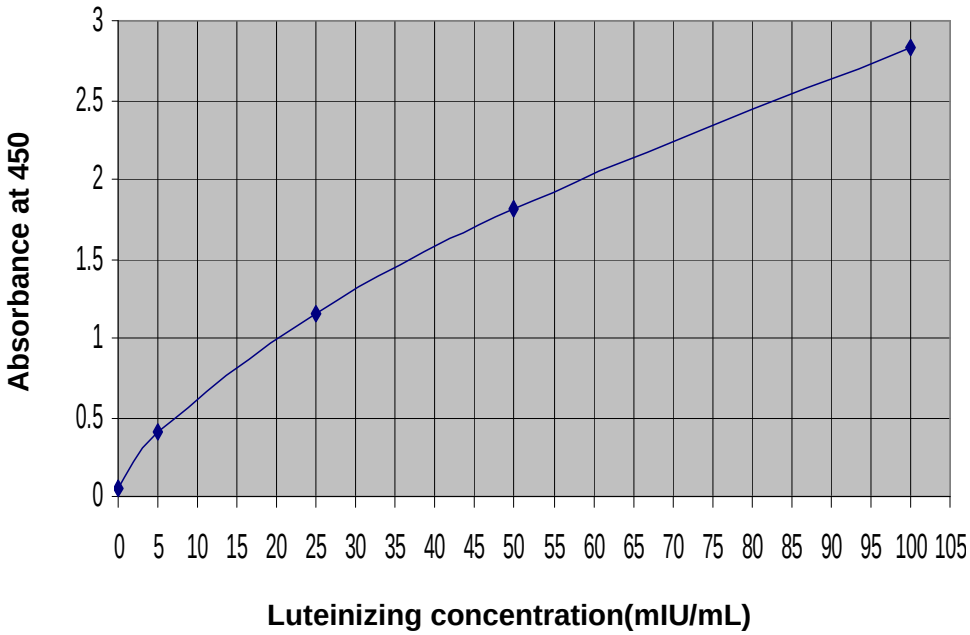

Figure 3.2. Luteinizing hormone standard curve

Normal reference values of LH for adult male

The normal range between 2.0-13.0 mIU/mL.

3.7.4.3 Follicle stimulating hormone (FSH) assay

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

Principle of the assay, kit components, **assay procedure and calculation of results were the same as in pages 23, 24 and 25 and instead of LH write FSH.**

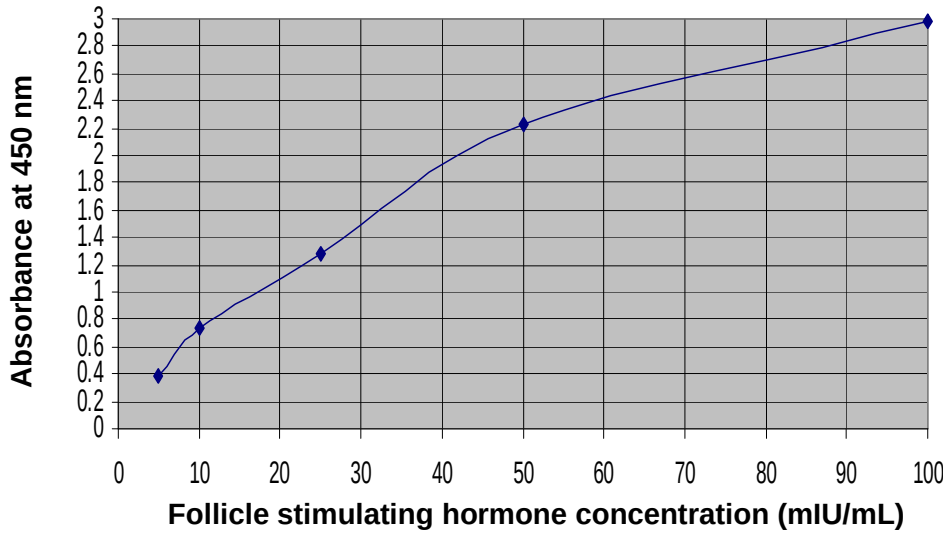

Figure 3.3. Follicle stimulating hormone standard curve

Normal reference value of FSH for adult female

The normal range between 2.5-10.0 mIU/mL.

3.7.4.4 Prolactin hormone assay

Prolactin hormone level was determined according to (Tietz, 1995) method using ELISA TECO kit for prolactin.

Principle of the assay

The essential reagents required for sandwich enzyme-linked immunoassay 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, a certain amount of prolactin hormone calibrator, patient specimen or control is first added to a microplate well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes of PRL) are added and the reactants mixed. Immobilization of the tagged PRL occurs through the interaction of streptavidin coated on the well and the added biotinylated monoclonal anti-PRL antibody. Upon mixing a reaction results between the native antigen contained in serum, the monoclonal biotinylated antibody and the enzyme-labeled antibody, without competition or steric hindrance, to from a soluble sandwich complex bound to the surface of microplate through the streptavidin-biotin system. 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 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 stripholder containing 96 microtitration wells coated with streptavidin, six prolactin reference standards with concentrations of approximately (0, 5.0, 10, 25, 50 and 100 ng/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. Twenty-five 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.
4. The wells were mixed for 30 seconds.
5. The wells were incubated for 60 minute at room temperature (~25°C).
6. Each well was aspirated and washed 3 times by added 300 µL of working wash solution.
7. One hundred µL of TMB reagent 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 minute at room temperature (~25°C).
9. Fifty 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 of results

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 prolactin in ng/mL from the stander curve (Figure 3.4).

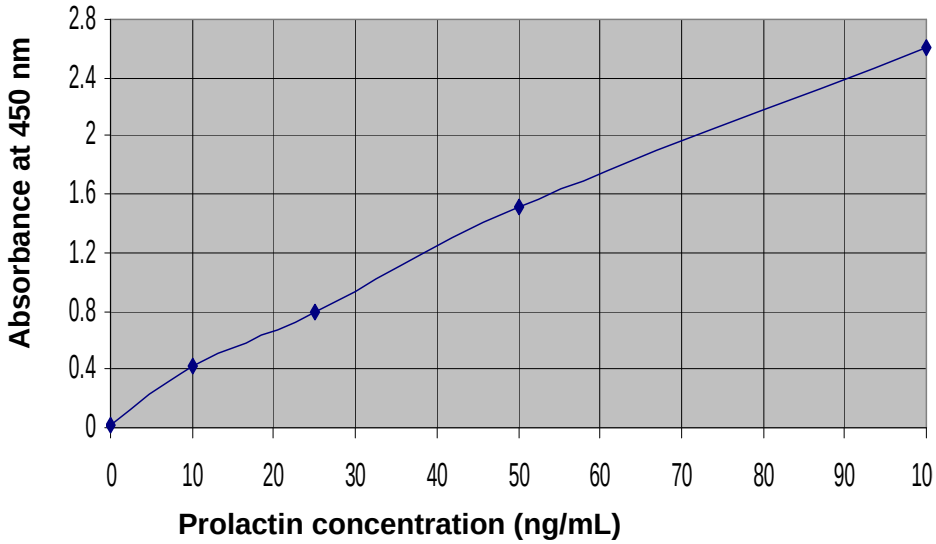

Figure 3.4. Prolactin hormone standard curve.

Normal reference values of prolactin for adult male

The normal range between 2.0-12.0 ng/mL.

3.8 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 (χ^2)** was used to identify the significance of the relations, associations, and interactions among various variables. **Yates's continuity correction test, $\chi^2_{(corrected)}$,** 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 patients and controls hormones. **The one-way ANOVA test** was applied.

The results in all the above mentioned procedures were accepted as statistical significant when the p-value was less than 5% ($p<0.05$).

Range as minimum and maximum values were used.

The percentage difference was calculated according to the formula:
Percentage difference equals the absolute value of the change in value, divided by the average of the 2 numbers, all multiplied by 100.
Percent difference = (| (V1 – V2) | / ((V1+ V2)/2)) X 100

Graphs were plotted using SPSS system version 18.0.

Chapter 4

Results

4.1 Personal characters of the study population

Table 4.1 illustrates the personal data of the study population. Age classification showed that 28 (51.9%) controls and 31 (57.4%) cases were 20-30 years old. Age group 31-40 years comprised 20 (37.0%) controls and 18 (33.3%) cases. Controls and cases aged 41-50 years old were 6 (11.1%) and 5 (9.3%), respectively. The difference between controls and cases in term of age distribution was not significant (χ^2=0.349, P=0.840). The mean ages of controls and cases were 33.5±7.6 and 32.7±6.3 years old with ranges of 22-50 and 24-50 years, respectively. The independent sample t-test also showed no significant difference between mean ages of controls and cases (t=0.378, P=0.707). Forty four (81.5%) controls compared to 20 (37.0%) cases having children (Figure 4.1), and the difference between controls and cases was significant (χ^2=22.091, P=0.000) indicating that smoking is a risk factor of male infertility. Analysis of the educational status of the study population showed that 24 (44.4%) controls and 26 (48.1%) cases had a university degree, 20 (37.0%) and 14 (25.9 %) finished secondary school, 8 (14.8%) and 10 (18.5%) had finished preparatory school and 2 (3.7%), 4 (7.4%) had passed primary school. The difference between various education levels of controls and cases was not significant (χ^2 corrected=0.978, P=0.807). It is worth mentioning that non of controls and cases were illiterate. The employed controls and cases were 27(50.0%) and 22 (40.7%) whereas 27 (50.0%) controls and 32 (59.3%) cases were unemployed (χ^2=0.934, P=0.334).

Table 4.1 Personal data of the study population.

Personal character	Controls (n=54)		Cases (n=54)		Statistical test	p-value
	No.	%	No.	%		
Age (Year)						
20-30	28	51.9	31	57.4	χ^2=0.349	0.840
31-40	20	37.0	18	33.3		
41-50	6	11.1	5	9.3		
Mean±SD	33.5±7.6		32.7±6.3		t = 0.378	0.707
Range	22-50		24-50			
Have children						
Yes	44	81.5	20	37.0	χ^2=22.091	0.000
No	10	18.5	34	63.0		
Education						
University	24	44.4	26	48.1	χ^2=0.978	0.807*
Secondary school	20	37.0	14	25.9		
preparatory school	8	14.8	10	18.5		
primary	2	3.7	4	7.4		
Employment						
Employed*	27	50.0	22	40.7	χ^2=0.934	0.334
Un-employed	27	50.0	32	59.3		

*p-value of χ^2 (corrected) test

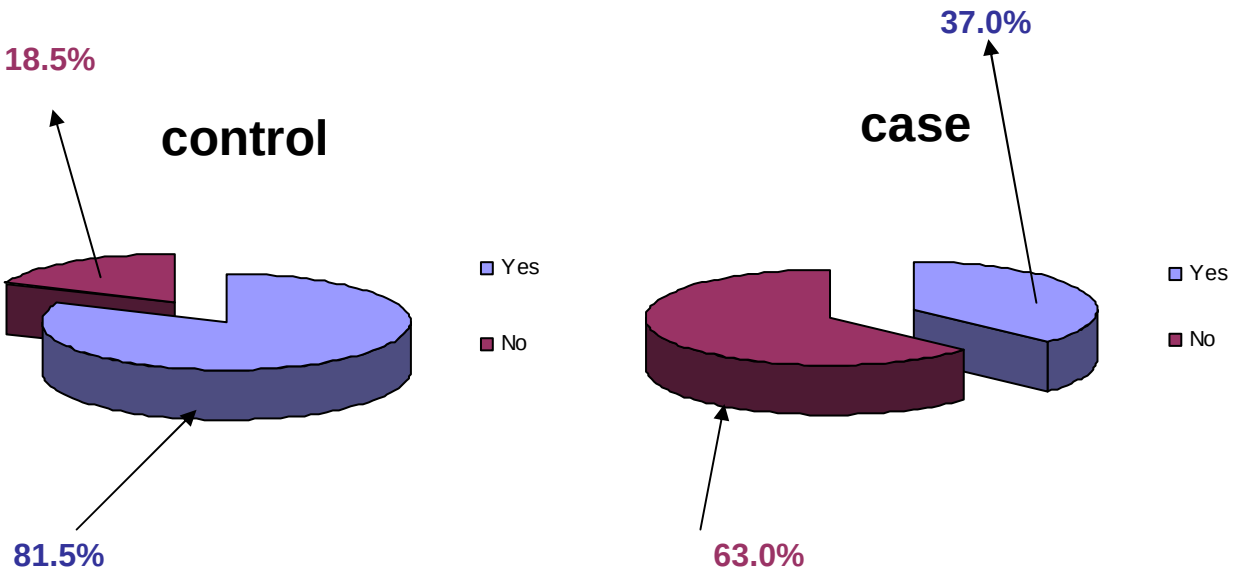

Figure 4.1. Distribution of controls and cases by having children

4.2 Medical history of the study population

The medical history of the study population is presented in Table 4.2. None of the controls 0 (0.0%) had trauma compared to 4 (7.4 %) cases. The difference between the two groups was not significant (χ^2 corrected=2.337, P=0.126). Regarding testes surgery, 6 (11.1%) controls had surgery in testes compared to 18 (33.3%) cases. The difference was significant (χ^2 =7.714, P=0.005). Controls who had semen and hormone analyses were 16 (29.9%) and 5 (9.3%) compared to 48 (88.9%) and 45 (83.3%) cases (χ^2 =39.273, P=0.000 and χ^2 =59.586, P=0.000).

Table 4.2 Medical history of the study population.

Medical history	Controls (n=54)		Cases (n=54)		Statistical test	p-value
	No.	%	No.	%		
Testes trauma						
Yes	0	0.0	4	7.4	χ^2=2.337	0.126*
No	54	100	50	92.6		
Testes surgery						
Yes	6	11.1	18	33.3	χ^2=7.714	0.005
No	48	88.9	36	66.7		
Semen analysis						
Yes	16	29.6	48	88.9	χ^2=39.273	0.000
No	38	70.4	6	11.1		
Hormone analysis						
Yes	5	9.3	45	83.3	χ^2=59.586	0.000
No	49	90.7	9	16.7		

*p-value of $\chi^2_{(corrected)}$ test

4.3 Smoking and fertility

4.3.1 Passive smoking among the study population

Table 4.3 demonstrates passive smoking among the study population. A total of 20 (37.0%) controls and 52 (96.3%) cases had been exposed to passive smoking compared to 34 (63.0%) and 2 (3.7%) who had not. The difference between the two groups was significant (χ^2 corrected= 40.042, P=0.000).

Table 4.3 Passive smoking among the study population.

Medical history	Controls (n=54)		Cases (n=54)		Statistical test	P-value*
	No.	%	No.	%		
Passive smoking						
Yes	20	37.0	52	96.3	χ^2=40.042	0.000
No	34	63.0	2	3.7		

*p-value of χ^2 (corrected) test

4.3.2 Type of smoking and frequency/day among cases

Type of smoking and frequency/day among cases are summarized in table 4.4. Forty six (85.2%) cases were cigarette smokers and 8 (14.8%) were nergella smokers. The number of cases who smoked cigarette <10, 10-20 and >20 cigarettes/day were 10 (21.7%), 16 (34.8%) and 20 (43.5%), respectively whereas those smoked nergella one time, 2 times and >2 times/day were 4 (50.0%), 2 (25.0%) and 2 (25.0%), respectively.

Table 4.4 Type of smoking and frequency/day among cases.

Type of smoking and number/day	Cases (n=54)	
	No.	%
Cigarette		
Yes	46	85.2
No	8	14.8
No. of cigarette/day		
<10	10	21.7
10-20	16	34.8
>20	20	43.5
Nergella		
Yes	8	14.8
No	46	85.2
No. of Nergella/day		
One time	4	50.0
2 times	2	25.0
>2 times	2	25.0

4.3.3 Duration of smoking among cases

Duration of smoking among cases is pointed out in Table 4.5. The number of cases who smoked for <5, 5-15 and >15 years were 14 (25.9%), 28 (51.9%) and 12 (22.2%), respectively.

Table 4.5 Duration of smoking among cases

Smoking duration (year)	**Cases (n=54)**	
	No.	**%**
<5	14	25.9
5-15	28	51.9
>15	12	22.2

4.3.3.1 Duration of smoking in relation to have children among cases

Table 4.6 and Figure 4.2 provide duration of smoking in relation to have children among cases. Ten (18.5%), 6 (11.1%) and 4 (7.4%) cases who have children smoked for <5, 5-15 and >15 years, respectively compared to 4 (7.4%), 18 (33.3%) and 12 (22.2%) who have no children (χ^2 corrected=7.267, P=0.026).

Table 4.6 Duration of smoking in relation to have children among cases

Smoking duration (year)	**Cases (n=54) Have children**		**χ^2**	**P-value***
	Yes (%)	**No (%)**		
<5	10 (18.5)	4 (7.4)		
5-15	6 (11.1)	18 (33.3)	7.267	0.026
>15	4 (7.4)	12 (22.2)		

* p value of $\chi^2_{(corrected)}$ test

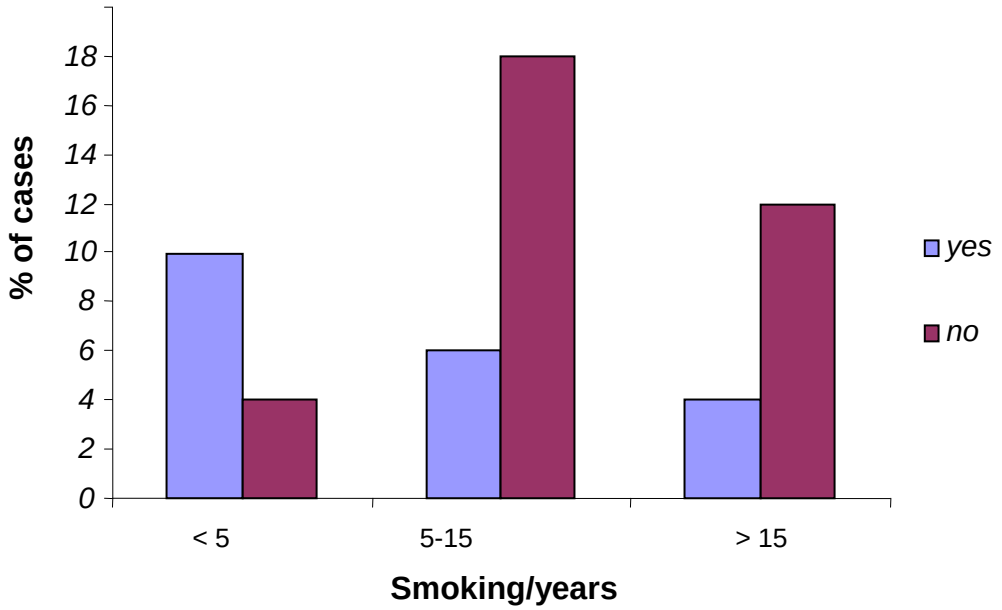

Figure 4.2 Duration of smoking in relation to have children among cases

4.3.4 Negative impact of smoking on sexual desire and erection among cases

Negative impact of smoking on sexual desire and erection among cases is given in Table 4.7. The number of cases reported negative impact of smoking on sexual desire was 16 (29.6%) where as 8 (14.8%) cases mentioned that smoking has negative impact on erection.

Table 4.7 Negative impact of smoking on sexual desire and erection of cases

Negative impact of smoking on sexual desire and erection of cases	Cases (n=54)	
	No.	**%**
Sexual desire		
Yes	16	29.6
No	38	70.4
Erection		
Yes	8	14.8
No	46	85.2

4.4 Semen parameters of the study population

Table 4.8 summarized semen parameters of the study population. The means of sample volume, total count and active sperm were significantly decreased in cases compared to controls with percentage difference of 41.2%, 103.5% and 65.1%, respectively (2.7±1.3, 16.7±16.1 and 22.0±16.3 v 4.1±1.9, 52.6±14.6 and 43.3 ± 11.2, with P=0.004, P=0.000 and P=0.000 respectively). In contrast, the mean of abnormalities and dead sperms were markedly increased in cases compared to controls showing percentage differences of 71.2% and 47.1%, respectively (23.0±9.9 and 57.9±16.2 v 10.9±6.0 and 35.8±10.2, respectively, P=0.000). On the other hand, no significant change was found in sluggish sperm levels between cases and controls (21.6±9.0 v 21.0±3.5, % difference=2.8%, P=0.754).

Table 4.8 Semen parameters of the study population

Semen parameter	**Controls (n=54)**	**Cases (n=54)**	**% Difference**	**t**	**P-value**
Sample volume (ml)	4.1±1.9	2.7±1.3	41.2	2.984	0.004
Range (min-max)	(2.5-10.0)	(1.0-7.0)			
Total count (million/ml)	52.6±14.6	16.7±16.1	103.5	8.502	0.000
Range (min-max)	(21.0-75.0)	(0.2-44.0)			
Abnormalities	10.9±6.0	23.0±9.9	71.2	5.371	0.000
Range *(min-max)*	(3.0-30.0)	(12.0-40.0)			
Active (million/ml)	43.3±11.2	22.0±16.3	65.1	5.514	0.000
Range *(min-max)*	(20.0-65.0)	(0.0-65.0)			
Sluggish (million/ml)	21.0±3.5	21.6±9.0	2.8	0.315	0.754
Range (min-max)	(10.0-25.0)	(10.0-40.0)			
Dead (million/ml)	35.8±10.2	57.9±16.2	47.1	5.948	0.000
Range (min-max)	(15.0-55.0)	(25.0-85.0)			

All values are expressed as mean±SD

P>0.05: non significant, P<0.05: significant

4.5 Hormonal profile of the study population

Hormonal profile of the study population is illustrated in Table 4.9. The mean levels of testosterone, LH, FSH and prolactin in cases showed no significant decrease compared to controls with percentage differences of 16.8%, 16.7%, 9.5% and 10.6%, respectively (4.9±2.9, 4.4±1.7, 5.0±2.4 and 9.8±3.1 v 5.8±2.0, 5.2±1.0, 5.5±1.1 and 10.9±2.1, with P=0.189, P=0.060, P=0.324 and P=0.274, respectively).

Table 4.9 Hormonal profile of the study population

Hormone	**Controls (n=54)**	**Cases (n=54)**	**% Difference**	**t**	**P-value**
Testosterone (ng/ml)	5.8±2.0	4.9±2.9	16.8	1.332	0.189
Range (min-max)	(3.0-12.9)	(1.8-16.2)			
LH (mIU/ml)*	5.2±1.0	4.4±1.7	16.7	1.934	0.060
Range (min-max)	(3.3-7.2)	(3.1-9.6)			
FSH (mIU/ml)**	5.5±1.1	5.0±2.4	9.5	0.997	0.324
Range (min-max)	(4.1-9.0)	(0.1-9.0)			
Prolactin (ng/ml)	10.9±2.1	9.8±3.1	10.6	1.175	0.274
Range (min-max)	(9.8-15.2)	(5.7-14.9)			

*LH: Luteinizing hormone, **FSH: Follicle stimulating hormone

All values are expressed as mean±SD

P>0.05: non significant

4.6 Number of cigarette/day in relation to semen parameters and hormonal profile of the cases

4.6.1 Number of cigarette/day in relation to semen parameters of the cases

Table 4.10 shows the number of cigarettes/day in relation to semen parameters of the cases. There is a successive significant decrease in the mean number of active sperms with increasing number of smoked cigarettes/day showing values of 40.0±18.7, 20.6 ±10.8 and 16.8±13.3 million/ml with <10, 10-20 and >20 cigarette/day, respectively, P=0.018 (Figure 4.3). However, the mean dead sperms was significantly increased showing values of 44.2±18.5, 54.1±8.7 and 66.4±15.7 million/ml with <10, 10-20 and >20 cigarettes/day, respectively, P=0.028 (Figure 4.4). In general, sperm abnormalities were increased whereas sample volume, total count and sluggish sperms were decreased with the increased number of cigarettes/day. However, such changes were not significant (P>0.05).

Table 4.10 Number of smoked cigarettes/day in relation to semen parameters of the cases

Semen parameter	**No. of smoked cigarettes/day**				
	<10	**10-20**	**>20**	**F**	**P-value**
Sample volume (ml)	2.9±1.2	3.3±1.7	2.3±1.2	1.408	0.268
Total count (million/ml)	20.9±10.7	15.6±12.8	12.0±6.2	1.348	0.282
Abnormalities	19.6±5.5	29.0±13.7	21.7±8.6	1.588	0.230
Active (million/ml)	40.0±18.7	20.6±10.8	16.8±13.3	4.914	0.018
Sluggish (million/ml)	23.8±12.1	25.3±4.8	17.8±6.4	3.190	0.073
Dead (million/ml)	44.2±18.5	54.1±8.7	66.4±15.7	4.284	0.028

All values are expressed as mean±SD

P>0.05: non significant, P<0.05: significant

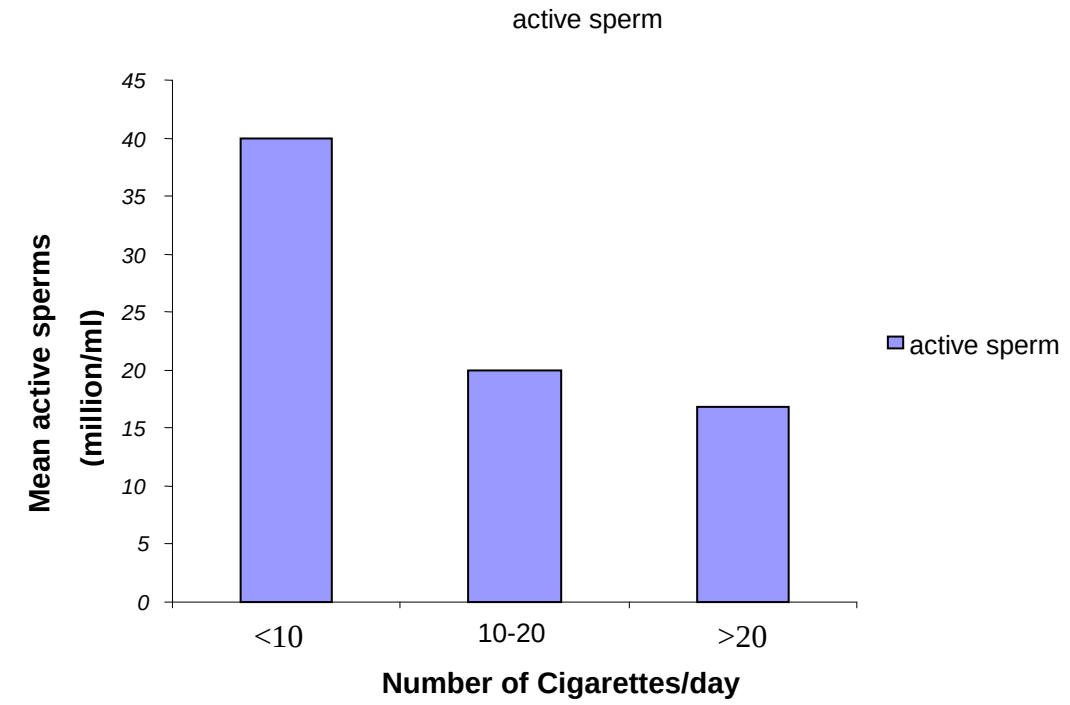

Figure 4.3 Number of smoked cigarette/day in relation to mean number of active sperms (million/ml).

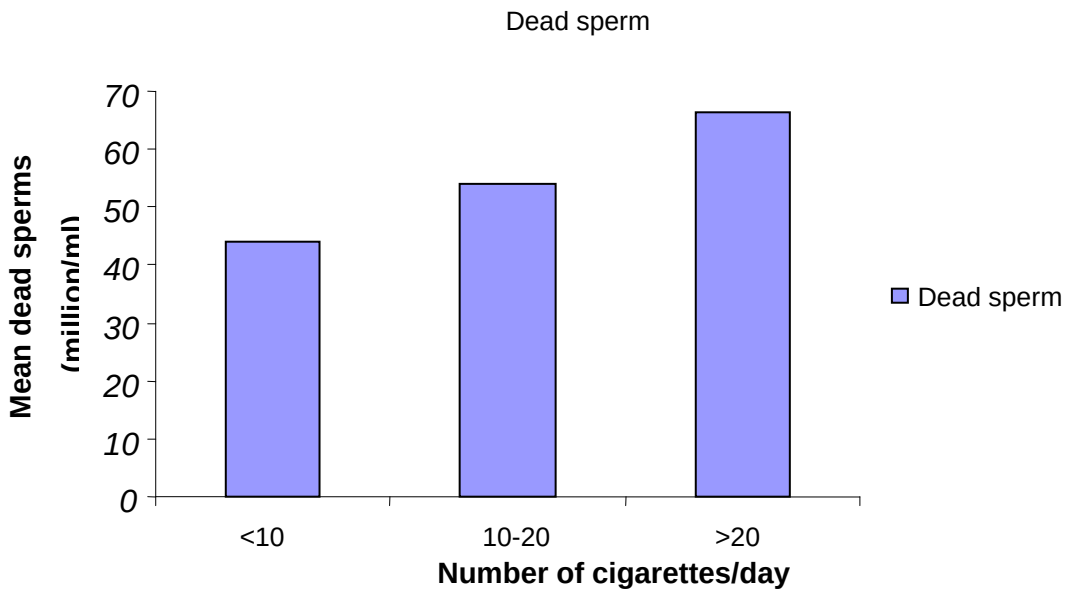

Figure 4.4 Number of smoked cigarette/day in relation to mean number of dead sperms (million/ml).

4.6.2 Number of cigarettes/day in relation to hormonal profile of the cases

The number of cigarettes/day in relation to hormonal profile of the cases is presented in Table 4.11. Testosterone and prolactin exhibited no significant increase with increasing the number of smoked cigarette/day showing maximum values of 5.7±4.3 and 10.3±2.9 with >20 cigarette/day (P=0.471 and P=0.373, respectively). In contrast, LH and FSH were gradually decreased registering maximum decreases of 3.4±1.4 and 4.7±2.7 (P=0.621 and P=0.875, respectively).

Table 4.11 Number of cigarettes/day in relation to hormonal profile of the cases

Hormone	**No. of cigarette/day**				
	<10	**10-20**	**>20**	**F**	**P-value**
Testosterone (ng/ml)	4.2±1.1	4.4±1.3	5.7±4.3	0.782	0.471
LH (mIU/ml)*	4.5±2.3	4.5±2.6	3.4±1.4	0.493	0.621
FSH (mIU/ml)**	5.4±2.3	4.8±2.8	4.7±2.7	0.135	0.875
Prolactin (ng/ml)	5.7±2.1	9.8±1.9	10.3±2.9	1.208	0.373

*LH: Luteinizing hormone, **FSH: Follicle stimulating hormone

All values are expressed as mean±SD

P>0.05: non significant

4.7 Duration of smoking in relation to semen parameters and hormonal profile among cases

4.7.1 Duration of cigarette smoking in relation to semen parameters of the cases

Table 4.12 illustrates duration of cigarette smoking in relation to semen parameters of the cases. There is an obvious significant decrease in the mean total count and active sperms with duration of smoking showing maximum decrease of 4.8±3.3 and 7.2±5.1 million/ml with >15 of smoking, P=0.021 and P=0.023, respectively (Figure 4.5 and Figure 4.6). In contrast, dead sperms

were significantly increased showing mean numbers of 49.1±14.7, 55.2±13.9 and 74.5±11.8 million/ml with <5, 5-15 and >15 years of smoking, respectively, P=0.007 (Figure 4.7). In general, sperm abnormalities were increased whereas sample volume and sluggish sperms were decreased with the increase smoking duration. However, such changes were not significant (P>0.05).

Table 4.12 Duration of cigarette smoking in relation to semen parameters of the cases

Semen parameter	Duration of cigarette smoking (Year)				
	<5	5-15	>15	F	P-value
Sample volume (ml)	3.0±1.1	2.8±1.6	2.1±0.7	0.726	0.494
Total count (million/ml)	28.7±25.9	15.8±7.8	4.8±3.3	4.584	0.021
Abnormalities	18.5±4.2	24.5±11.0	24.4±11.4	0.824	0.452
Active (million/ml)	30.1±20.5	24.3±13.6	7.2±5.1	4.419	0.023
Sluggish (million/ml)	20.7±8.0	23.4±8.2	18.3±12.1	0.680	0.516
Dead (million/ml)	49.1±14.7	55.2±13.9	74.5±11.8	6.114	0.007

All values are expressed as mean±SD

P>0.05: non significant, P<0.05: significant

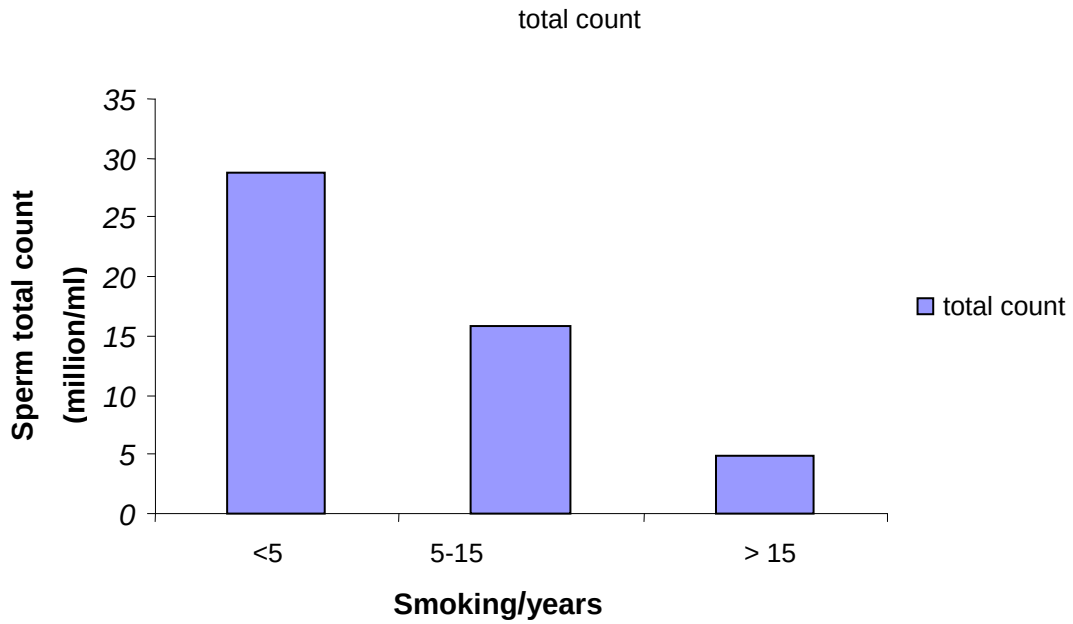

Figure 4.5. Duration of cigarette smoking in relation to total count of sperms (million/ml) among cases

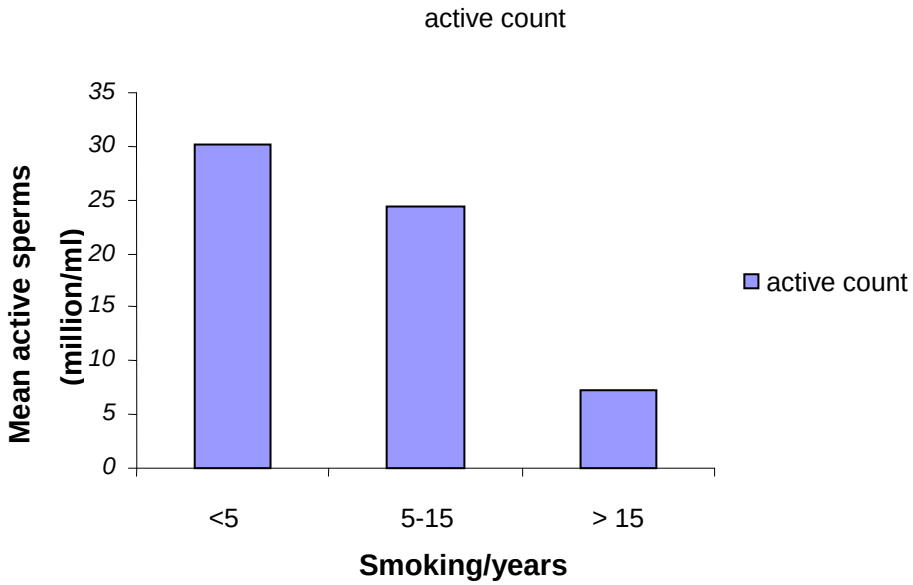

Figure 4.6. Duration of cigarette smoking in relation to active sperm count (million/ml) among cases

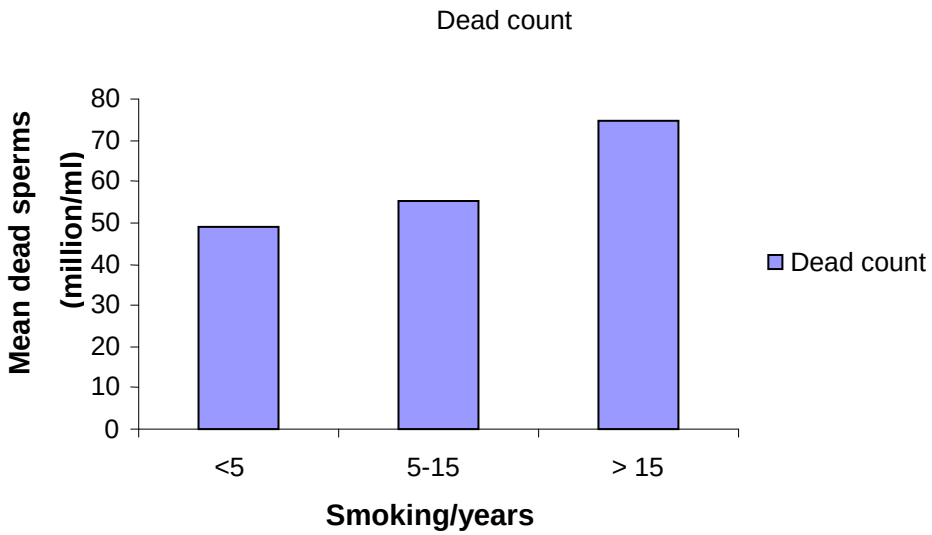

Figure 4.7. Duration of cigarette smoking in relation to dead sperms (million/ml) among cases

4.7.2 Duration of cigarette smoking in relation to hormonal profile among cases

The duration of cigarette smoking in relation to hormonal profile among cases is shown in Table 4.13. Testosterone exhibited no significant decrease with increasing smoking duration showing maximum decrease of 4.3±1.2 with >15 years of smoking (P=0.876). In contrast, LH, FSH and prolactin showed no significant increase with maximum increases of 5.4±2.5, 6.0±2.3 and 10.0±3.3 with >15 years of smoking (P=0.280, P=0.313 and P=0.987, respectively).

Table 4.13 Duration of smoking in relation to hormonal profile of the cases

Hormone	**Duration of smoking (Year)**				
	<5	**5-15**	**>15**	**F**	**P-value**
Testosterone (ng/ml)	5.0±1.9	5.0±3.8	4.3±1.2	0.133	0.876
LH (mIU/ml)*	3.8±1.6	3.8±1.8	5.4±2.5	1.362	0.280
FSH (mIU/ml)**	4.0±1.8	5.1±2.6	6.0±2.3	1.220	0.313
Prolactin (ng/ml)	9.5±4.5	9.7±3.3	10.0±3.3	0.013	0.987

*LH: Luteinizing hormone, **FSH: Follicle stimulating hormone

All values are expressed as mean±SD

P>0.05: non significant

Chapter 5

Discussion

It is believed that wrongly in the Middle East both virility and fertility are typically tied to manhood. Fertility is the natural capability to produce offspring. Total fertility rate (TFR) - the total number of children a woman would bear during her lifetime if she were to experience the prevailing age-specific fertility rates of women. TFR equals the sum for all age groups of 5 times each Age-specific fertility rate (Garrett, 2001). Patterns of male fertility vary greatly among regions and even within regions. A combination of social habits e.g. cigarette smoking, environmental conditions, and genetics is suspected to contribute to this variation (Kidd et al., 2001). In Gaza strip there are under-reporting of male fertility or even no study link between smoking and fertility in men. Therefore, the present study is the first to evaluate the risk of cigarette smoking on male fertility in Gaza Governorate, Gaza Strip.

5.1 Personal characteristics and medical history of the study population

The present study was case control design comprising 54 controls (non smokers) and 54 cases (smokers). The mean ages of controls and cases were 33.5±7.6 and 32.7±6.3 years old, respectively. Age distribution in our study was an independent factor to affect male fertility. Similar result was reported (Kumtepe et al., 2003). The number of cases who had children was significantly lower than controls indicating that smoking could be a risk factor of male fertility. However education and employment were not found to be associated with male fertility. Concerning medical history of the study population, the number of cases who had testes surgery was higher than controls implying that testes surgery may have some extent of a negative impact on male fertility which seems to be exacerbated by smoking. This finding was in agreement with that reported by Collodel et al. (2009) who found that smoking cigarettes increases the effect of varicocele on sperm morphology. Regarding previous semen and

hormone analyses, the number of cases who seeking such analyses was higher than controls. Therefore, in my opinion cases had lower rate of fertility which may be more likely link to smoking.

5.2 smoking and fertility

5.2.1 Passive smoking among the study population

The number of cases who exposed to passive smoking was significantly higher than controls indicating that passive smoking may be associated with male fertility. This finding coincides with that mentioned by American Society for Reproductive Medicine (2003) which reported that the harmful effect of passive smoke on the fertility of female partners and the evidence that smoking adversely affects sperm quality suggest that smoking in men should be regarded to as an infertility risk factor. It has been shown that passive exposure to environmental tobacco smoke resulted in measurable seminal nicotine and cotinine levels correlated with the degree of reported exposure (Pacifici R. et al., 1995). It is accepted that nicotine and its metabolite cotinine may lead to poor semen function and resultant infertility (Arabi and Shareghi, 2005).

5.2.2 Type of smoking, frequency/day, duration and negative impact on sexual desire and erection

The present result showed that the majority of cases were cigarette smokers and around half of them smoked more than 20 cigarette/day. In addition, more than half of the cases smoked for 5-15 years. These circumstances may put them at a high risk of male infertility. This view was supported by the finding that the longer duration of smoking, the less number of cases having children. These results are in agreement with that found by Collodel et al. (2009) who demonstrated that the sperm motility, sperm concentration, and fertility index decreased and the percentage of sperm pathologic features increased as the number of cigarettes smoked daily increased. According to Centers for Disease Control and Prevention (2009), sperm from chronic smokers were 75 % less fertile than sperm from nonsmokers. Men who were lighter smokers suffered less severe decreases in fertility. In addition, significant negative correlation was obtained between smoking duration and percentage of sperm motility (Hussein

et al., 2011). Concerning negative impact of smoking, data presented here showed that smoking affect sexual desire and erection in about quarter of cases. Difficulties in getting pregnant may be from the effects of the nasty chemicals in cigarette smoke that affect the man's sperm and libido (Quitters Guide, 2008). In addition, it was reported that cigarette smoking raised risk of erectile dysfunction (Miron et al., 2002 and He et al., 2007).

5.3 Semen parameters of the study population

The mean sample volume, total count and active sperm were significantly decreased whereas the mean levels of abnormalities and dead sperm were markedly increased in cases compared to controls. These findings on semen quality are in agreement with that documented by Wong et al., (2000), Kunzel et al., (2007) and Hussein et al., (2011). Nicotine in tobacco and its metabolite cotinine may lead to poor semen function and resultant infertility (Arabi and Shareghi, 2005). Cigarette smoke contains also carbon monoxide which may affect male reproduction via direct effect on the testicular function and spermatogenesis (Hanrahan et al., 1996).

When semen parameters were related to the number of smoked cigarettes/day and duration of smoking, the present results showed a significant decrease in the mean number of active sperms whereas the mean of dead sperms was significantly increased with increasing number of smoked cigarettes/day and smoking duration. In addition, total sperm count was significantly decreased only with increasing smoking duration. However, the other semen parameters were not significantly affected. Such results were in concurrent with that addressed by Zhang et al., (2000) who found significant negative correlation between sperm density, viability and forward progression with the amount and duration of cigarette smoking. Additionally, Collodel et al. (2009) pointed out that the sperm motility, sperm concentration, and fertility index decreased and the percentage of sperm pathologic features increased as the number of cigarettes smoked daily increased.

5.4 Hormonal profile of the study population

The mean levels of testosterone, LH , FSH and prolactin in cases showed no significant decrease compared to controls. Similar results were obtained by Attia et al. (1989) and English et al. (2001). However, our findings were in contradiction with that mentioned by Field et al. (1994) who reported that cigarette smoking had increased serum levels of testosterone by 9% and with that of Ramlau-Hansen et al. (2007) who found a positive dose–response relationship between smoking and testosterone, LH and the LH/free testosterone ratios. This contradiction was frequently encountered in literature among different population and may be related to different life style and environment. Also, the number of smoked cigarettes/day and duration of smoking had no significant effect on hormonal profile. In conclusion, cigarette smoking affects fertility by its main negative impact on semen parameters rather than hormonal profile at least in our patients.

Chapter 6

Conclusions and Recommendations

6.1 Conclusions

* The mean ages of controls and cases were 33.5±7.6 and 32.7±6.3 years, respectively.

* The number of cases who had children was significantly lower than controls.

* The number of cases who had testes surgery, previous semen and hormone analysis was higher than controls.

* The number of cases who exposed to passive smoking was significantly higher than controls.

* The majority of cases were cigarette smokers and around half of them smoked more than 20 cigarettes/day. In addition, more than half of the cases smoked for 5-15 years. The longer duration of smoking, the less number of cases having children.

* Smoking had negative impact on sexual desire and erection in about quarter of cases.

* The means of sample volume, total count and active sperm were significantly decreased whereas the means of abnormalities and dead sperm were markedly increased in cases compared to controls.

* Relation of semen parameters to the number of smoked cigarettes/day and duration of smoking showed a significant decrease in the number of active sperms whereas dead sperms was significantly increased with increasing

number of smoked cigarettes/day and smoking duration. In addition, total sperm count was significantly decreased only with increasing smoking duration.

* The mean levels of testosterone, LH , FSH and prolactin in cases showed no significant decrease compared to controls. Also, the number of smoked cigarettes/day and duration of smoking had no significant effect on hormonal profile.

* Finally, cigarette smoking affects fertility by its main negative impact on semen parameters rather than hormonal profile at least in our patients.

6.2 Recommendations

* Health education programs should be launched on the importance of quitting smoking and its positive impact on male fertility in terms of improving semen parameters, sexual desire and erection.

* Risk of cigarette smoking on male fertility must be explained to couples interested in getting pregnant in vitro via IVF "*In Vitro Fertilization*" to optimize the success rate.

* Exposure to passive smoking must be avoidable.

***** Frequent check up of semen parameters is advisable.

Chapter 7

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Appendices

Annexes

Annex1

Palestinian National Authority
Ministry of Health
Helsinki Committee

السلطة الوطنية الفلسطينية
وزارة الصحة
لجنة هلسنكي

(7)

التاريخ 6/6/2011

Name:

الاسم: أدهم جادالحق

I would like to inform you that the committee has discussed your application about:

نفيدكم علماً بأن اللجنة قد ناقشت مقترح دراستكم حول:-

" Risk evaluation of cigarette smoking on adult male fertility in Nuseirat area, Gaza Strip."

In its meeting on June 2011

و ذلك في جلستها المنعقدة لشهر 6 2011

and decided the Following:-

و قد قررت ما يلي:-

To approve the above mention research study.

الموافقة على البحث المذكور عاليه.

Signature
توقيع

Member	Member	Chairperson
عضو	عضو	

Conditions:-

- Valid for 2 years from the date of approval to start.
- It is necessary to notify the committee in any change in the admitted study protocol.
- The committee appreciate receiving one copy of your final research when it is completed.

Annex 2

Serial number:

Smoking questionnaire

Yes **No**

Personal data

1- Age ----------

2- Are you married?

☐ ☐

If yes , since ----------

If yes, Do you have children

☐ ☐

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

3- Education

University ☐ Secondary ☐ Preparatory ☐

Primary ☐ Illiterate ☐

4- Occupation

Employed ☐ Unemployed ☐

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

Yes **No**

Medical history

5- Do you have history of other disease?

☐ ☐

If yes define the disease ----------

And numerate drugs if present----------,----------,----------

6- Do you exposed to any testicular trauma?

☐ ☐

7- Do you have any surgery on your testes like "varicoceles"?

☐ ☐

If there are another operations numerate them ----------,----------, ----------

8- Do you have any of sexual transmitted disease?

☐ ☐

9- Did you check your sperm before?

☐ ☐

If yes, when----------

10- Did you check your testosterone, FSH and LH hormones before?

☐ ☐

If yes, when----------

Yes **No**

Smoking data

11- Are you a smoker?

☐ ☐

If yes

Cigarette ☐ Narjela ☐ Pipe ☐

If cigarette (No. of cigarette/day)

<10 (Light) ☐ 10-20 (Moderate) ☐ >20 Heavy ☐

If Narjela (Times/day)

One ☐ two ☐ >two ☐

If Pipe (Times/ day)

One ☐ two ☐ >two ☐

12- How long you have been smoked?

☐ ☐ ☐

<5 years 5-15 years >15 years

13- Does your wife smoke?

☐ ☐

14- Is the nature of your profession resulting any kind of gas?

☐ ☐

Yes **No**

15- Have you noticed that there is a negative impact of smoking on sex drive?

☐ ☐

16- Have you noticed that there is a negative impact of smoking on erectile Function?

☐ ☐

17- Do you sit down just next to the smokers?

☐ ☐

18- Do you deal with materials other than smoking?

☐ ☐

If yes, numerate them ---------- , ----------,----------