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CERTIFICATE

This is to certify that the thesis entitled "**EFFECT OF CHRONIC ALCOHOLISM AND SMOKING IN MALE REPRODUCTIVE FUNCTION"** submitted to The Tamilnadu Dr.M.G.R. Medical University, Chennai is a record of original research work done by **Mr K R MUTHUSAMI,** during the period of his study from April 1999 to February 2005 under my guidance and supervision for the award of the Degree of Doctor of Philosophy in Clinical Biochemistry. I further certify that this research work has not previously formed the basis for the award of any other Degree, Diploma, Associateship, Fellowship or other similar title to any candidate of any university.

(Dr P CHINNASWAMY)

DECLARATION

I do hereby declare that the thesis entitled '**EFFECT OF CHRONIC ALCOHOLISM AND SMOKING IN MALE REPRODUCTIVE FUNCTION"** submitted to The Tamilnadu Dr.M.G.R. Medical University, Chennai for the award of the Degree of Doctor of Philosophy in Clinical Biochemistry is a record of original and independent research work done by me during the period from April 1999 to February 2005 under the supervision and guidance of **Dr P CHINNASWAMY** and this research work has not previously formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or other similar title to any candidate of any university.

Signature of the Candidate

(K R MUTHUSAMI)

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotrophic Hormone
ALP	Alkaline Phosphatase
ANOVA	Analysis Of Variance
CFAS	Calibrator For Automated System
CO	Carbon Monoxide
CVD	Cardiovascular Disease
COPD	Chronic Obstructive Pulmonary Disease
COT	Cotinine
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosterone-Sulphate
DNA	Deoxyribonucleic Acid
ECL	Electrochemiluminescence
ECLIA	Electrochemiluminescence Immunoassay
EOP	Endogenous Opioid Peptides
ELISA	Enzyme-Linked Immunosorbent Assay
E2	Estradiol
EtOH	Ethanol
EDTA	Ethylenediaminetetraacetic Acid
FSH	Follicle Stimulating Hormone
GGT	Gamma Glutamate Transaminase
GPT	Glutamate Pyruvate Transaminase
GnRH	Gonadotropin-Releasing Hormone
HCT	Haematocrit
Hgb	Haemoglobin
HDLc	High Density Lipoprotein Cholesterol
HCN	Hydrogen Cyanide
HPG	Hypothalamic – Pituitary – Gonadal
IGF	Insulin-like Growth Factor
LDLc	Low Density Lipoprotein Cholesterol
LH	Luteinizing Hormone
LHRH	Luteinizing Hormone Releasing Hormone
MCHC	Mean Corpuscular Erythrocyte Haemoglobin Concentration
MCH	Mean Corpuscular Erythrocyte Haemoglobin
MCV	Mean Corpuscular Erythrocyte Volume

mRNA	Messenger Ribonucleic Acid
MAO B	Monoamine Oxidase B
NAD	Nicotinamide Adenine Dinucleotide
NADH	Nicotinamide Adenine Dinucleotide (reduced)
NADPH	Nicotinamide Adenine Dinucleotide Phosphate (reduced)
NIC	Nicotine
NO	Nitric Oxide
PCV	Packed Cell Volume
PTH	Parathormone
Plt	Platelet
P	Progesterone
PRL	Prolactin
ROS	Reactive Oxygen Species
RBC	Red Blood Cells
SHBG	Steroid Hormone-Binding Globulin
TIF	Testicular Interstitial Fluid
T	Testosterone
TGF	Transforming Growth Factor
StAR	Steroid Acute Regulatory protein
VLDLc	Very Low Density Lipoprotein Cholesterol
WBC	White Blood Cells
WHO	World Health Organization.

1.0 INTRODUCTION

Infertility is a global problem and the methods used for its prevention and treatment diametrically differ in various parts of the world. Infertility is seldom, if ever, a physically debilitating disease. It may, however, severely affect the couple's psychological harmony, sexual life and social function (Insler and Lunenfeld, 1993). Benoff et al, (2000) have reported that human sperm concentration and male fertility are declining, and have renewed interest in the role of environmental exposures in the etiology of human male fertility.

1.1. PROBABILITY OF MALE INFERTILITY

The chances of a normal couple conceiving are estimated to be 20% to 25% per month, 75% by six months and 90 % by one year (Spira, 1986; Thonneau et al, 1991). Approximately 20% of cases of infertility are caused entirely by a male factor, with an additional 30% to 40% of cases involving both male and female factors. Therefore, a male factor is present in one half of infertile couples. It is estimated that around 12% of male infertility is untreatable, 18% is treatable. Among the treatable 0.6% of male infertility is caused by reversible toxins like alcohol, smoke and pesticides (Mosher and Pratt, 1991; Thonneau et al, 1991; Baker, 2001; Farah and Blackwell, 2001).

1.2. DRUG ADDICTION

Drug abuse, addiction and overdose are serious public health problems. A national Comorbidity Survey conducted in 1995 discovered that 7.5% of US residents 15 to 54 years old had a history of drug dependence. Common drugs of abuses are namely A) Alcohol (ethanol), B) Opioids, C) Cocaine, D) Marijuana and E) Nicotine (tobacco use - cigarette smoking, chewing tabacco, pipes and cigars and bidi) (Messing, 2001; Kane and Kumar, 2004; Burns, 2005; Schuckit, 2005).

1.3. ALCOHOLISM

Disorders of alcoholism have been prevalent across all societies. The pattern of alcohol usage varies depending on age, religion, education, type of drink and other socio-demographic characteristics (Room et al, 2002). Alcohol use is increasing in developing countries. Since 1970, 47% of developing countries in transition and 35% of developed countries have increased their consumption of absolute alcohol per adult. Alcohol causes 4% of the total Disability Adjusted Life Years (DALYs) and alcohol use disorders account for 1.4% of the total burden of disease, according to recent estimates (Gupta et al, 2003). Alcohol has been consumed in India for centuries. A number of mythological and religious books have highlighted the role it played in society. The pattern of drinking in India has undergone a change from occasional and ritualistic use to being a social event. Today, the common purpose of consuming alcohol is to get drunk (Mohan et al, 2001). Large or nationally representative epidemiological studies on alcohol consumption have not been carried out in India due to resource constraints. However, there have been a number of studies conducted on smaller populations in different regions of the country (Gupta et al, 2003).

Initially the alcoholics may demonstrate a high tolerance to alcohol, consuming more and showing less adverse effects than the others. The person commonly loses control over drinking and is increasingly unable to predict how much alcohol will be consumed on a given occasion. Physical addiction to the drug may occur, leading to drinking round the clock to avoid withdrawal symptoms. In the interim, the neurons require ethanol to function optimally, and the individual can be said to be physically dependent. This is distinct from psychological dependence, a concept indicating that the person is psychologically uncomfortable without the alcohol (Schuckit, 2005).

The effects on major organ systems are cumulative and include a wide range of digestive-system disorders such as ulcers, inflammation of the pancreas and cirrhosis of the liver. The central and peripheral nervous systems can be permanently damaged. In advanced cases, abstinence from alcohol may result in a serious withdrawal syndrome, commonly known as delirium tremens, characterized by symptoms ranging from shaking limbs to hallucination and blackouts. By abusing ethanol the people are also voluntarily expose themselves to the hazards of cardiovascular system, respiratory system, haematopoietic system, skeletal muscle, fetal alcohol syndrome, cancer and reproductive system. Even low doses of alcohol have a significant effect on many organ systems including reproductive system (Kane and Kumar, 2004; Schuckit, 2005).

Acute and chronic alcohol consumption also appears to lower plasma testosterone synthesis. Van Thiel et al, (1983) have demonstrated that ethanol acts as a Leydig cell toxin. Ethanol increases the metabolic clearance rate of testosterone with an increase in hepatic 5 alpha-reductase activities and also increase the conversion of androgens into estrogens. Those patients in whom follicle stimulating hormone (FSH) levels decline demonstrate a spermatogenesis whereas those in whom FSH levels remain elevated are unlikely to demonstrate spermatogenesis (Kader and Rostom, 1991). Sperm may be selectively affected by various toxic substances throughout the process of spermatogenesis to spermiogenesis (Insler and Lunenfeld, 1993).

1.4. PREVALENCE OF CIGARETTE SMOKING

The Indian Council of Medical Research has carried out epidemiological studies on smoking in India, which have revealed that there are 184 million tobacco consumers in India of which 20 % smoke cigarettes, 40 % smoke beedis and 40 % chew tobacco in various

forms. 55,000 children in India take up this habit annually. Indians smoke 90 billion cigarettes annually which costs about 180 billion rupees. 8,00,000 people die each year in India due to tobacco related diseases, that is 2200 deaths per day, 90 per hour (Fisher, 1990; West et al, 2000; Glaxo Welcome, 2000).

Smoking also interacts with the other environmental and occupational exposures in an addictive or synergistic fashion. The most important example of such synergism is the increase in risk of lung cancer in cigarette smokers exposed to asbestos. Smoke from the average cigarette contains around 4,000 chemicals, some of which are highly toxic and atleast 43 of which cause cancer. The major diseases caused by cigarette smoking are coronary heart disease, cerebrovascular disease, aortic aneurysm, chronic airways obstruction, cancer, sudden infant death syndrome, infant respiratory distress syndrome, low birth weight at delivery and male impotence (Kane and Kumar, 2004; Burns, 2005).

1.5. REPRODUCTIVE TOXICITY

After long-term and prolonged (>6 months) exposure to toxic substances, changes are seen in motility, number and morphology of normal spermatozoa in the ejaculate. A decrease in sperm motility is one of the first manifestations of exposure to toxic chemicals. Numerous studies have demonstrated that some environmental toxic agents may have adverse effects on the male reproductive system, either by affecting neuro-endocrine function or by directly affecting the process of spermatogenesis resulting in poor semen quality. (Robins et al, 1997; Celis et al, 2000).

1.6. AIM OF THE PRESENT STUDY

Several studies on the effects of alcohol or cigarette consumption on human seminal quality have been reported. Martini et al, (2004) and Marinelli et al, (2004) have reported that no study exists to highlight the effect of alcohol and smoking on male reproductive function.

In the present study the 1). Effect of Alcohol, 2). Effect of smoking and 3). Effect of alcohol and smoking on human male endocrinology and production of semen have been evaluated.

In the present study “Effect Of Chronic Alcoholism And Smoking In Male Reproductive Function” the following Hormones are estimated and Semen is analyzed.

1.7. HORMONAL ASSAY IN BLOOD

- Follicle Stimulating Hormone (FSH)
- Luteinizing Hormone (LH)
- Testosterone (T)
- Progesterone
- Estradiol (E_2)
- Prolactin (PRL)

1.8. ANALYSIS OF SEMEN

Seminal analysis is carried out to assess the sperm count, motility and morphology.

1.9. SIGNIFICANCE OF THE PRESENT STUDY

Alcohol and smoking are the two important pleasure-seeking habits of the human society especially among middle-aged male all over the world. Majority of people drink alcohol along with smoking. Some

people consume alcohol without smoking cigarettes. But people rarely smoke cigarettes without an alcoholic drink.

Studies on the effect of cigarette smoking on male reproductive hormones are very few and the available study reports have given contradictory reports. The study on effects of smoking on testicular estradiol and progesterone hormones are little. Studies on effect of smoking on semen are available, but those too have controversial findings. Apart from the above, the effect of alcoholism and smoking together and the comparative effects of alcohol and smoke on male reproductive function are rare.

The previous studies on cigarette smokers and alcoholics revealed that the number of cigarettes smoked per day, duration of cigarette smoking without break, with and without alcoholic drink, the volume and the duration of alcohol consumption, alcohol and cigarette smoke induced disease status of the subjects were not given more emphasis.

The present study has classified in depth the volume, the duration and the variety of alcohol consumption and smoking, drugs of abuse and the general health status of subject's evaluated bio-chemically and haematologically. Physicians examined and certified the subject's health status based on the blood results and physical examination. The screened healthy middle-aged male subjects were included in the present study to know the exclusive effects of chronic alcoholism, chronic smoking and chronic alcoholism with smoking together on reproductive hormones, semen quality and their reproductive functional activity. Comparative effects of alcoholism and smoking on the above subjects are also done to know the severity of the toxicity between these two habits.

1.10. SIGNIFICANCE OF PARAMETERS INCLUDED IN THE PRESENT STUDY

Hypothalamic, pituitary and testicular hormones maintain the normal reproductive function in male. The hypothalamus secretes gonadotropin-releasing hormone (GnRH), which stimulates the pituitary gland, which in turn secretes FSH and LH. The FSH and LH reach the testis through the blood circulation. The LH stimulates the testicular Leydig cells, which synthesize the male reproductive steroid hormone testosterone. As an intermediate product in steroidogenesis pathway, a small amount of progesterone is also synthesized and secreted in testis. By way of aromatization from testosterone and androstenedione a small amount of estradiol is also synthesized and secreted in testis. The testosterone further reaches the testicular seminiferous tubule and along with the FSH the testosterone stimulates the seminiferous tubule Sertoli cells and spermatogonia. Prolactin pituitary peptide hormone, also acts on the seminiferous tubule along with testosterone and FSH. The spermatogonium undergoes a series of mitosis and meiosis cell divisions and finally the male gamete sperm is produced in the testicular seminiferous tubules. This process is called spermatogenesis. The spermatids from the seminiferous tubules moves towards reti testis, epididymis, vas deferens and finally at the time of sexual excitement the mature sperm along with the secretions of accessory sex organs ejaculated in the form of semen.

The pituitary LH, FSH and Prolactin and testicular testosterone, progesterone and estradiol hormone's normal synthesis and normal function are very essential for the stimulation and maintenance of male reproductive function including spermatogenesis. Semen normal volume, sperm count, motility, viability and morphology are very important to fertilize the ovum. In the present study, more emphasis is given to study the above parameters in the healthy middle aged male

chronic alcoholics, smokers and alcoholics with smoking to know the effect of alcoholism, smoking and alcoholism with smoking on male reproductive function and also to know which of the above habit is having more toxicity in the middle aged male reproductive function.

2.0 REVIEW OF LITERATURE

2.1. MALE REPRODUCTIVE PHYSIOLOGY

2.1.1. MALE REPRODUCTIVE FUNCTION

Male reproductive function is controlled by the reproductive axis, which has three tiers of organization, the hypothalamus, the pituitary gland and the testis. The hypothalamus and pituitary axis produce an endocrine signaling molecule that acts as a secretagogue for hormone secretion at the target level. Hypothalamic neurons are located in the preoptic area with axons projecting to the median eminence secrete gonadotropin-releasing hormone (GnRH) into a portal system of blood vessels leading to the pituitary (Schlegel and Hardy, 2002).

The anterior pituitary gland or adenohypophysis contains gonadotrope cells that are specialized for the secretion of gonadotropins. Secretory activity of the gonadotropes is stimulated by GnRH. The two gonadotropins secreted by pituitary gonadotropes are luteinizing hormone (LH) and follicle stimulating hormone (FSH). The two gonadotropins then enter the blood stream and are borne to the testis where LH stimulates testosterone (T) production by Leydig cells while FSH through stimulation of Sertoli cells supports spermatogenesis in the seminiferous epithelium. The rates of T secretion and sperm production are fine tuned by a network of negative feedback relationships between testis and the upper levels of reproductive axis (hypothalamus and pituitary axis). Testosterone and its metabolite estradiol suppress the secretory activity of GnRH neurons and gonadotropes (Schlegel and Hardy, 2002). (Fig 1 and 2).

2.1.2. ROLE OF FSH AND LH IN MALE REPRODUCTION

Gonadal function and reproduction in men and other mammalian species are controlled primarily by anterior pituitary hormones, which

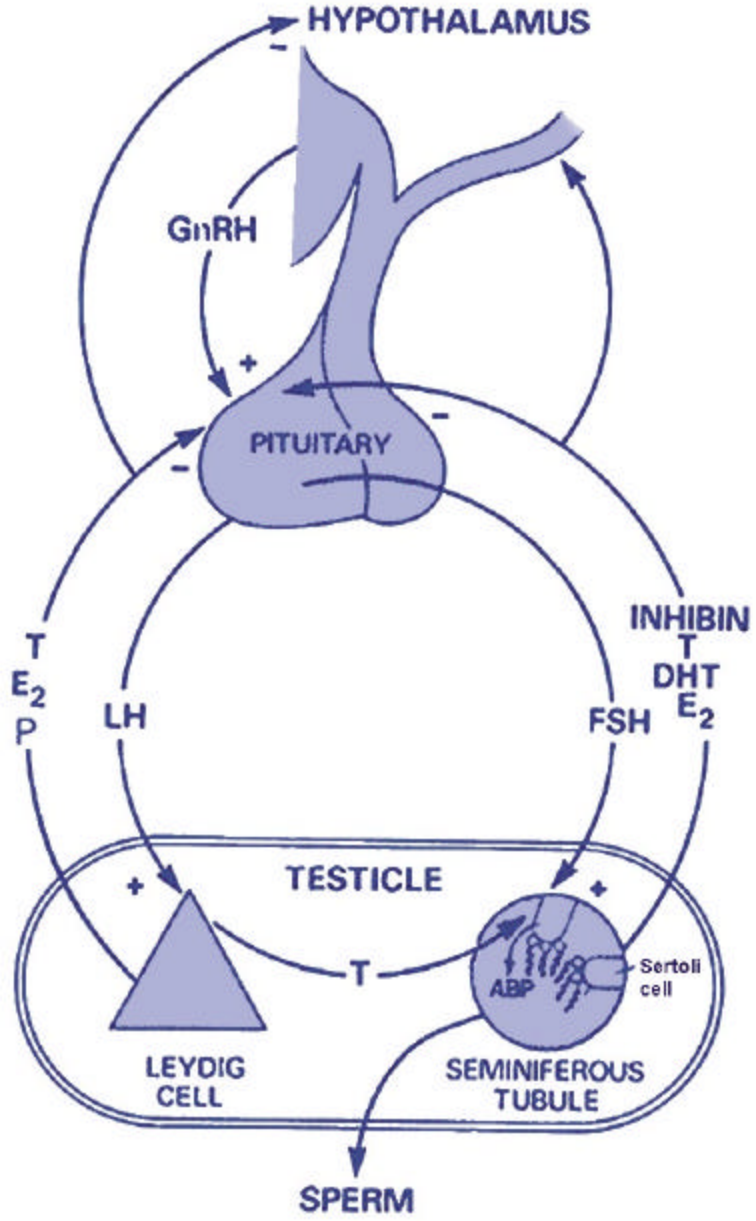


Figure1. Hypothalamic-pituitary-testicular axis.

GnRH = gonadotropin-releasing hormone; LH=luteinizing hormone; FSH = follicle-stimulating hormone; T = testosterone; DHT = dihydrotestosterone; ABP=androgen-binding protein; E2 = estradiol; P=Progesterone; + = positive influence; - = negative influence. (Braunstein, 2001)

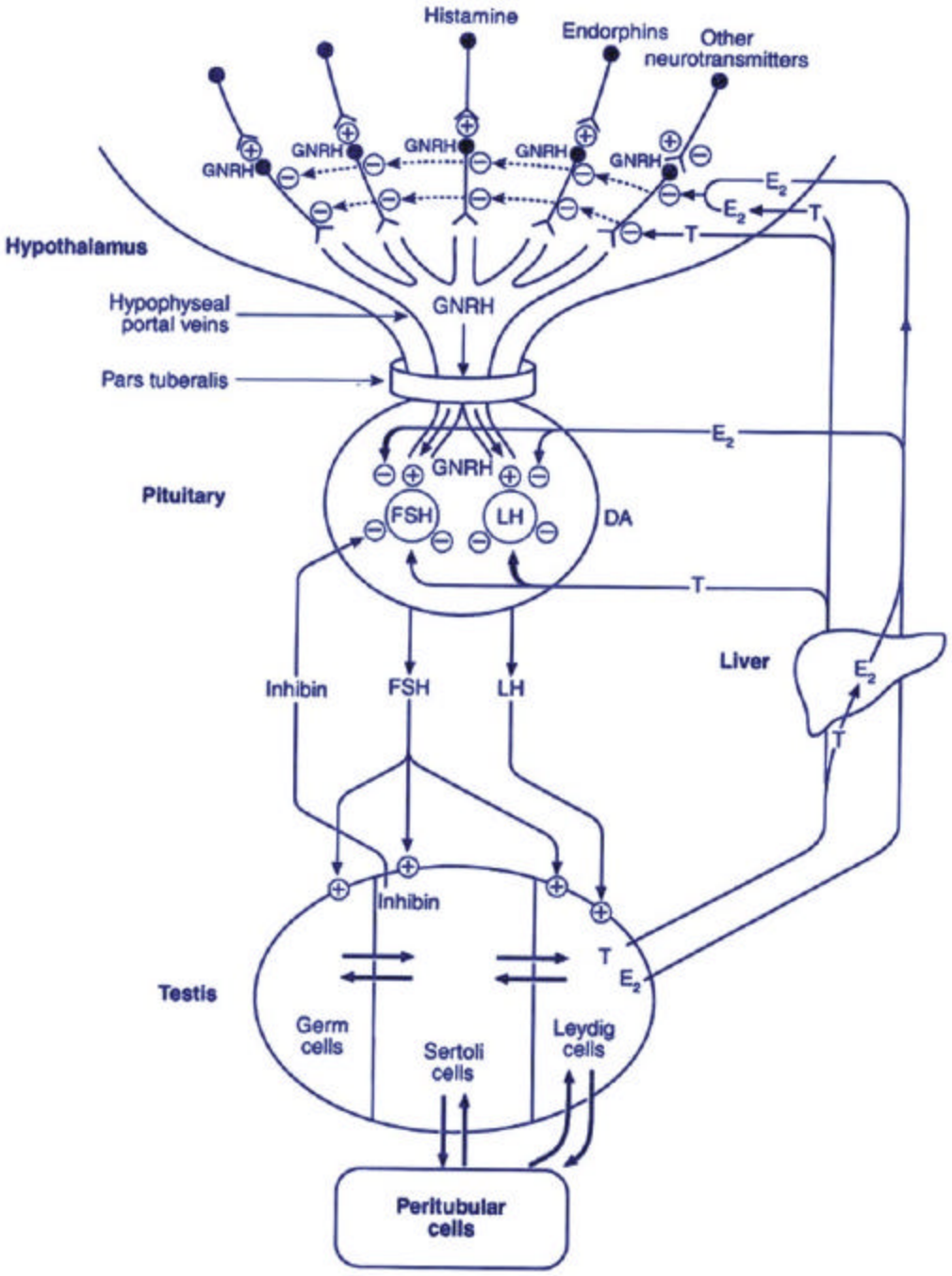


Fig. 2

Feedbacks in the hypothalamus-hypophyseal-testes compartments.
(Paz et al, 1993)

bind to specific receptors in the testis to regulate steroidogenesis and gametogenesis. These actions are exerted predominantly through the pituitary gonadotropins, follicle stimulating hormone, luteinizing hormone and the peptide hormone prolactin. FSH acts on the Sertoli cells in the testis to promote germ cell development in the gonads. LH acts on the steroidogenic cells of the testis to regulate local and peripheral concentrations of the gonadal steroid hormones that are essential for normal sexual development and reproductive function (Catt and Dufau, 1991). In men, LH stimulates testosterone production from the interstitial cells (Leydig cells) of the testes. Maturation of spermatozoa requires both LH and FSH. FSH stimulates testicular growth and enhances the production of the androgen binding protein by the Sertoli cells which is necessary for sustaining the maturing sperm cell. This androgen-binding protein causes high local concentrations of testosterone near the sperm, an essential factor in the development of normal spermatogenesis (Aron et al, 2001).

2.1.3. THE ROLE OF PROLACTIN IN MALE REPRODUCTION

In the testis, prolactin has a modulating action on testosterone production by controlling the levels of precursors available for conversion to testosterone under the influence of LH. Analogous to its actions in the ovarian cell, prolactin also promotes the expression of LH receptors in the Leydig cells of the testis. In men, elevated prolactin levels are often associated with secondary hypogonadism with normal or slightly reduced gonadotropin and testosterone levels. Increased prolactin levels cause a reduction in the conversion of testosterone to dihydrotestosterone, which may contribute to the hypogonadism in hyperprolactinemic men (Magrini et al, 1976). Although the pathophysiology is complex, excess prolactin is often accompanied by impaired reproductive function in both men and women. Such negative effects of hyperprolactinemia appear to be due to the associated defect

in gonadotropin secretion and to the peripheral actions of high prolactin levels on gonadal function (Catt and Dufau, 1991).

2.1.4. TESTIS

In healthy young men, the ovoid testis measures 15 to 25 ml in volume (Prader, 1966) and has a longitudinal length of 4.5 to 5.1 cm (Winter and Faiman, 1972) and the average width is 2.6 cm. The testes are located within the scrotum which not only serves as protective envelope but also helps to maintain the testicular temperature, approximately $2^{o}C$ below the abdominal temperature (Braunstein, 2001).

2.1.4.1. TESTICULAR STRUCTURE

The testicular parenchyma is surrounded by a capsule made up of three layers; the outer visceral layer of the tunica vaginalis, the tunica albuginea and the inner most layer of the tunica vasculosa. The tunica albuminea contains large numbers of branching smooth muscle cells that course through the predominantly collagenous tissue (Braunstein, 2001). (Fig. 3).

Extension of the tunica albuginea into the testicle as fibrous septa results in the formation of approximately 250 pyramidal lobules, each of which contains coiled seminiferous tubule and these structures account for 80% to 90% of testicular mass (Braunstein, 2001). The seminiferous tubules are large “V” shaped tubules, both ends of which usually terminate in the rete testis. Lennox and Ahmad (1970) have estimated that the combined length of the 600 to 1200 tubules in the human testis is approximately 250 metres. The rete testis coalesces to form the 6 to 12 ductuli efferentes which act as conduits to carry testicular fluid and spermatozoa into the caput epididymis (Schlegel and Hardy, 2002; Braunstein, 2001).

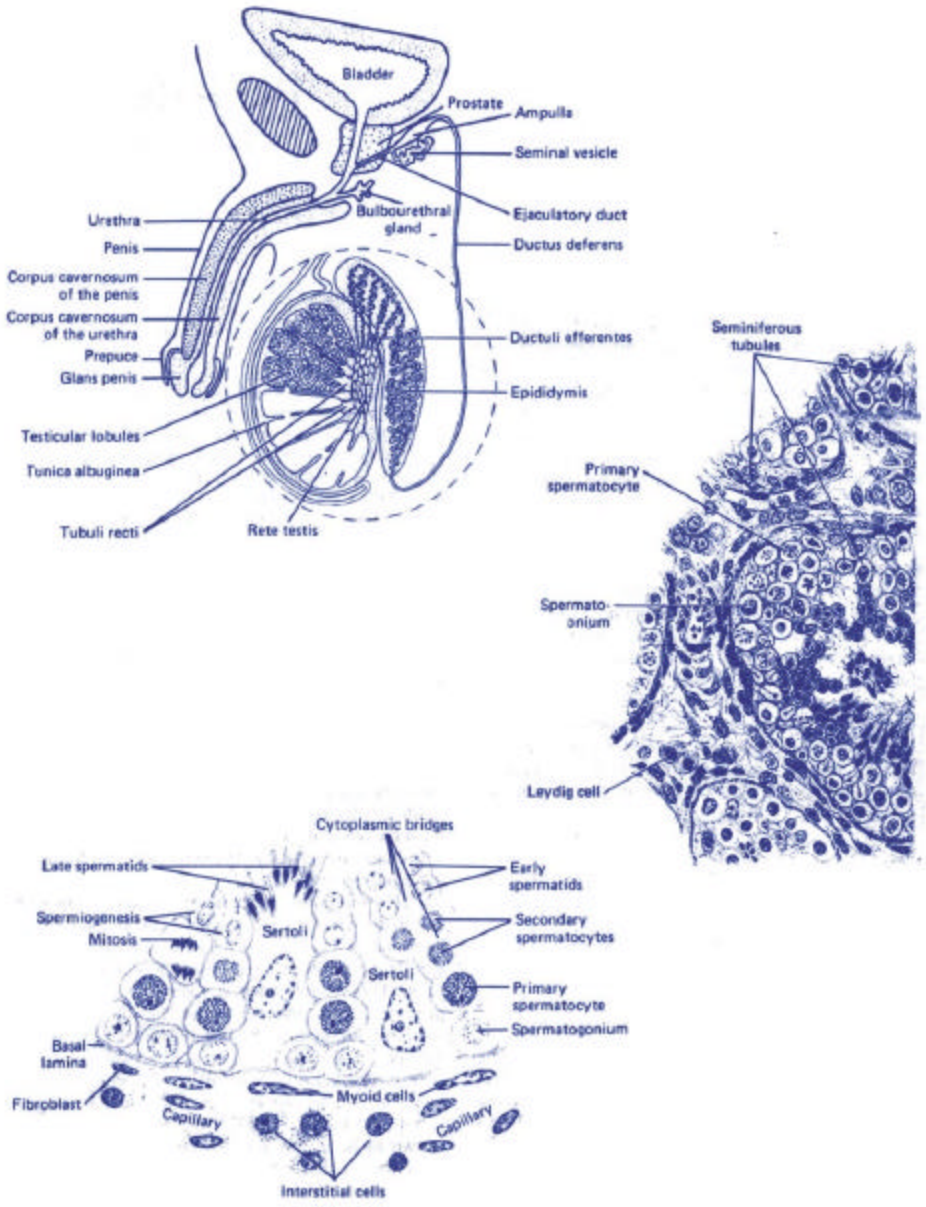


Figure 3. Male genital system. *Top:* The testis and the epididymis are in different scales from the other parts of the reproductive system. Observe the communication between the testicular lobules. *Bottom:* Structural organization of the human seminiferous tubule and interstitial tissue. This figure does not show the lymphatic vessels frequently found in the connective tissue. *At right:* Section of human testis.

(Braunstein, 2001)

Within each septum are individual seminiferous tubules which contain the developing germ cells as well as interstitial tissue. Interstitial tissue is composed of Leydig cells, mast cells and macrophages as well as nerves, blood and lymph vessels. In human, interstitial tissue takes up 20% to 30% of the total testicular volume. The testis has no somatic innervations but receives autonomic innervations primarily from the intermesenteric nerves and renal plexus. These nerves run along the testicular artery to the testis (Burger, 2001; Braunstein, 2001).

2.1.4.2. TESTICULAR FUNCTION

The two principal functions of the male hypothalamic-pituitary-gonadal axis are to produce regulated quantities of physiologically relevant steroid hormones and to produce healthy male gametes under diverse environmental conditions. The two corresponding activities of the testes are segregated automatically viz., androgen biosynthesis which occurs in the Leydig cells and spermatogenesis which is accomplished in the seminiferous tubules. The hypothalamus and the anterior pituitary gland participate integrally in the regulation of these functions via respective secretion of gonadotropin-releasing hormone by specific brain stem neurons and of the two gonadotropins by the specific adenohypophyseal cells (Veldhuis, 1991; Schlegel and Hardy, 2002).

Testosterone is responsible either directly or indirectly for embryonic differentiation of male external and internal genitalia, male secondary sexual development at puberty, maintenance of libido and potency in the adult male. The seminiferous tubules comprise the bulk of the testis and are responsible for the production of approximately 30 million spermatozoa per day during male reproductive life (Braunstein, 2001).

Both Leydig cells and seminiferous tubules of the testicular components are interrelated and both require an intact hypothalamic pituitary axis for initiation and maintenance of their function. In addition, several accessory genital structures are required for the functional maturation and transport of spermatozoa. Thus disorders of the testes, hypothalamus, pituitary or accessory structures only result in abnormalities of androgen or gamete production, infertility or a combination of these problems (Braunstein, 2001).

2.1.4.3. TESTICULAR BLOOD CIRCULATION

The human testicular parenchyma is provided with approximately nine ml of blood per 100 mg of tissue per minute (Pettersson et al, 1973). The arterial supply to the human testis and epididymis is derived from the internal spermatic artery, the deferential artery and the external spermatic or cremasteric artery. The spermatic artery arises from the abdominal aorta just below the renal artery (Schlegel and Hardy, 2002).

After traversing a complicated capillary network, blood enters multiple testicular veins that form an anastomotic network, the pampiniform plexus. The pampiniform plexuses coalesce to form the internal spermatic veins. The right spermatic vein drains directly into the vena cava and the left enters the renal vein (Braunstein, 2001).

The extra cellular fluid bathing the Sertoli cells and germinal cells flows from the seminiferous tubules into the rete to form rete testis fluid, which is transported into the caput epididymis (Schlegel and Hardy, 2002).

2.1.4.4. LEYDIG CELLS

Stereologic analysis has showed that a human testis from a 20-year old man contained approximately 700 million Leydig cells. Leydig cells alone account for 5% to 12% of the total volume of the human

testis (Kaler and Neaves, 1978; Burger, 2001; Schlegel and Hardy, 2002).

The Leydig cell is responsible for the bulk of testicular steroid production. Testosterone, synthesized from the steroid precursor cholesterol, is the principal steroid produced by the human testis although numerous C_{18}, C_{19} and C_{21} steroids are also produced. It is unclear at present whether the bulk of cholesterol used for testosterone biosynthesis is derived from blood plasma or from de novo biosynthesis (Schlegel and Hardy, 2002).

2.1.4.5. MECHANISM OF ACTION OF LH

In Leydig cells binding of LH to plasma-membrane receptors is followed by stimulation of adenylate cyclase activity and elevation of intracellular cyclic adenosine monophosphate (cAMP) levels. The increase in cAMP formation leads to activation of protein kinase in the cytoplasm of the Leydig cells and subsequent phosphorylation of proteins that are believed to regulate the early steps in steroidogenesis. The activation of protein kinase and phosphorylation of components that influence cholesterol transport or cholesterol side-chain enzyme system or both is an important part of the sequence leading to increased steroidogenesis. Cholesterol from the metabolically active pool must be transported into the mitochondria, where the cholesterol side chain cleavage enzyme converts cholesterol into pregnenolone. Movement of cholesterol to the inner membrane of the mitochondrion is conducted by two transport proteins: steroid acute regulatory proteins (StAR) and peripheral benzodiazepine receptor. LH binding alicits new protein synthesis in the Leydig cells, and the newly synthesized StAR contains a signal sequence that enables the protein to be threaded through the outer mitochondrial membrane (Stocco, 2000). The peripheral benzodiazepine receptor forms a channel for cholesterol in the

mitochondrial membrane. The two proteins interact together and form a close association in testosterone synthesis (West et al, 2001; Schlegel and Hardy, 2002). Pregnenolone must then be transported out of the mitochondrial membrane into the smooth endoplasmic reticulum where it is converted into testosterone. Testosterone probably then diffuses across the cell membrane and is trapped in the extra cellular fluid at blood plasma by steroid binding macromolecules. The actions of LH on steroid biosynthesis also are dependent upon synthesis of RNA and proteins, including the P-450 enzymes. Efflux of chloride ions, influx of calcium and release of arachidonic acid from phospholipids all play a role in the acute stimulation of testosterone synthesis (Burger, 2001; Schlegel and Hardy, 2002) (Fig 4).

2.1.4.6. GONADAL STEROID HORMONE SYNTHESIS

The three steroids of primary importance in male reproductive function are testosterone, dihydroxytestosterone, and estradiol. From a quantitative standpoint, the most important androgen is testosterone. Over 95% of the testosterone is secreted by the testicular Leydig cells; the remainder is derived from the adrenals. In addition to testosterone, the testes secrete small amount of the potent androgen dihydrotestosterone (DHT) and the weak androgens dehydroepiandrosterone (DHEA) and androstenedione (Braunstein, 2001).

Dihydrotestosterone and estradiol are derived not only by direct secretion from the testes but also in peripheral (80%) tissues by conversion of androgen and estrogen precursors secreted by both the testes and the adrenals (Braunstein, 2001).

The Leydig cells are stimulated by the gonadotropins primarily by LH to synthesize testosterone from acetate and cholesterol. The

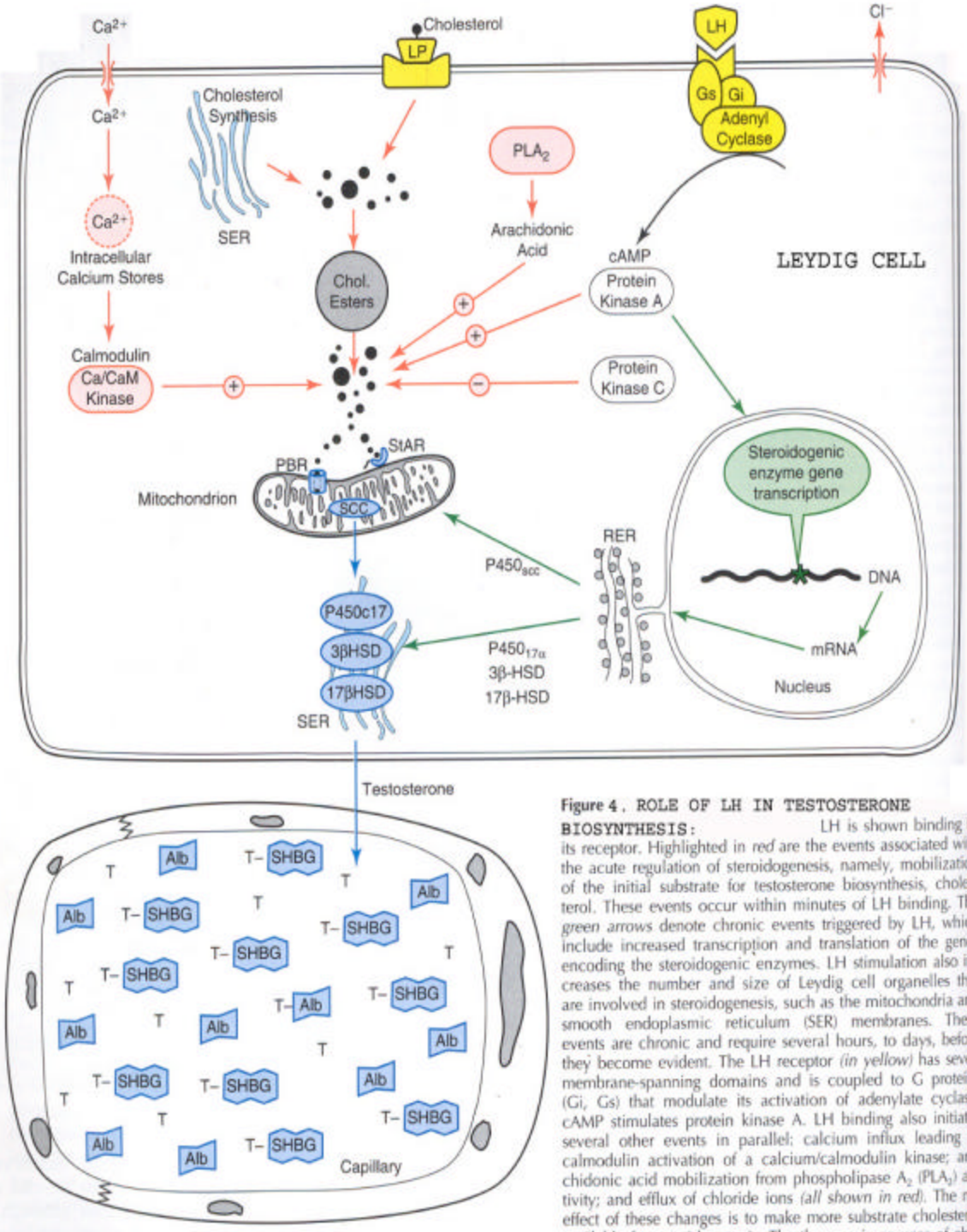


Figure 4. ROLE OF LH IN TESTOSTERONE BIOSYNTHESIS: LH is shown binding to its receptor. Highlighted in *red* are the events associated with the acute regulation of steroidogenesis, namely, mobilization of the initial substrate for testosterone biosynthesis, cholesterol. These events occur within minutes of LH binding. The *green arrows* denote chronic events triggered by LH, which include increased transcription and translation of the genes encoding the steroidogenic enzymes. LH stimulation also increases the number and size of Leydig cell organelles that are involved in steroidogenesis, such as the mitochondria and smooth endoplasmic reticulum (SER) membranes. These events are chronic and require several hours, to days, before they become evident. The LH receptor *(in yellow)* has seven membrane-spanning domains and is coupled to G proteins (Gi, Gs) that modulate its activation of adenylate cyclase. cAMP stimulates protein kinase A. LH binding also initiates several other events in parallel; calcium influx leading to calmodulin activation of a calcium/calmodulin kinase; arachidonic acid mobilization from phospholipase A_2 (PLA_2) activity; and efflux of chloride ions *(all shown in red)*. The net effect of these changes is to make more substrate cholesterol available for steroidogenesis. The three main sources of cholesterol in the Leydig cell are (1) externally, from blood-borne lipoprotein and internalization of cholesterol/lipoprotein receptor complexes, (2) de novo synthesis from acetate, and (3) stored cholesterol esters in lipid droplets. Maintenance of cholesterol stores is part of the normal resting function of the Leydig cell; LH stimulation evokes cholesterol mobilization through cholesterol esterase activity. The free cholesterol then associates with steroid acute regulatory protein (StAR) for transport to the inner membrane of the mitochondrion. A key part of the transport mechanism is the signal sequence *(depicted as a blue, threadlike tail)* that enables StAR protein to pass through mitochondrial membranes. Peripheral benzodiazepine receptor (PBR) forms a channel in the mitochondrial membranes and also facilitates cholesterol entry. The cholesterol side-chain cleavage enzyme (cytochrome $P450_{scc}/\Delta^{5\rightarrow4}$ isomerase, $P450_{scc}$) cleaves cholesterol at C21 to form pregnenolone. This and subsequent steps of steroidogenesis are shown in *blue*. Pregnenolone diffuses out of the mitochondrion to the SER. In the Δ^5 pathway of human Leydig cells, the ordering of steroidogenic enzymes in the SER is as shown: cytochrome P-450 17α-hydroxylase/C_{17-20} lyase (P450c17) → 3β-hydroxysteroid dehydrogenase (3β-HSD)→ 17β-hydroxysteroid dehydrogenase (17β-HSD). Rodent Leydig cells have a different ordering of these enzymes, with 3β-HSD acting first (the Δ^4 pathway). Testosterone (T) diffuses out of the Leydig cell and associates quickly with binding proteins, including albumin (Alb) and, primarily, sex hormone–binding globulin (SHBG) in the circulation. **(Schlegel and Hardy, 2002).**

spermatic vein concentration of testosterone is 40 to 50 ìg/dl and is approximately 75 times more concentration than the level detected in the peripheral venous serum (Hammond, 1978), which is approximately 600 ng/dl. Other androgens also leave the testes by means of the spermatic vein and these include androstanediol, androstenedione (3 ìg/dl) and DHT (0.4 ìg/dl). Therefore the concentrations of these androgens are much lower in the spermatic vein than in those of testosterone (Partin and Rodriguez, 2002).

The conversion of cholesterol into steroid hormones involves hydroxylation reactions that require nicotinamide adenine dinucleotide phosphate (NADPH) and activated oxygen (O_2), which is accomplished by a cytochrome p-450 (O'Malley and Strott, 1991). The regulated step in the process of steroid hormone biosynthesis is the conversion of cholesterol to pregnenolone and pregnenolone to progesterone. In progesterone and pregnenolone pathway the enzymes 20-22-desmolase, 3β -hydroxysteroid dehydrogenase, Δ^5, Δ^4- isomerase, 17-hydroxylase, 17-20- desmolase, 17- ketoreductase and 5á- reductase produce C-19 androgenic steroid hormones testosterone and DHT. Testosterone by action of aromatase enzyme produces C-18 estrogenic steroids estrone and estradiol (O'Malley and Strott, 1991; Burger, 2001; Braunstein, 2001). (Fig. 5).

Estrogen excess may be endogenous or exogenous. Patients with estrogen excess often have bilateral gynecomastia, erectile dysfunction and atrophic testes. One of the most common causes of estrogen excess is morbid obesity because fat cells contain the enzyme aromatase that converts testosterone to estradiol (Schneider et al, 1979). Estradiol normally stimulates hepatic production of sex steroid hormone-binding globulin (SHBG), which would lower the amount of bioavailable testosterone (Sigman and Jarow, 2002).

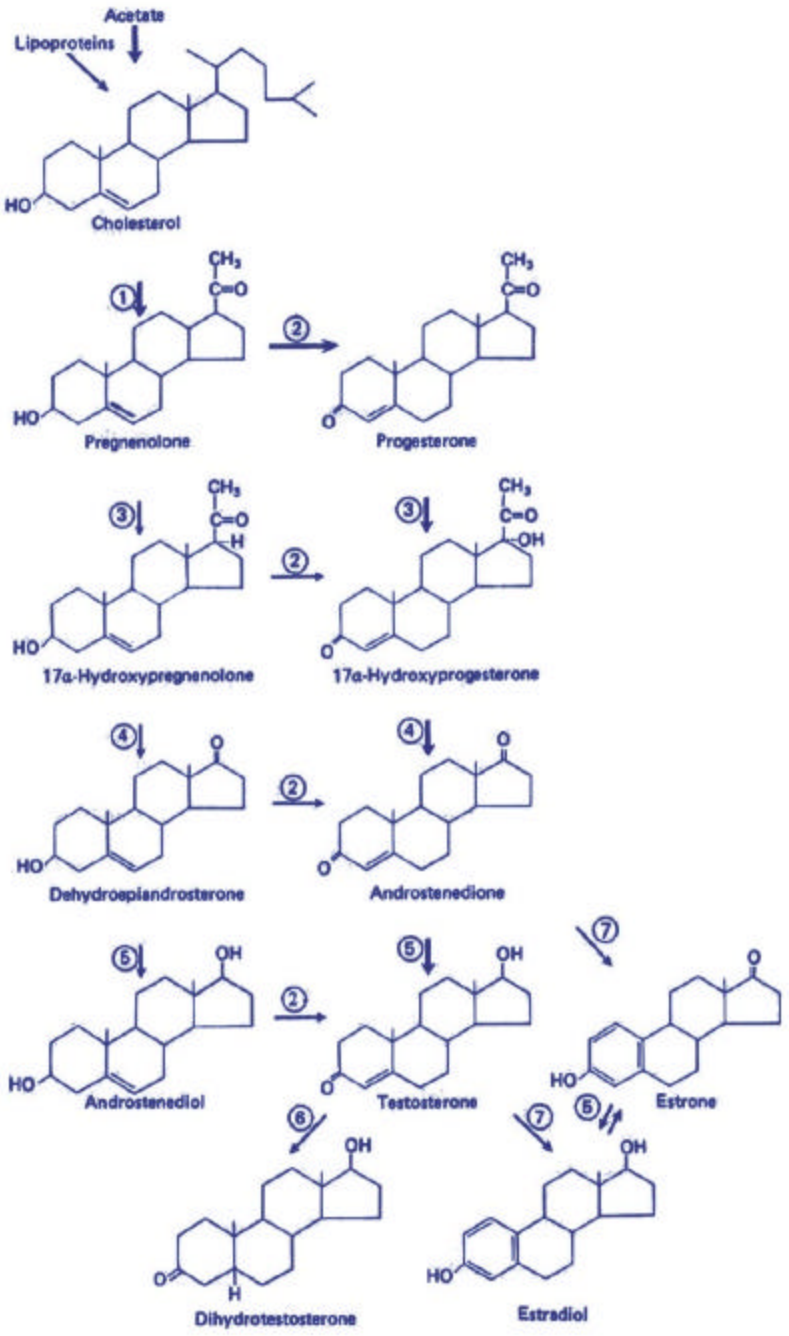


Figure 5. Pathways for testicular androgen and estrogen biosynthesis. Heavy arrows indicate major pathways. Circled numbers represent enzymes as follows: ① = 20,22-desmolase; ② = 3β-hydroxysteroid dehydrogenase and Δ^5,Δ^4-isomerase; ③ = 17-hydroxylase; ④ = 17,20-desmolase; ⑤ = 17-ketoreductase; ⑥ = 5α-reductase; ⑦ aromatase.
(Braunstein, 2001)

In the blood, androgens and estrogens exist in either a free state or bound to serum proteins. About 38% of testosterone is bound to albumin and 60% of the testosterone is bound to sex hormone binding globulin. This protein is synthesized in the liver and is distinct from the androgen binding protein secreted by the Sertoli cells. About 2% of the circulating testosterone is not bound to serum proteins and is able to enter cells and exerts its metabolic effects. Most circulating testosterone is converted primarily by the liver into various metabolites such as androsterone and etiocholanolone, which after conjugation with glutamic acid or sulfuric acid are excreted in the urine as 17-ketosteroids (Burger, 2001; Braunstein, 2001).

2.1.4.7. TESTOSTERONE AND DIHYDROTESTOSTERONE ACTION

Testosterone leaves the circulation and rapidly traverses the cell membrane by passive diffusion. In almost all cells in the body testosterone can enter the nucleus. This steroid hormone has a specific soluble, oligomeric receptor protein (mobile receptor) either in the cytosol and / or inside the nucleus. This brings about conformational changes and also changes in the surface charge of the receptor protein to favour its binding to the nuclear chromatin attached to nuclear matrix. The receptor-steroid complex is translocated to the nuclear chromatin and binds to a steroid-recognizing acceptor site called the, hormone-receptor element (HRE) of a DNA strand on the upstream side of the promotor site for a specific steroid responsive gene. The consequent change in the intracellular concentration of m-RNA alters the rate of synthesis of a structural, enzymatic, carrier or receptor protein coded by it. This results in ultimate cellular effects in the spermatogenesis (Partin and Rodriguez, 2002; Chatterjea and Shinde, 2002).

In most androgen target cells, testosterone is enzymatically converted to the more potent androgen dihydrotestosterone (DHT) by

the microsomal enzyme 5 á reductase. DHT then binds to a specific intracytoplasmic receptor protein. After binding the DHT, receptor complex is translocated into the nucleus where it undergoes transformation, allowing it to bind to the nuclear chromatin. The interaction of the androgen receptor complex with the chromatin results in the synthesis of messenger RNA (mRNA) which is eventually transported to the cytoplasm where it directs the transcription on the new protein synthesis (Burger, 2001; Braunstein, 2001).

2.1.4.8. FUNCTIONS OF STEROID HORMONES

A variety of biologic effects of androgens have been defined in males. They are essential for appropriate differentiation of the internal and external male genital system during fetal development. During puberty, androgen mediated growth of the scrotum, epididymis, vas deferens, seminal vesicles, prostate and penis occurs. The functional integrity of these organs require androgens. Androgens stimulate skeletal muscle growth and growth of the larynx, which results in deepening of the voice and of the epiphyseal cartilaginous plates which results in the pubertal growth spurt. Both ambisexual (pubic and axillary) hair growth and sexual (beard, moustache, chest, abdomen and back) hair growth are stimulated by the activity of sebaceous gland. Other effects include stimulation of erythropoiesis and social behavioral changes (Burger, 2001; Braunstein, 2001).

2.1.4.9. REGULATION OF STEROIDOGENESIS

Plasma testosterone concentration reaches adult levels (2.6 to 8.5 ng/ml) at the age of 17 years. The adult level is maintained until late middle age and then decreases slowly. After the age of 70, plasma testosterone levels decline while sex hormone binding globulin (SHBG) concentration increases. This results in a larger drop in free testosterone concentration (Griffin and Wilson, 1983; Burger, 2001).

Testosterone production in the Leydig cells is under feedback control involving the hypothalamic-pituitary-testis axis. GnRH is released in a pulsatile manner from the hypothalamus into the hypothalamic-hypophyseal portal system and stimulates the hypophysis (pituitary) to release LH and FSH. FSH regulates LH receptors by paracrine modulation of tubular function (Dufau, 1988). Testosterone, either directly or through its metabolites estradiol or DHT, decreases secretion of LH from the pituitary gland. Testosterone also decreases the mean LH level by reducing the frequency of discharge mediated by the hypothalamus (Bardin, 1986; Burger, 2001) (Fig 1 and 2).

2.1.5. SEMINIFEROUS TUBULES AND SERTOLI CELLS

The size of seminiferous tubules in the adult averages 165 ìm in diameter and are composed of Sertoli cells and germinal cells. The Sertoli cells line the basement membrane and form the tight junctions with other Sertoli cells. Through extension of cytoplasmic processes the Sertoli cells surround developing germ cells and provide an environment essential for germ cell differentiation. In addition, these cells have been shown to be responsible for the movement of germ cells from the base of the tubule toward the lumen and for the release of mature sperm into the lumen. Finally in response to FSH or testosterone, the Sertoli cells secrete androgen-binding protein, a molecule with high affinity for androgens. This substance, which enters the tubular lumen, provides a high concentration of testosterone to the developing germinal cells during the process of spermatogenesis (Braunstein, 2001).

2.1.5.1 MECHANISM OF ACTION OF FSH

In testis, FSH acts primarily on the seminiferous tubule maturation and the control of spermatogenesis. The presence of FSH is necessary

for the initiation of spermatogenesis, but the maintenance of normal spermatogenesis requires the presence of a high intratesticular androgen concentration, which in turn depends upon the steroidogenic action of LH in the Leydig cells. The actions of FSH on the seminiferous tubule are exerted through effects of the hormone upon Sertoli cells, which constitute the target cells for FSH in the testis. In Sertoli cells FSH binds and interact with cell membrane receptors and followed by activation of adenylate cyclase in the cell membrane and increased intracellular levels of cAMP. This in turn leads to activation of cAMP-dependent protein kinase in the cytoplasm and consequent phosphorylation of various proteins that mediate synthesis of DNA and cell proteins inhibin, activin, folliststin, plasminogen activator and androgen binding protein in the Sertoli cell. It is likely that FSH and testosterone act on the Setoli cells and maintenance of spermatogenesis (Catt and Dufau, 1991; Burger, 2001; Schlegel and Hardy, 2002).

2.1.5.2. MALE GAMETES

More than a dozen different types of germ cells have been described in males. Broadly they can be classified as spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa. Spermatogenesis occurs in an orderly fashion with the spermatocytes derived from the spermatogonia via mitotic division. Through meiotic division the spermatids are formed. They contain a haploid number of chromosomes. The interval from the beginning of spermatogenesis to the release of mature spermatozoa into the tubular lumen is approximately 74 days. Although there is little variation in the duration of the spermatogenic cycle, a cross section of a seminiferous tubule will demonstrate several stages of germ cell development (Braunstein, 2001).

2.1.5.3. SERTOLI CELLS

These are epithelial cells of mesodermal embryonic origin. They are arranged in a continuous single layer lining the inner aspect of the seminiferous tubule. Sertoli cells are found in a constant number at sexual maturation. The cells are characterized by several unique morphological features. They have an irregular outline with cytoplasmic projections surrounding adjacent germ cells and an irregularly shaped nucleus containing a prominent nucleolus. Adjacent Sertoli cell membranes have unique tight junctional complexes (Kerr, 1989; Ritzen et al, 1989).

The Sertoli cells rest on the basement membrane of the seminiferous tubule and each cell extends to the inner tubular lumen. Germinal cells are arranged between the Sertoli cells engulfed by their cytoplasmic projections. The undifferentiated spermatogonia are located near the basement membrane and the more advanced forms are arranged at successively higher levels nearer to the tubular lumen. Sertoli cells are more than nursing cells to the adjacent germinal cells. They play a crucial role in regulation of spermatogenesis (Paz et al, 1993; Burger, 2001).

Two types of inter Sertoli junctions have been identified. They are 'tight' and 'less intimate' junctions. The tight junction fuses with outer membranes of neighbouring Sertoli cells (Fewcett, 1975). Intramembranous granules, bundles of actin-like microfilaments and cisternae of endoplasmic reticulum are present at the junction sites (Ritzen et al, 1989). The specialized functional complexes between adjacent Sertoli cells are believed to be the principal site of this blood-testis barrier. Tight junctions of Sertoli cells subdivide the seminiferous tubule into basal and adluminal compartments (de Kretser et al, 1972; Chemes et al, 1977; Burger, 2001).

It has been shown that the Sertoli cells are the target for a number of gonadal toxins and these causes the alteration of cytoskeleton which maintains the functions of Sertoli cells. The damage of Sertoli cells secondarily leads to degeneration of the germ cell within 24 hours (Creasy et al, 1983; Farghali et al, 1993).

2.1.6 AUTOCRINE AND PARACRINE REGULATIONS

Production of a factor that acts locally on cells other than those that produce them, the action is called paracrine action. The factor can also act on the cell in which it is produced, this phenomenon is referred as autocrine action. In addition to the endocrine control, there has been increasing awareness as to the existence of a vigorous and essential paracrine activity in the testicular tissue. In the mammalian testis, very active paracrine and autocrine regulatory processes takes place between cells of the interstitial tissue (macrophages and Leydig cells) and the seminiferous tubules (Sertoli, germinal and peritubular myoid cells). The direct effect of the factors and materials secreted and acting on each of the cells was studied and reviewed in animals and in humen (Paz et al, 1993; Schlegel and Hardy, 2002) (Fig 6).

Insulin-like growth factor-I (IGF-I) is an important permissive factor involved in the maintenance and activity of most cells in the testis (Cailleau et al, 1990). All major cells of the testicular tissue produce and secrete IGF-I. Insulin-like growth factor-I has an important role in the facilitation of deoxyribonucleic acid (DNA) synthesis followed by proliferation of the cell. Most of the testicular cell type require cell proliferation (except for Sertoli cells that terminate their proliferation prior to puberty). The local production and action of growth factors are therefore required to regulate testicular cell differentiation and growth. The interstitium of the testis tissue contains macrophages and lymphocytes. The macrophages produces a peptide called interleukin-Iβ

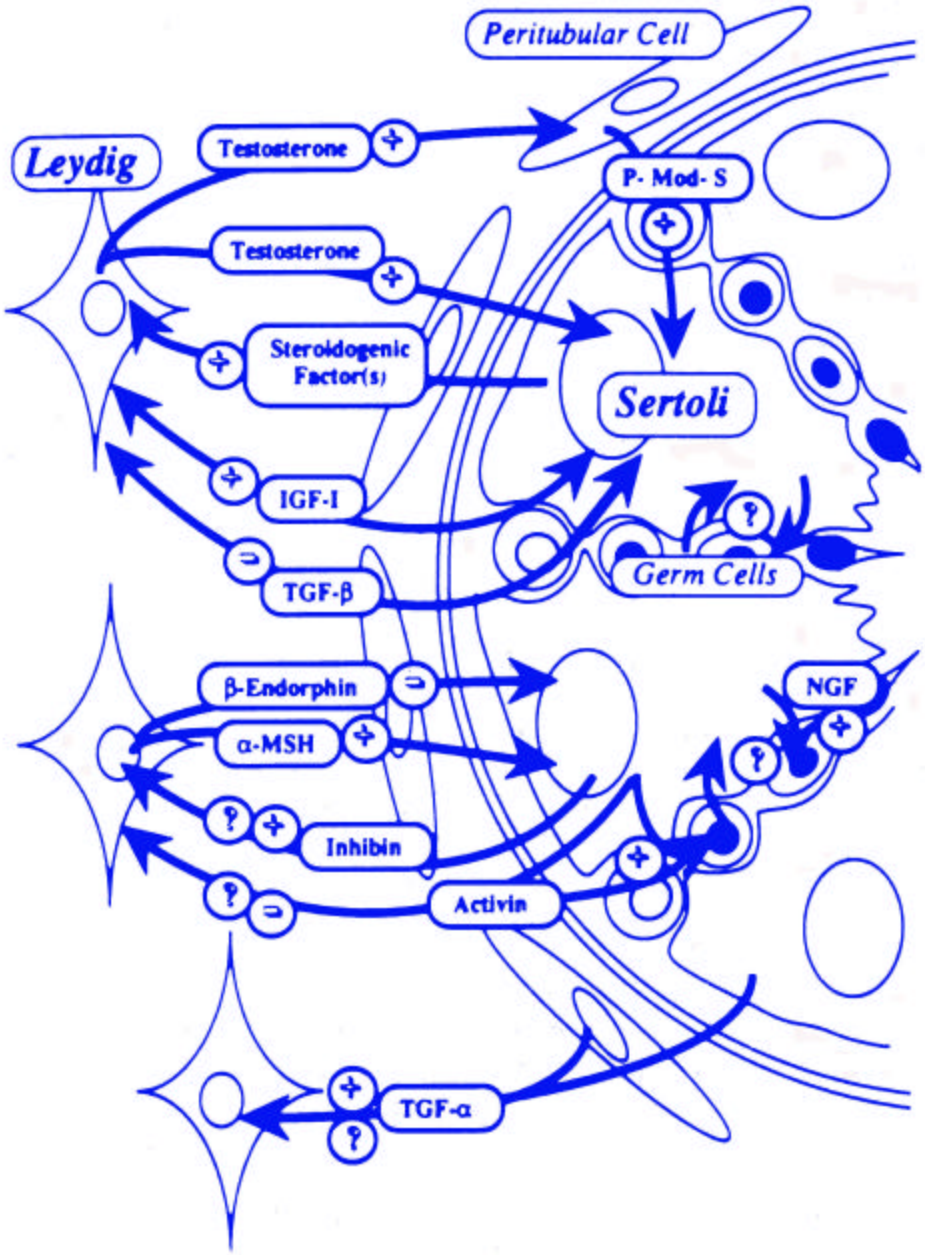


Figure 6. Paracrine regulation of testicular functions.
(Paz et al, 1993).

(IL-Iβ). This peptide at maximal effective doses stimulates steroidogenesis in vitro in isolated rat Leydig cells and implicates these cells in paracrine control of testicular function (Paz et al, 1993).

2.1.6.1 SERTOLI-GERM CELL INTERACTIONS

Sertoli cells appear to be the primary somatic cell to interact directly with the developing germinal cells. An individual Sertoli cell can be in contact with 47 adjacent germinal cells at various stages of development. A rapid rate of remodelling between the cell populations in the seminiferous tubule is necessary in order to maintain spermatogenesis at a daily production rate of millions of sperm cells (Paz et al, 1993; Schlegel and Hardy, 2002).

Sertoli cells have been shown to metabolize glucose to lactate and pyruvate which supply energy to the cells. In addition, different transport proteins, capable of transferring nutritional components from the interstitial space into the tubule and germinal cells, a process which is critical for germ-cell survival. Sertoli cells are known for their capacity to synthesize transferrin, which in turn delivers iron to the cells in the tubule through a receptor-mediated process. Transferrin and the receptor complex are endocytosed, and the iron is released. This function of Sertoli cells is essential in order to enable spermatogenesis to continue in a rapid, synchronized, well-controlled order. A similar transport mechanism was shown for copper by ceruloplasmin (copper transfer). Vitamins (especially vitamin A) and cofactors are also transported into the germinal cell line during spermatogenesis (Paz et al, 1993).

Spermatogenesis is under the control of FSH and testosterone (via LH), which also regulate Sertoli-cell function. On the other hand, germ cells can regulate Sertoli-cell activity via structural and paracrine avenues. The nature of the germ-cell factors involved in these

regulations is unknown. Some studies point to the importance of nerve growth factor (NGF), since it is produced by spermatids and affects Sertoli cells (Paz et al, 1993).

Inhibin is a glycoprotein secreted by Sertoli and Leydig cells. This is a dimer of α- and β- subunits that act to preferentially suppress pituitary FSH secretion both in vivo and in vitro (de Kretser and Robertson, 1989). The β- subunits may also form dimmers termed activin, which, in contrast to inhibin, stimulates FSH secretion. Activin formed in gonads is carried by a binding protein which is structurally similar to follistatin. It is suggested that this binding protein has an autocrine or paracrine function in the gonad (Paz et al, 1993; de Kretser et al, 2000; Schlegel and Hardy, 2002).

Androgen binding protein (ABP) was one of the first Sertoli cell secretory products identified. However even now the function of ABP can only be surmised. It may be an intracellular carrier of androgen within the Sertoli cell. ABP production has proved to be an excellent marker to test the hormonal regulation of Sertoli cell function in vitro. However, the measurement of ABP or other Sertoli cell product as a marker of Sertoli cell function has yet to have a demonstrable role in the evaluation of male infertility (Schlegel and Hardy, 2002).

2.1.6.2 LEYDIG-SERTOLI CELL INTERACTIONS

Leydig cells are the major source of androgens in the testis, and are under strict control of LH. The androgens produced regulate male behavior and accessory sex-gland function in the usual endocrine manner and support peritubular-myoid and Sertoli cell functions and spermatogenesis by way of paracrine activity. Sertoli-cell function is also known to be regulated by β-endorphin synthesized by Leydig cells under LH control. These observations suggest that β-endorphin regulates inhibin secretion by inhibiting FSH receptors coupled to

adenyl cyclase. Leydig cells also secrete an oxytocin-like immunoreactive material which influences the contractibility of the seminiferous tubule by acting on the peritubular-myoid cells (Paz et al, 1993). Melsert et al, (1988) reported that albumin present in the TIF is evidently an important regulator of testosterone production. In studies in vitro utilizing conditioned culture medium, factors secreted by Sertoli cells were identified and characterized as stimulators (IGF-I, inhibin, Transforming growth Factor-β (TGF-β)) and inhibitors (TGF-α, estradiol, interleukin-I) of Leydig-cell steroidogenesis. Thus, a Leydig cell-stimulating factor of tubular origin acts as a paracrine regulatory molecule responsible for the effects of FSH on Leydig-cell function. It can be concluded that a bi-directional paracrine short-loop feedback exists in the testis, which regulates the activities of Leydig-, Sertoli- and germ-cell populations (Paz et al, 1993; Schlegel and Hardy, 2002).

2.1.6.3 PERITUBULAR-LEYDIG-SERTOLI CELL INTERACTIONS

Peritubular cells are stromal cells that surround the seminiferous tubule, and are in contact with the basal surface of the Sertoli cells. These cells provide the structural integrity for the tubule and also appear to be involved in contraction of the tubule. Both the peritubular and Sertoli cells are responsible for the secretion of the extracellular matrix, which plays a crucial role in maintenance of the integrity of the tubule and the efficiency of the blood-testis barrier. Human Sertoli cells cultured in vitro in the presence of peritubular cells were stimulated and produced increased amounts of transferrin. Peritubular cells also influence Leydig-cell function by secreting paracrine factors which include IGF-I, TGF-α and TGF-β. The peritubular cells respond to Leydig cell androgen stimulation by secretion of a protein named P-Mod-S, which is important for the maintenance of proper Sertoli-cell activity and spermatogenesis (Skinner et al, 1988). Unfortunately, the physiologic role of these effector molecules and the mechanism by

which they regulate Sertoli cell function remain to be elucidated (Paz et al, 1993; Schlegel and Hardy, 2002).

The relevance of the paracrine regulation in idiopathic male infertility can be explained on the grounds that in these men most measurable testicular parameters are in the normal range, including reproductive hormones. Nevertheless, sperm production is abnormal. It is possible that in these cases some elements of the nature of paracrine regulation are not physiologically effective, causing an imbalance in the fine regulation of spermatogenesis, which leads to infertility (Paz et al, 1993).

2.1.7. SPERMATOGENESIS

The epithelium of the seminiferous tubules is populated by cells that give rise to approximately 123 x 10^6 spermatozoa daily in the human male (Amann and Howards, 1980). This process of sperm production is called spermatogenesis. It involves a proliferative phase during which spermatogonia divide either to replace their number (stem cell renewal) or to produce daughter cells committed to become spermatocytes; a meiotic phase when spermatocytes undergo reduction division, resulting in haploid spermatids and a spermatogenic phase when spermatids undergo a dramatic metamorphosis in size and shape to form mature spermatozoa (DiZerga and Sherins, 1981; Schlegel and Hardy, 2002). (Fig 7 and Fig 8.)

2.1.7.1. GERM CELL TYPES

Histological examination of the human testis reveals large numbers of germ cells arranged among Sertoli cells and extending from the basement membrane to the lumen of the seminiferous tubule. Morphologic analysis has revealed the presence of atleast 13 recognizable germ cell types in human testis. These cells are thought to

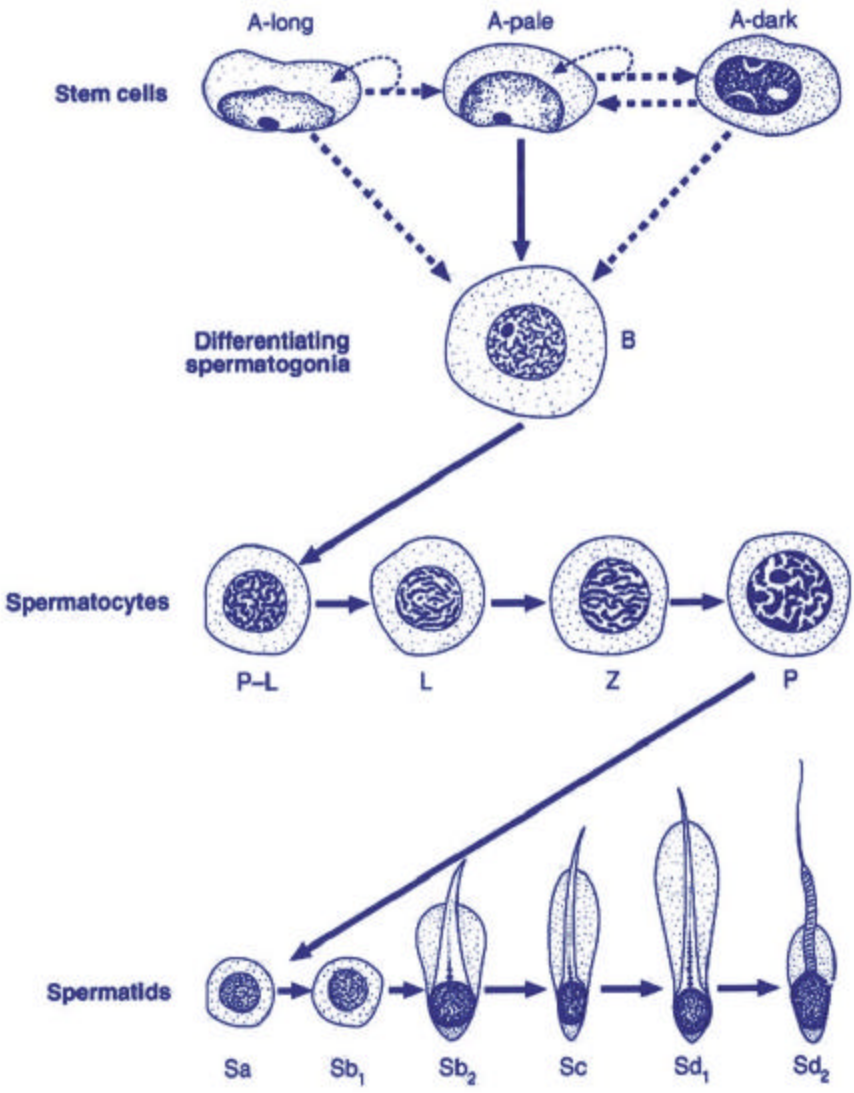


Fig. 7
Types of germ cells during the three processes of human spermatogenesis. Relationships between stages are indicated by arrows. Uncertain relationships are indicated by broken lines.
(Paz et al, 1993)

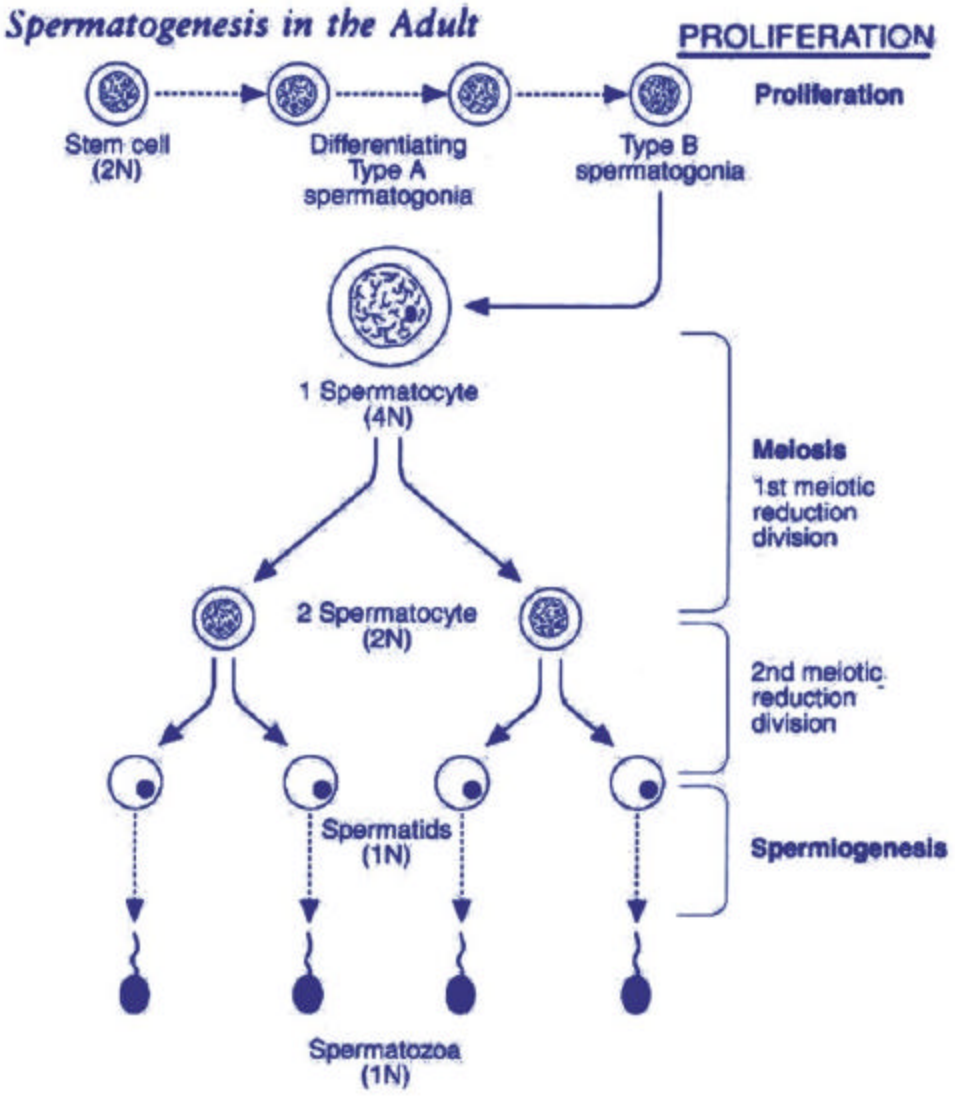


Fig. 8 Description of the three processes: proliferation, meiosis and spermiogenesis. During spermatogenesis, the DNA of the primary spermatocyte is replicated, creating the first spermatocyte with twice the normal complement of DNA (4N). Two subsequent meiotic reduction divisions produce the spermatids (1N). (Paz et al, 1993)

represent different steps in the developmental process. Proceeding from the least to the most differentiated they were named dark type A spermatogonia (A-dark), pale type A spermatogonia (A-pale), type B spermatogonia (B), preleptotene primary spermatocytes (P-L), leptotene primary spermatocytes (L), zygotene primary spermatocytes (Z), pachytene primary spermatocytes (P), secondary spermatocytes (II) and Sa, Sb_1, Sb_2, Sc, Sd_1 and Sd_2 spermatids (Schlegel and Hardy, 2002). (Fig 7 and Fig 8).

2.1.7.2. SPERMATOGENESIS PROCESS

Spermatogenesis can be subdivided into four successive processes.

1. Undifferentiated spermatogonia- proliferative process
2. Spermatogonial differentiation
3. Spermatocyte development- meiosis
4. Spermatid development- spermiogenesis and spermiation.

1. UNDIFFERENTIATED SPERMATOGONIA- PROLIFERATIVE PROCESS

Pale type A spermatogonia are located in the basal compartment of the seminiferous tubules that is formed by over reaching Sertoli-Sertoli tight junction. These Ap spermatogonia divide at 16-day intervals in the human to form B spermatogonia which are committed to become preleptotene spermatocytes (Paz et al, 1993).

2. SPERMATOGONIAL DIFFERENTIATION

The final spermatogonial division generates preleptotene spermatocytes which enter a resting phase. Towards the end of this period the preleptotene spermatocyte begins to synthesize deoxyribonucleic acid (DNA) for meiosis and subsequently enter the

long meiotic prophase. This process occurs in the basal compartment of the tubule (Paz et al, 1993).

3. SPERMATOCYTE DEVELOPMENT - MEIOSIS

Just prior to the first meiotic division, primary spermatocytes replicate their DNA and contain twice the normal amount (4N). After the first meiotic division, each secondary spermatocyte contains a haploid number of chromosomes. But the total amount of DNA in each daughter spermatocyte is equal to that of a normal somatic cell (2N) since each chromosome is in a double structure. During the second meiotic division each double structural chromosome divides so that each daughter cell (spermatid) contains 23 chromosomes (Paz et al, 1993).

4. SPERMATID DEVELOPMENT - SPERMIOGENESIS AND SPERMIATION

During spermiogenesis the products of meiosis, the round Sa spermatids metamorphose into mature spermatids. During this metamorphosis, extensive changes occur in both the spermatid cytoplasm and the nucleus. These changes include loss of cytoplasm, formation of the acrosome, formation of the flagellum and migration of cytoplasmic organelles to positions characteristic of the mature spermatozoon (Schlegel and Hardy, 2002).

2.1.7.3. TYPES OF SPERM MORPHOLOGICAL DEFECTS

Based on the measurement criteria given in Figure 9, the following various discriminatory morphological sperms (Fig. 10) were observed.

1). A Long Acrosomal Region (Long Headed Sperm).

2). Short Acrosomal Region (Tapered Headed Sperm).

3). Neck Disorders (Neck Defective Sperms).

Head
Length: 5–6 microns
Width: 2.5 – 3.5 microns

Acrosome: 40% – 70% of head

Midpiece
Width ≤ 1 micron
Length 1.5 x head length

Tail
Approximately 45 microns long
Uniform
Thinner than midpiece
Uncoiled
Free from kinks

Cytoplasmic droplets
Less than 1/2 of head area
In midpiece only

Figure 9 Criteria for normal sperm morphology by rigid criteria. (Sigman and Jarow, 2002)

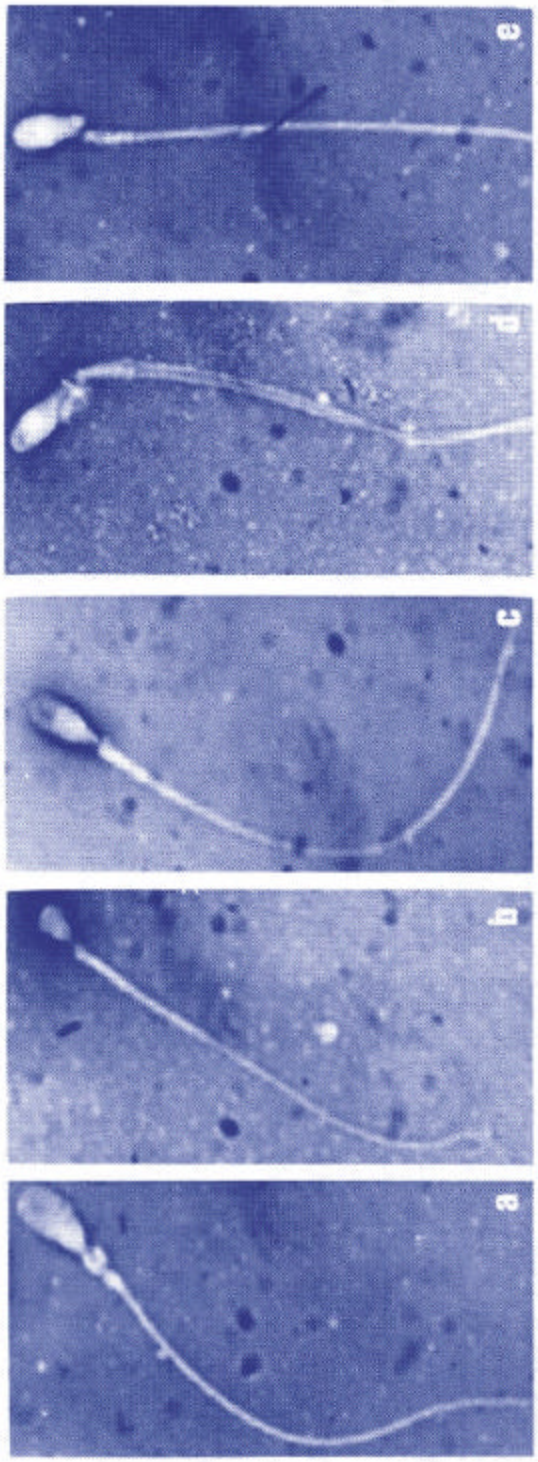


Fig.10 Photomicrographs illustrating the various discriminatory morphological sperm characteristics as observed by light microscopy. **a** long acrosomal region; **b** short acrosomal region; **c** long postacrosomal region; **d** tapering cell; **e** neck disorder. (× 1500 (Glezerman and Bartoov, 1993)

4). Long Tapering Tail Region (Tail Defective Sperms) (Glezerman and Bartoov, 1993).

2.2. MALE REPRODUCTIVE DYSFUNCTION

2.2.1. GONADOTOXINS

2.2.1.1. ENVIRONMENTAL TOXINS AND OCCUPATIONAL EXPOSURES

Exogenous chemicals are absorbed after ingestion, inhalation, or skin contact, and then distributed to various organs. Chemicals are frequently metabolized, often by multiple enzymatic pathways, produce multiple products that may be more toxic or less toxic than the parent chemical. One or more of these products then interacts with the target macromolecule, resulting in a toxic effect (Kane and Kumar, 2004).

Heindel and Treinen (1989) have estimated over 100 chemicals, toxic to the male reproductive systems in rodents. Spermatogenesis has been shown to be adversely affected by exposure to lead, arsenic, hydrocarbons, cadmium, pesticide kepone, ionizing radiation, amebicide, soil fumigants and 2-bromopropane, a recently introduced substitute for chloroflurocarbons (Kim et al, 1999; Celis et al, 2000; Telisman et al, 2000). A recent study contains compelling evidence that even moderate exposure to lead has a negative impact on the quality of human semen (Telisman et al, 2000; Benoff et al, 2003). The toxic agents may have adverse effects on the male reproductive system, resulting in poor quality of semen, either by affecting neuroendocrine function or by directly affecting the process of spermatogenesis (Robins et al, 1997; Celis et al, 2000).

2.2.1.2. RADIATION EXPOSURE

Because of the high rate of cell division, the germinal epithelium is radiosensitive. Spermatids are more resistant than spermatogonia or

spermatocytes. Leydig cells are reasonably resistant to radioactivity. Therefore testosterone levels usually remain normal after radiation exposure. Serum FSH level is increased after radiation but may revert to normal after a return of spermiogenesis. Exposure of over 65 cGy radiation usually results in azoospermia (Hahn et al, 1982; Sandeman, 1966). Quality of semen will usually return to baseline, within 2 years after radiation therapy for seminoma (Ohl and Sonksen, 1996). Approximately one fourth of patients may become permanently infertile due to radiation treatment (Fossa et al, 1989).

2.2.1.3. HEAT

There is an experimental evidence that the heat exposure may be detrimental to spermatogenesis. Occupational heat exposure affects sperm morphology which must be considered to be a significant risk factor for male infertility (Thonneau et al, 1998). A very small prospective randomized study has found lower sperm counts in those wearing tight underwear as opposed to loose shorts (Tiemessen et al, 1996).

2.2.1.4. MEDICATIONS

Erectile disorders, reduced sperm counts and diminished semen volume have been noted in patients on antipsychotic drugs. Early maturation arrest at the primary spermatocyte stage may be induced by high doses of nitrofurantoin. Sulfasalazine treatment for ulceratic colitis induces reversible defects in sperm concentration and motility. There is an evidence that calcium channel blockers cause a reversible functional defect in sperm (Benoff et al, 1994).

2.2.1.5. ABUSE OF ILLEGAL SUBSTANCE

Marijuana (Cannabis) causes impotence and low sperm count. Use of Marijuana and associated substances such as hashish lowers

testosterone levels and has an adverse effect on sexual desire (libido). Marijuana has also been reported to reduce serum testosterone and gonadotropin levels in adults. Decreased serum testosterone levels, gynecomastia and a decrease in sperm concentration have been demonstrated in patients with heavy marijuana use (Hembree et al, 1976). The use of cocaine causes impotence and other health problems. Amphetamines such as aped are also having similar effects. Opium derivatives like heroin can decrease libido and damage nervous system contributing to impotence (Morganstern and Abrahms, 1994).

2.2.1.6 ACTION OF ALCOHOL

Alcohol is ingested in alcoholic beverages such as beer, wine, and distilled spirits (Brandy and Whiskey). Congeners found in alcoholic beverages, including low-molecular weight alcohol (e.g., methanol and butanol), aldehydes, esters, histamine, phenols, tannins, iron, lead and cobalt may contribute to the adverse health consequences associated with heavy drinking. A blood alcohol concentration of 80 to 100 mg/dl is the legal definition for driving under the influence of alcohol. Approximately 44 ml of ethanol are required to produce this blood alcohol level in 70-kg person. This is equivalent to 176 ml of fortified wine or 88 ml of brandy and whiskey. In occasional drinker, a blood alcohol level of 200 mg/dl produces inebriation, with coma, death and respiratory arrest at 300 to 400 mg/dl. Habitual drinkers can tolerate blood alcohol levels up to 700 mg/dl. Chronic use of alcohol results in psychologic and physical dependence (Kane and Kumar, 2004; Schuckit, 2005).

Alcohol is absorbed from mucous membranes of the mouth and oesophagus in small amounts, from the stomach and large bowel in modest amounts and from the proximal portion of the small intestine the major site. The rate of absorption is increased by rapid gastric

emptying, by absence of proteins, fats and carbohydrates and by carbonation (Schuckit, 2005).

The intoxicating effects of alcohol appear to be due to actions at a number of neurotransmitter receptors and transporters. Alcohol enhances γ-aminobutyric acid A ($GABA_A$) receptors and inhibits N-methyl-D-aspartate (NMDA) receptors. In vitro studies suggest that additional effects involve inhibition of adenosine uptake and a translocation of the cyclic AMP-dependent protein kinase catalytic subunit from the cytoplasm to the nucleus. Neurons adapt quickly to these actions, and thus different effects may be present during chronic administration and withdrawal (Schuckit, 2005). The metabolism of ethanol is directly responsible for most of its toxic effects. In addition to its acute action as a central nervous system depressant, chronic ethanol use can cause a wide range of systemic effects (Kane and Kumar, 2004; Schuckit, 2005).

2.3. EFFECT OF ALCOHOL CONSUMPTION

2.3.1. ALCOHOL AND SOCIAL PROBLEMS

Alcohol is responsible for 50 % of traffic accidents and industrial accidents. One of the earlier homicide studies has found that a majority of murderers or their victims have been drinking prior to commiting the crime (US Congress, 1983). Rape is an extremely difficult crime to investigate. In a study of violent crime in Western Canada, 60 percent of rapes in a 12 months period were considered to be alcohol related; either the victim or the defendant had been found drunk (Gerson and Preston, 1979).

Alcohol is clearly associated with disrupted family functioning but the marital and family problems may precede the alcoholism. The rate of separation and divorce among alcoholics and their spouses is seven

times more than that of the general population. The abuse of children by their parents and the spouses by their marital partners has received increasing attention in the media and in the professional literature. Summaries of unstructured or semistructured interviews with children of alcoholics suggest that these children feel rejected by their parents, and are guilty of parent's alcoholism (US Congress, 1983).

In men who consume alcohol, both the testes and the liver are directly affected by ethanol. Testicular atrophy is commonly found in chronic alcoholics. Testicular specimens demonstrate peritubular fibrosis and a reduction in the number of germ cells. Free testosterone levels are often decreased where as total testosterone levels may be within the normal range secondary to elevated levels of testosterone-estradiol-binding globulin. Patients may demonstrate erectile dysfunction and gynecomastia as well as a decrease in general virilization (Mendelson et al, 1977). Gonadotropins levels may or may not be increased because pituitary function is suppressed. Studies on the acute consumption of alcohol in nonalcoholics demonstrate fallen testosterone levels (Gordon et al, 1976). No correlation has been found between sperm count or motility and the level of ethanol consumption in groups of infertile men (Marshburn et al, 1989; Dunphy et al, 1991). In addition the chance of couples conceiving (fecundity) has not been found to be associated with alcohol consumption in men (Goverde et al, 1995; Curtis et al, 1997; Olsen et al, 1997).

2.3.2. EFFECT OF ALCOHOL ABUSE ON HUMAN SYSTEMS

Cancers of mouth, tongue, pharynx and esophagus are more common in alcoholics than in nonalcoholics. Wynder (1975) has suggested that a synergistic effect between alcohol and tobacco may be responsible. After either acute or chronic alcohol consumption, the small intestine may demonstrate structural injury. Alcohol excess inhibits the

transport of electrolyte, amino acids and glucose across the small intestine (US Congress, 1983; Kuo and Shanbour, 1978). Alcohol when used heavily, may have detrimental effects on the heart. Cardiac myopathy a damage to the heart muscle often occurs after 10 or more years of heavy drinking. Alcohol also affects involuntary smooth muscle contractions. Muscular weakness and severe pain from muscle cramping are fairly common in heavy drinkers. Excessive drinking causes blood abnormalities like enlarged red blood cells and anemia. Many alcoholics also have reduced white blood cell counts. These cells play an important role in the body's immune response (US Congress, 1983; Kane and Kumar, 2004; Schuckit, 2005).

Excessive alcohol use can cause chronic pancreatitis in which the functional impairment persists even in the absence of alcohol intake. More than 75 % of the cases of chronic pancreatitis in the United States occur in alcoholics (Sarles, 1974). It is unclear whether liver disease associated with alcohol consumption is a direct effect of the alcohol or of the malnutrition that often accompanies alcoholism. The landmark Work, of Lieber (1981) and his colleagues established that alcohol is directly toxic to the liver. There are three specific subtypes of hepatic pathology associated with alcohol use: 1. alcoholic fatty liver, 2. alcoholic hepatitis and 3. alcoholic cirrhosis. Alcoholics with liver disease may have enlarged kidneys with increased fat, protein and water (US Congress, 1983; Kane and Kumar, 2004; Schuckit, 2005).

2.3.3. EFFECT OF ALCOHOL ON ENDOCRINE FUNCTION

The effect of alcohol on hypothalamus, pituitary and adrenal axis have been studied and found that there is a significant raise in the ACTH (Adrenocorticotrophic Hormone) and Cortisol (Inder et al, 1995). The effect of alcohol on hypothalamus, pituitary and thyroid axis was studied and found that there is no significant effect on the axis (Jackson

et al, 1990). Serum parathormone (PTH) may also be affected by alcohol. Despite of the decrease in serum levels of calcium and magnesium, serum PTH and 1,25- $(OH)_2$ vitamin D were also low. Laitinen et al, (1991) found that an acute administration of ethanol to normal subjects resulted in decrements in serum levels of ionized calcium and PTH and augmented calcium and magnesium excretion (Adler, 1992).

2.3.4. ALCOHOL AND SEXUAL DISORDERS

2.3.4.1. EFFECT OF ALCOHOL ON ACCESSORY SEX ORGANS

Sexual disorders have been reported in men who are on long term alcohol use, ranging from 8% to 58 % (Schiavi, 1990). Lemere and Smith (1973) reported that 8% of 17,000 patients treated for alcoholism had impotence.

Whalley, (1978) reported that 54% of hospitalized alcoholic men and 24% of healthy controls had erectile impotence. Jensen, (1984) reported that 63% of married alcoholic men and 10% of controls had sexual dysfunctions especially lack of sexual desire. It has been reported that high levels of blood alcohol cause reduced sexual stimulation, ability to enjoy orgasm and retarded ejaculation (Mulligan et al, 1988; Rosen, 1991; Gumus et al, 1998).

Ethanol produces pathological changes in the male reproductive tract. The changes are, reduced weight of testicular and accessory sex organ, decreased spermatogenesis, increased desquamation of immature germ cells into the lumina of seminiferous tubule and increased frequency of inactive seminiferous tubules (Willis et al, 1983). The mass and volume of seminiferous tubules decrease with the

increased alcohol consumption. The most sensitive tissue to chronic alcohol induced injury in testis is the germinal tissue (Karhunen et al, 1984).

2.3.5. EFFECT OF ALCOHOL ON REPRODUCTIVE ENDOCRINOLOGY

Long term ethanol treatment diminishes the circular and intratesticular testosterone levels, causing a hypogonadism in humans and animals. Proposed mechanisms of the adverse effects of ethanol are 1. decreased gonadotropin secretion, 2. direct suppression of testicular function and 3. altered metabolism of androgens in the liver and other peripheral organs. Ethanol inhibits the action of the C17- 20 lyase enzyme or decreases the same in the steroidogenic pathway of testosterone biosynthesis (Eriksson et al, 1983; Pohl et al, 1987; Akane et al, 1988). Ethanol consumption also produces low concentrations of luteinizing hormone (LH) and LH-Releasing Factor when compared with plasma testosterone levels (Schade et al, 1983).

Alcohol appears to exert dual effect on the hypothalamic – pituitary – gonadal (HPG) axis by directly inhibiting testicular steroidogenesis and by sliding the release of Luteinizing hormone releasing hormone (LHRH)/LH from the hypothalamic-pituitary axis (Little et al, 1992). Chronic ethanol diet decreases pituitary LH and FSH release by subtle mechanisms modifying hypothalamic GnRH release, but does not suppress their synthesis and maintenance of GnRH receptors. Ethanol consumption lowers the levels of testicular T and serum T by reducing the serum LH and testicular LH receptors (Salonen and Huhtaniemi, 1990).

Recently Kim et al, (2003) reported that chronic ethanol treatment in the male rat decreased the number of GnRH mRNA-containing cells

in the preoptic area and levels of GnRH mRNA in the hypothalamus. The decrease in GnRH mRNA level resulted in the reduction of GnRH secretion onto portal vessel and inhibited synthesis and release of pituitary LH.

2.3.5.1. EFFECT OF ALCOHOL ON HYPOTHALAMIC ENDOGENOUS OPIOID PEPTIDES (EOP)

Alcohol suppresses the secretion of LHRH in the hypothalamus by activating endogenous opioid peptide including beta-endorphin systems (Burns et al, 1989; Adams et al, 1991) which tonically inhibits the secretion of LHRH (Cicero, 1980; Kalra and Leadem, 1984). Alcohol also directly suppresses the biosynthesis of testosterone at the gonadal level of the HPG axis (Cicero, 1981; 1982).

It is well established that alcohol inhibits the HPG axis at the hypothalamus through inhibition of luteinizing hormone releasing hormone (LHRH) release and at the testes through direct inhibition of steroidogenesis. Various reports have suggested that alcohol stimulates the release of endogenous opioid peptides in male gonadal portion and in brain (Douglass et al, 1987; Adams et al, 1991). Alcohol's inhibition of LHRH secretion in the hypothalamus is mediated through the stimulation of EOP secretion in the hypothalamus. In vivo evidence also suggests that EOP exerts inhibitory autocrine/paracrine effects on testosterone secretion and testicular interstitial fluid (TIF) formation (Adams et al, 1991; Adams et al, 1997).

2.3.6. EFFECT OF ALCOHOL ON PROLACTIN

Ethanol acts directly on the central nervous system by increasing plasma prolactin levels. High prolactin levels have been shown to decrease LH secretion (Sanchis et al, 1985) and lowered LH values may result in reduced testosterone levels (Cicero and Badger, 1976;

Cicero et al, 1979). Chronic alcohol administration has resulted in the decrease in plasma testosterone and LH levels. Along with these modification, there was an increase in basal plasma estrogen and prolactin levels (Mateos et al, 1987).

Acute alcohol intoxication induced dysfunction of pituitary-gonadal axis hormones, pituitary adrenal axis hormones and prolactin were studied in adult men and was found that acute alcohol had produced a high increase in plasma prolactin, corticotropin (ACTH), dehydroepiandrosterone-sulphate (DHEA-S) and cortisol and decrease in plasma luteinizing hormone and testosterone (Frias et al, 2002). Alcohol ingestion increased prolactin levels and decreased testosterone levels, but did not change other hormone levels in men (Ida et al, 1992).

2.3.7. EFFECT OF ALCOHOL ON TESTICULAR FUNCTION

Severe germ cell injury and seminiferous tubule atrophy are the characterized findings in men who take alcohol for prolonged periods. Prolonged consumption of alcohol causes impaired spermatogenesis, decreased seminiferous tubular volume and gross testicular atrophy. Ethanol impairs Leydig cell function which is manifested principally as a reduced synthesis and secretion of testosterone. This leads directly to a loss of male secondary sex characteristics. The initial Leydig cell injury induced by ethanol, increased gonadotropin secretion (Rosenblum et al, 1989).

The effects of alcohol on the function of the HPG axis in humans have been well documented. A suppression of plasma or serum testosterone levels after acute alcohol administration is the most consistent and predominant effect reported. This effect appears to reflect a direct inhibition of testicular steroidogenesis (Cicero, 1982; Ellingboe, 1987).

Impotence, testicular atrophy, gynecomastia and loss of sexual interest are often associated with alcoholism in men. Both acute and chronic alcohol intoxication result in dose-dependent suppression of plasma testosterone levels in normal men. Alcohol induced suppression of male testosterone appears to be due to a direct effect on the biosynthetic processes in testes (Valimaki et al, 1984; Mello et al, 1985). Increased LH levels after alcohol-induced suppression of testosterone in man (Mendelson et al, 1978; Valimaki et al, 1984) is consistent with established mechanisms of negative feedback of LH secretory activity (McCann, 1974). Alcohol decreases the volume of testicular interstitial fluid (TIF) and the TIF testosterone concentration (Adams and Cicero, 1991; Adams et al, 1991).

Several investigators have reported that the alcohol readily penetrates the testis (Salonen and Eriksson, 1989), acts directly on Leydig cells and interferes with steroidogenesis (Akane et al, 1988; Orpana et al, 1990a; Adams et al, 1997; Rivier, 1999). Serum levels of testosterone are found to be decreased and estradiol levels increased in chronic alcoholic men (Gomathi et al, 1993).

Acute alcohol treatment simultaneously stimulates the secretion of immunoreactive beta-endorphin-E, the EOP into testicular interstitial fluid and decreases testosterone secretion. Ethanol induced increase EOP have been shown an inhibitory effect on the release of LHRH and LH from the hypothalamic-pituitary neuro endocrine axis (Adams and Cicero, 1991; Adams et al, 1991; Burns et al, 1989; Gianoulakis, 1990). Taken as a whole, these data suggest that the effects of alcohol on the HPG axis mediated changes in the EOP and control of functional activity in the hypothalamus, pituitary and testes (Adams and Cicero, 1991).

2.3.7.1. EFFECT OF ALCOHOL ON ALCOHOL AND STEROIDOGENESIS ENZYMES

Ethanol (EtOH) inhibition sites in vitro are the reactions from pregnenelone to progesterone and androstenedione to testosterone. The enzymes are nicotinamide adenine dinucleotide (NAD^+) dependent 3-beta-hydroxy steroid dehydrogenase/ Oxisteroid isomerase and NADPH-dependent 17-ketosteroid reductase respectively (Akane et al, 1988; Orpana et al, 1990b).

Ethanol inhibits testicular steroidogenesis by suppressing atleast two steps in the pregnenolone to progesterone pathway catalyzed by (1) NAD^+ dependent 5-ene-3-beta hydroxysteroid dehydrogenate / isomerase and (2) the 17-hydroxy progesterone to androstenedione step catalyzed by the NAD^+ independent 17-20 lyase (Akane et al, 1988).

Ethanol misusers may develop gonadal disorders including structural testicular changes and a decrease in testicular and serum levels of testosterone which may be involved in the hypogonadism and feminization phenotype. Ethanol and its metabolite acetaldehyde cause a reduction in the luteinizing hormone binding to the Leydig cells (Bannister and Lowosky, 1987; Adler, 1992). Ethanol also directly affects activities of serum steroidogenic enzyme (Salonen and Huhtaniemi, 1988; 1990; Emanuele et al, 1989; 1991; Shi et al, 1998).

The metabolism of Ethanol probably by rising the nicotinamide adenine dinucleotide (NADH)/NAD^+ ratio in different cell compartments is responsible for the direct testicular inhibition of testosterone biosynthesis in Leydig cells (Orpana et al, 1990b). At the level of testicular interstitial cells, the ethanol-induced decrease of NADH/NAD^+ ratio might suppress the NAD^+ dependent steps of

testicular steroidogenesis (Eriksson et al, 1983; Akane et al, 1988). Alcohol may act via metabolic mechanisms such as alterations in the NAD^+/NADH equilibrium in Sertoli and germ cells and also act by disturbing the function of mitochondria in Leydig cells (Orpana et al, 1990a, 1990b).

2.3.7.2. EFFECT OF ALCOHOL ON LEYDIG CELL StAR PROTEIN

The StAR protein synthesized in steroidogenic Leydig cells of testis plays an essential role in the regulation of rate-limiting step in steroid hormone synthesis. StAR mRNA is expressed in the rat Leydig cells and is induced by gonadotropic hormones such as FSH, LH and forkolin via a cAMP second messenger. The chronic administration of ethanol inhibited the gene expression of StAR in the Leydig cells of male rat. Therefore the depression of serum testosterone by prolonged ethanol intake seemed to be mediated by a decrease in StAR mRNA level. (Kim et al, 2003).

2.3.7.3. EFFECT OF ALCOHOL ON TESTICULAR ESTRADIOL

Masters and Johnson (1970) have reported that in 35 out of 213 men with secondary impotence, the erectile disorder has occurred as a direct result of alcohol intake. Hyperprolactinemia is frequently observed in chronic alcoholics with and without liver disease. Being hypogonadal, chronic alcoholic men may show signs of hyperoestrogenization and have elevated plasma oestrone levels (Van Thiel and Lester, 1979; Valimaki and Ylikihri, 1983). Low plasma androgens and high estrogen and prolactin levels have been noted repeatedly in sexually dysfunctional alcoholic men (Schiavi, 1990). The hypersecretion of prolactin, β-endorphin and pituitary-adrenal axis hormones may also be involved in alcohol-induced dysfunction of pituitary-gonadal axis hormones in human adults (Frias et al, 2000).

Aromatase converts testosterone to estradiol. Aromatase is likely to be increased by ethanol in the study since ethanol treated animals have elevated estradiol levels. This ethanol induced rise in circulating estrogen has been seen in human and animal studies (Emanuele et al, 1999a).

2.3.7.4. EFFECT OF ALCOHOL ON BLOOD - TESTIS BARRIER

Ethanol has been shown to be a testicular toxin at the ultrastructural, biochemical, hormonal and morphological levels. Anatomically ethanol produces testicular atrophy as a result of a marked loss of germ cells. This reduction in germ cell numbers is a consequence of ethanol-induced reduction in testosterone synthesis, an alteration in vitamin A metabolism, lipid peroxidation and a disruption of the blood testes barrier (Farghali et al, 1993; Chiao and Van Thiel, 1983).

2.3.8. EFFECT OF ALCOHOL ON CATECHOLAMINES AND TESTOSTERONE

Catecholamines that are released by stress stimulate testosterone secretion in vitro (Anakwe et al, 1985) but catecholamines given in vivo can decrease plasma testosterone (Damber and Janson, 1978). In relation to TIF formation, catecholamines affect vascular smooth muscle and were found to decrease testicular blood flow (Free and Jaffe, 1972). Alcohol also stimulates corticosterone release from the adrenals and corticosteroids appear to inhibit testosterone secretion through testicular glucosteroid receptors (Feek et al, 1989; Adams et al, 1991). Both Nitric Oxide (NO) and Ethanol inhibit HPG function especially testosterone production in the male. It is conceivable that NO may mediate some of Ethanol actions on the HPG axis, because it mediates the sedative-hypnotic effect of Ethanol (Adams et al, 1994).

2.3.9. EFFECT OF ALCOHOL ON METABOLIC CLEARANCE OF TESTOSTERONE

The fall in testosterone in alcoholics is likely owing to decreased secretion as well as increased metabolism. A number of in vitro studies have established that Ethanol and its metabolite acetaldehyde can decrease testosterone secretion. The ability of Ethanol to increase the clearance of testosterone has been established in healthy humans without liver disease (Orpana et al, 1990a; Emanuele et al, 1999a).

2.3.10. ALCOHOL AND LIVER DISEASE INDUCED REPRODUCTIVE CHANGES

Alcoholic liver cirrhosis has significantly elevated serum estrone, estradiol, follicle stimulating hormone, luteinizing hormone and prolactin, whereas serum testosterone is not significantly different from that of controls. The incidence of gynaecomastia (38%), cutaneous spiders (67%), testicular atrophy (24%) and reduced axillary hair (71%) has been observed in man with alcoholic cirrhosis (Bahnsen et al, 1981).

Alcoholic cirrhotic man often presents with testicular atrophy, azoospermia, reduced body hair, reduced beard growth, reduced prostatic size, as well as gynecomastia, arterial spiders, female escutcheon, female body habitus and sexual dysfunction. These symptoms suggest an endocrine imbalance of sexual hormones but both normal and decreased plasma testosterone concentrations and normal and increased plasma estrogen concentrations have been observed. Alcoholic cirrhotic man have decreased albumin and increased concentrations of sex hormone binding globulin (SHBG) which may alter the metabolism and action of sex steroids (Gluud, 1988; Rosenfield and Moll, 1983). Alcoholic males with cirrhosis have been reported to have a decreased testosterone concentration and an

increased level of luteinizing hormone (Jaan et al, 1997). Clinical manifestations of gonadal failure such as loss of libido, infertility, impotence and testicular atrophy are commonly observed in chronic alcoholics both with (Valimaki et al, 1982) and without (Bertello et al, 1982) cirrhosis of the liver (Iranmanesh et al, 1988).

2.3.11. WITHDRAWAL EFFECTS OF ALCOHOL

Alcohol reduces serum testosterone levels which gets increased after the withdrawal of alcohol and there is also a recovery of the lost circadian rhythm (Castilla-Garcia et al, 1987; Ruusa and Bergman, 1996).

2.3.12. EFFECT OF ALCOHOL ON SEMEN AND IT'S FUNCTIONAL CONSTITUENTS

Ethanol produces significant decrease in the percentage of sperm motility, straight line velocity and curvilinear velocity. Alcohol causes a significant decrease in the number of spermatozoa with normal morphology and an increase in irreversible tail defects (Donnelly et al, 1999). In chronic alcoholics, there is a marked reduction in sperm concentration, forward motility and increase in the number of spermatozoa with morphological abnormalities (Gomathi et al, 1993).

The sperm of ethanol consuming animals has exhibited alterations in their spermatozoa concentration, abnormal motility and morphology and a decrease of the fecundation capability (Anderson et al, 1983). It has been reported that ethanol users may exhibit sperm alterations such as changes in the count, morphology and viability of the spermatozoa (Gomathi et al, 1993; Goverde et al, 1995). Alcohol exerts a dose-related toxic effect on testicular function. Spermatogenesis disruption and a primary testicular insufficiency and compensatory

increase of FSH and LH secretion have been observed in alcoholics (Villalta et al, 1997).

Alcohol is one agent that has been suspected of contributing to testicular damage (Anderson et al, 1989). Hypogonadism, disturbances of spermatogenesis, peritubular fibrosis and infertility have been shown to occur in chronic alcoholics. The main alcohol-related morphological disorder of the testis is spermatogenic arrest (Pajarinen and Karhunen, 1994).

2.4. CIGARETTE SMOKING

The use of tobacco leaf to create and satisfy nicotine addiction was introduced to Columbus by Native Americans and spread rapidly to Europe. Use of tobacco products, including cigarettes, cigars, pipes and snuff, is associated with more mortality and morbidity than any other personal, environmental, or occupational exposure. About 45 million people in the United States smoke 480 billion cigarettes each year. Cigarette smoking in United States, resulting in an annual economic loss of 157 billion dollars from health-related costs (Arora et al, 2001; Kane and Kumar, 2004; Burns, 2005).

Tobacco use is one of the major preventable cause of death and disability worldwide. The emergence of tobacco related diseases is a burgeoning public health problem. According to recent WHO estimates, 4.9 million deaths annually are attributed to tobacco. This figure is expected to rise to 8.4 million by the year 2020. Currently about one-fifth of all worldwide deaths attributed to tobacco occur in India, more than 800000 people die and 12 million people become ill as a result of tobacco use each year. The deaths attributable to tobacco, in India, are expected to rise from 1.4% of all deaths in 1990 to 13.3% in 2020 (Arora et al, 2001)

India is the world's second largest producer of tobacco. The country manufactured 94 billion cigarettes in 2001. In the country, other forms of tobacco use are more prevalent than cigarette smoking, particularly among women. According to the National Sample Survey in 1993-94, among persons aged 10 years and older, 29.3% of rural males, 20.2% of urban males, 2.3%of rural females and 0.7% of urban females smoked cigarettes or bidis. Whereas for other forms of tobacco such as snuff, chewing tobacco, burnt tobacco, powder and paste, 19.3% rural males, 9.9% urban males, 9.3% rural females and 4.3% urban females in the same age-group used these (Arora et al, 2001).

Nicotine is the principal constituent of tobacco responsible for its addictive character. Addicted smokers regulate their nicotine intake by adjusting the frequency and intensity of their tobacco use both to obtain the desired psychoactive effects and avoid withdrawals. Other forms of tobacco use are moist snuff deposited between the cheek and gum, chewing tobacco, pipes and cigars, and recently bidi and clove cigarettes. All the forms of burned tobacco generate toxic and carcinogenic smoke similar to that of cigarette smoke. Filtered cigarettes with lower machine-measured yields of tar and nicotine have been recommended as offering lower disease risk. However there is no meaningful disease reduction benefit for filtered cigarette smokers (Kane and Kumar, 2004; Burns, 2005).

Tobacco chewing constitutes one of the forms of smokeless tobacco. The habit also appears to be common in young amateur and professional baseball athletes and in other parts of the world, such as India, China, and the southeast Asia region. In India, chewing tobacco is systematically associated with socioeconomic markers at the individual and household level. Individuals with no education are 2.69

times more likely to smoke and chew tobacco than those with a postgraduate education. In general, smokeless tobacco is substantially less harmful than smoking (Said et al, 2005).

2.4.1. CIGARETTE SMOKE AND ITS CONTENTS

Mainstream of cigarette smoke inhaled by the smoker is composed of a particulate phase and a gas phase; tar is the particulate phase without water or nicotine. There are 0.3 to 3.3 billion particles per milliliter of mainstream of smoke and more than 4000 constituents, including 43 known carcinogens. In addition to these chemical carcinogens, cigarette smoke contains carcinogenic metals such as arsenic, nickel, cadmium and chromium. The gas phase contains carbon monoxide, respiratory irritants such as nitrogen dioxide and formaldehyde and cilio toxins such as hydrogen cyanide (Kane and Kumar, 2004; Burns, 2005).

Each cigarette contains about 5-15 mg of nicotine (NIC) and from each cigarette smoked, about 1 mg of NIC is absorbed to the blood stream. Inhaled NIC is swiftly oxidized to its main metabolite, cotinine (COT). COT is eliminated more slowly since it has a half-life of 19 hours. NIC is having a half-life of 2 hours. Cigarette smoke contains a mixture of harmful components such as carbon monoxide (CO), hydrogen cyanide (HCN), ammonia, volatile hydrocarbons, alcohol, aldehydes, tar, benzo(a)pyrene, nitrosamines and ketones (Gandini et al, 1997).

Carbon monoxide has 200 times higher affinity for hemoglobin than oxygen does. Thus, carbon monoxide exposure decreases the delivery of oxygen to the peripheral tissues. Nicotine the important alkaloid constituent of cigarette smoke readily crosses the blood-brain barrier and stimulates nicotine receptors in brain. The inhaled agents in cigarette may act directly on the mucous membranes, may be

swallowed in saliva, or may be absorbed into the bloodstream from the abundant alveolar capillary bed. By various routes of delivery, the constituents of cigarette smoke act on distant target organs and cause a variety of systemic diseases (Kane and Kumar, 2004; Burns, 2005).

2.4.2. HEALTH HAZARDS OF CIGARETTE SMOKING

Smoking increases the risk for all four major types of lung cancer squamous, small cell, large cell and adenocarcinoma. The greatest numbers of deaths attributable to cigarette smoking are due to lung cancer, ischemic heart disease and chronic obstructive lung disease. Cigarette smoking is associated with a significantly increased risk of lung cancer, laryngeal cancer, oral cancer, esophageal cancer, bladder and kidney cancer, carcinoma of pancreas, stomach cancer, cervical cancer and haematopoietic cancer. Lung cancer is caused by multiple carcinogens and promoters in cigarette smoke. The lung cellular changes are dose related and the incidence of lung cancer is directly related to the number of cigarettes smoked (Fiore, 1992; Kane and Kumar, 2004; Burns, 2005).

Cigarette smoking is a multiplicative risk factor with hypertension and hypercholesterolemia for development of coronary artery disease and arteriosclerosis. It is also a multiplicative risk factor for acute myocardial infarction and stroke. Smoking may contribute to cardiac arrest by increasing platelet adhesion and aggregation, triggering arrhythmia and by causing an imbalance between the demand for oxygen and supply to the myocardium. Cigarette smoking is especially hazardous in the workplace. Cigarette smoke may act as a vector to transport other hazardous agents into the lungs, such as radon gas in miners (Kane and Kumar, 2004; Burns, 2005).

NIC is quickly absorbed through the respiratory tract, mucosa of mouth and skin. About 80% to 90% of the NIC is metabolized mainly by

the liver and also by the kidney and the lungs. NIC and its metabolites have been detected in serum, urine, saliva and human milk (Pacifici et al, 1993) and more recently it has been found at significant levels in smokers seminal plasma (Gandini et al, 1997; Kane and Kumar, 2004; Burns, 2005).

2.4.3. CIGARETTE SMOKING AND MORTALITY

The American Cancer Society has estimated that cigarettes are responsible for more than 440,000 deaths in the United States each year. The risks of dying from lung cancer are 23 times higher for male smokers and 11 times higher for female smokers than nonsmokers. Research has shown that mothers who smoke, give birth more frequently to premature or underweight babies, probably because of a decrease in blood flow to the placenta. Babies born to mothers who smoke during pregnancy are also at increased risk for sudden infant death syndrome (Kane and Kumar, 2004; Burns, 2005).

2.4.4. EFFECT OF CIGARETTE SMOKING ON REPRODUCTIVE FUNCTION

Many smokers and smokeless tobacco users are at increased risk of reduced sexual function. Smoking can cause the early onset of menopause among women, incontinence and reduced fertility. It may also cause reduced fertility among men (Close et al, 1990; Moskova and Popov, 1993). The highest prevalence of smoking is observed in 46% of young adult male smokers, between 20 and 39 years of age, during their effective reproductive period (Langgassner, 1999). According to a meta-analysis of 20 different study populations worldwide, the sperm count is found to be reduced by 13% to 17% in smokers (Vine et al, 1994). It is reported that smoking is related to aneuploidy in spermatozoa (Rubes et al, 1998; Harkonen et al, 1999).

Paternal smoking is found to be associated with childhood brain cancer (McCredie et al, 1994; Norman et al, 1996), neuroblastoma and rhabdomyosarcoma. There is a recent report that preconception smoking is associated with an increased risk of childhood cancer (Rubes et al, 1998). Epidemiological studies in women of reproductive age have shown that cigarette smoking can delay conception by 2 months (Zenzes, 1995) and advance the start of menopause by 2 years. The literature also suggests that women who smoke have a spontaneous abortion (Armstrong et al, 1992).

Smoking is found to significantly reduce the ejaculated volume of semen by altering accessory glands (Pacifici et al, 1993; Vine et al, 1996; Gandini et al, 1997; Rubes et al, 1998; Zenzes et al, 1999; Zhang et al, 2000; Chia et al, 2000; Kunzle et al, 2003). The increasing incidence of testis cancer in Copenhagen has been temporarily halted among men born between 1939 and 1945 during the wartime tobacco shortage (Clemmesen, 1997).

Eighty-three percent of alcoholics are found to be smokers compared to 34% of the nonalcoholics. Alcoholism is estimated to be 10 times more common among smokers than nonsmokers (DiFranza and Guerrera, 1990). Sperm density of smokers is 13% to 17% lower than that of non-smokers. Cigarette smoking is associated with lowered sperm density (Vine et al, 1994).

2.4.5. EFFECT OF CIGARETTE SMOKING ON REPRODUCTIVE ENDOCRINE FUNCTION

Numerous studies have demonstrated that some environmental toxic agents may have adverse effects on the male reproductive system, either by affecting neuroendocrine function or by directly

affecting the process of spermatogenesis resulting in poor semen quality (Robins et al, 1997; Celis et al, 2000). The mean estradiol level is higher in smokers than in non-smokers whereas the levels of testosterone and dehydroepiandrosterone (DHEA) are similar and do not differ significantly in smokers when compared to non-smokers. Smokers have also lower mean levels of LH, FSH and PRL than non-smokers. Smokers with low prolactin levels have also low sperm motility. Changes in endocrine profile due to cigarette smoking can reduce the fertility (Ochedalski et al, 1994).

Mendelson, (2003) studied the effects of cigarette smoking and reported that low nicotine cigarette smoking did not change the levels of LH, T and prolactin hormones. LH and prolactin increased significantly after high nicotine cigarette smoking. Testosterone levels did not change significantly even after high nicotine cigarette smoking. The effects of cigarette smoking on testosterone levels in human males demonstrate that testosterone levels are lower in healthy male smokers than in age matched non-smokers (Shaarawy and Mahmoud, 1982; English et al, 2001). Smoking is detrimental to testicular steroidogenesis due to acute reversible reduction in plasma testosterone (Briggs 1973; Handelsman et al, 1984).

It has been shown that plasma testosterone levels of male smokers are lower than non-smokers. It has been suggested that decreased plasma testosterone levels may adversely affect spermatogenesis (Anderson et al, 1984; Yeh et al, 1989). Plasma levels of FSH and LH of smoke-exposed group do not change significantly. Direct toxic effects of smoking on spermatogenic cells decrease plasma levels of testosterone and FSH (Yardimci et al, 1996).

Epidemiological studies of cigarette smokers have suggested an increased prevalence of impotence and impaired fertility. The

mechanism of several dysfunction in man is commonly associated with low testosterone levels (Anderson et al, 1984). Cigarette smoking is associated with modest reduction in semen quality including sperm concentration, motility and morphology. Smoking has also been associated with increased levels of estrone and estradiol (Vine, 1996). Nicotine and cotinine have also been shown to directly reduce testosterone levels in isolated Leydig cells (Patterson et al, 1990).

2.4.6. EFFECT OF CIGARETTE SMOKING ON QUALITY OF SEMEN

Smokers have been found to have significantly decreased sperm density and motility than nonsmokers. Similarly, Rantala and Koskimies (1987) compared the quality of semen of 60 smoking and 50 nonsmoking men undergoing infertility investigation. They too reported that there was a significant lower total sperm count among the heavy smokers. Cigarette smoking is associated with a reduction in sperm quality and viability (Sofikitis et al, 1995; Vine, 1996; Rubes et al, 1998).

Cigarette smoking is possibly only a minor risk factor for male subfertility (Wong et al, 2000). Rubes et al, (1998) reported an increase in the percentage of “round-headed” sperm and increased frequencies of sperm disomy among smokers as compared with nonsmokers which are associated with an increased risk of male factor subfertility (Syms et al, 1984) and abnormal reproductive and developmental outcomes (Little and Vainio, 1994).

In addition to nicotine and tars, cigarette smoke contains carbon monoxide and radioactive particles which may have detrimental effects on the testis. Radioactive particles carried in cigarette smoke have been identified in the testis of smokers (Kaufman et al, 1983) and it is known that spermatogonia are extremely sensitive to radiation (Clifton and Bremner, 1983). Smoking is a risk factor which decreased libido and impotence in humans. Smoking affects the sperm fertilizing process.

For example, sperm capacitates the acrosome reaction, binding to the zona pellucida and penetration through the zona pellucida. This effect is probably mediated through alteration in spermatogenesis and the epididymal sperm maturation process (Yamamoto et al, 1998).

Quality of spermatozoa obtained from non-smokers was found to be superior than that of smokers. Exposure of spermatozoa from the non-smokers to seminal plasma from the smokers resulted in a significant reduction in sperm viability (Zavos et al, 1998a). Smokers had significantly poorer sperm density, a lower percentage of viability, a lower percentage of normal sperm morphology and the lower percentage of motile sperm (Merino et al, 1998). Cigarette smoking among teenagers was associated with increases in disomic sperm and decrease in specific aspects of semen quality. Such defects may affect male fertility and may increase future chances of fathering offspring with aneuploidy syndromes (Rubes et al, 1998).

2.4.6.1. EFFECT OF CIGARETTE SMOKING ON SPERM MORPHOLOGY

The normal morphology of spermatozoa has decreased over the last 15 to 20 years of cigarette smoking (Benvold et al, 1991). Carlsen et al, (1992) reported a significant decrease in sperm count and semen volume during the last 50 years in smokers. These findings have led to the suggestion that changes in life style and environmental factors are responsible for these phenomena. A number of exogenous and environmental factors are reportedly found to influence semen quality (Goverde et al, 1995). The incidence of morphologically abnormal sperm in semen was higher in smokers than in non-smokers. The incidence of abnormal sperms appeared to be correlated with the number of cigarettes smoked per day (Evans et al, 1981; Viczian, 1988; Vine et al, 1994).

Changes in the number and the arrangement of axonemal microtubules were noted in the smokers when compared to non-smokers. Smoking a large quantity of cigarettes per day severely affected the ultra structure of the flagellum and more specifically it affected the axoneme of the human spermatozoon (Zavos et al, 1998b).

2.4.6.2. EFFECT OF SMOKING ON MUTATION OF SPERM DNA AND CHROMATIN

Smoking may induce higher proportions of genetically defective sperm since cigarette smoke contains more than 30 chemical agents known to be mutagens, clastogens, aneugens and carcinogens. Chromosome aberrations in male smokers exhibit higher levels of oxidative DNA adducts in their sperm nuclei which may be indicative of potential genetic damage (Rubes et al, 1998). Smokers have a significantly higher percentage of spermatozoa with DNA fragmentation than non-smokers. This suggests that smoking can have a subtle impact on male reproduction. (Sun et al, 1997; Potts et al, 1999; Zenzes, 2000; Sergerie et al, 2000; Shi et al, 2001).

The fact that cigarette smoke is a known somatic cell mutagen and carcinogen, is a major concern and that smoking may adversely affect reproductive health (Vine, 1996). In addition, cigarette smoking has been correlated with poor sperm function in sperm penetration assays (Sofikitis et al, 1995; Mak et al, 2000). Furthermore, paternal smoking has been associated with a significant increase in the percentage of spermatozoa with DNA damage (Sofikitis et al, 1995; Shen et al, 1997) and a higher risk of birth defects and childhood cancers (Zhang et al, 1992; Saleh et al, 2002).

Sperm chromatin is a highly organized, compact structure consisting of DNA and heterogeneous nucleoproteins. Packing of sperm

chromatin may also serve to reprogramme the paternal genome and set the appropriate genes to be expressed in the early stages of embryo development. Thus, the achievement of a correct chromatin packing level seems essential to express fully the fertilizing capacity of sperm (Braun, 2001). Smoking might have impact upon the results of sperm chromatin structure assay (Potts et al, 1999).

2.4.7. EFFECT OF SMOKING ON REACTIVE OXYGEN SPECIES OF SEMEN

Under aerobic conditions, human cells including spermatozoa and white blood cells produce reactive oxygen species (ROS) such as superoxide radicals (O^{2-}), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-). These ROS induce peroxidative damage to sperm lipid membranes and produces detrimental effects on sperm metabolism, morphology, motility and fertilizing capacity have been demonstrated. A greater proportion of infertile men demonstrate elevated levels of seminal ROS as compared with populations of fertile men (Sigman and Jarow, 2002). Saleh et al, (2002) recently reported that the cigarette smoking increases levels of ROS in semen and having important implications in fertilizing potential of sperm.

2.5. SIGNIFICANCE OF ROUTINE BLOOD INVESTIGATIONS

The proper goal oriented evaluation of a man proactive and complaining of sexual dysfunction requires physical examination and complete evaluation of laboratory risk factors. It may include blood glucose, lipid profile (including cholesterol, triglycerides, high density lipoprotein cholesterol (HDLc), low density lipoprotein cholesterol (LDLc), very low density lipoprotein cholesterol (VLDLc), renal function tests, liver function tests, L-gamma glutamyl gransferase (GGT) and

complete blood cell counts. In diabetes mellitus, hyperlipidemia, renal failure, liver failure, anemia and in infections have frequently been associated with diminished erectile function, impaired libido, sperm count and infertility (Lue, 2002; Kirby, 2004).

3.0 MATERIALS AND METHODS

3.1. STUDY SUBJECTS

The study entitled “Effect of Chronic Alcoholism and Smoking in Male Reproductive Function” was performed at Kasthuriba Gandhi Memorial de-addiction Center, Coimbatore, India. One thousand seven hundred alcoholics reported were screened for non smoking alcoholism and alcoholism with smoking. The non alcoholic smokers and controls were obtained from Health Exhibition registry in which 6000 people registered in Kasthuriba Gandhi Memorial de-addiction Center.

The study population consisted of alcoholics with and without smoking and smokers with and without alcohol drinking. Patients consuming drugs like valium, pethidine, cannabis and marijuana along with alcohol were excluded from the study. All the subjects underwent a general medical examination by the physician before subjecting for this study. Age, sex, marital status, details of fertility, diet, details regarding alcohol consumption, past medical illness, treatment history, smoking details, sexual behavior and frequency were obtained through questionnaire.

3.1.1. EXPERIMENTAL DESIGN

Subjects falling under the age group of 20 to 50 years, married, free from diseases and medications were included for the present study. Based on the questionnaire, the inclusion and exclusion criteria were fixed for the four study groups. All the biochemical investigations planned in the study were done before de-addiction treatment started.

The individuals included in the study were categorized into the following four groups:

GROUP I. Control (n = 30)

GROUP II. Alcoholics (n =32)

GROUP III. Smokers (n = 30)

GROUP IV. Alcoholics with smoking (n = 34)

3.1.1.1. GROUP I - CONTROL

The volunteers who were free from diseases and non consumption of alcohol drinks and smoking were included as control subjects in the current study.

3.1.1.2. GROUP II - ALCOHOLICS

Volunteers who consumed minimum of 180 ml of alcoholic drinks like brandy and whiskey (both 40% to 50% alcohol content) per day for minimum 5 days a week for one-year without smoking were included in this group.

3.1.1.3. GROUP III - SMOKERS

Volunteers who were only smokers smoking 20 or more cigarettes per day for more than one year were included in this group.

3.1.1.4. GROUP IV - ALCOHOLICS WITH SMOKING

Subjects consuming a minimum of 180 ml of alcoholic drinks like brandy and whiskey (both 40% to 50% alcohol content) per day for 5 days a week for a minimum period of one year in addition to smoking 20 or more cigarettes per day for the same period were included in this group.

3.1.2. INCLUSION CRITERIA

The subjects of the above groups who were healthy, free from diseases like diabetes mellitus, hypertension, renal failure, liver failure, anemia, malnutrition and chronic infections were included for the present study.

3.2. COLLECTION OF SAMPLES

3.2.1. SEMEN COLLECTION AND DELIVERY

The semen samples were collected after a minimum of 48 hours but not longer than seven days of sexual abstinence. The semen sample was collected by masturbation in ethylene trioxide sterilized wide mouth container. Subject's number, the period of abstinence, the date and time of collection and the interval between collection and analysis were recorded (World Health Organization, 1999). The samples were delivered to the laboratory within half an hour from the time of collection. The samples were protected from high temperatures and the sampling guidelines were strictly observed. In addition to the semen sample, blood samples were collected for investigating the various haematological and biochemical parameters.

3.2.2. BLOOD

Ten ml of venous blood was collected from a prominent vein and 5 ml of blood was transferred into a clean 10 ml conical centrifuge tube without any anticoagulant. The remaining 5 ml of blood was added to vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) and mixed well. The blood collected without any anticoagulant was allowed to clot at room temperature. The clot was allowed to contract and then centrifuged. The serum was transferred to a dry clean screw capped tube. The serum was kept in the deep freezer at -72^{o} C till further use (Varley et al, 1980a).

3.3. BIOCHEMICAL PARAMETERS

Blood parameters like glucose, creatinine, bilirubin, cholesterol, triglycerides, high density lipoprotein cholesterol (HDLc), low density lipoprotein cholesterol (LDLc), gamma- glutamyltransferase (GGT),

glutamate pyruvate transaminase (GPT), alkaline phosphatase and hormones like follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone, estradiol, progesterone and prolactin were estimated. The Roche Diagnostics, Germany, International Quality Control materials (Precinorm–U, Precipath-U, Precicontrol–U1 and U2) were used for the standardization of biochemical parameters.

3.3.1. ESTIMATION OF BLOOD GLUCOSE

Blood glucose was estimated by glucose oxidase peroxidase method (GOD-POD) using Hitachi-912, Roche diagnostics, Germany, Autoanalyser. This modified GOD-POD method is based on the work of Trinder, (1969). (The detailed principle is given in Appendix - I)

3.3.2. ESTIMATION OF CREATININE

Creatinine was estimated by Jaffe Method using Hitachi-912, Roche diagnostics, Germany, Autoanalyser (Foster-Swanson et al, 1994). (The detailed principle is given in Appendix - II)

3.3.3. ESTIMATION OF SERUM TOTAL BILIRUBIN

Serum bilirubin was estimated by 2-5 dichlorophenyl diazonium tetrafluroborate (DPD) method developed by Wahlefeld et al, (1972) using Hitachi-912, Roche diagnostics, Germany, Autoanalyser. (The detailed principle is given in Appendix - III)

3.3.4. ESTIMATION OF CHOLESTEROL

Serum cholesterol was estimated by using cholesterol oxidase-peroxidase method using Hitachi-912, Roche diagnostics, Germany, Autoanalyser. Cholesterol was determined enzymatically using cholesterol esterase and cholesterol oxidase (Greiling and Gressner, 1995; Trinder, 1969). (The detailed principle is given in Appendix - IV)

3.3.5. ESTIMATION OF TRIGLYCERIDE (TG)

Serum triglyceride was estimated with Hitachi-912, Roche diagnostics, Germany, Autoanalyser by glycerol phosphate oxidase peroxidase (GPO-PAP) method (Siedel et al, 1993; Trinder, 1969). (The detailed principle is given in Appendix - V)

3.3.6. ESTIMATION OF SERUM HIGH DENSITY LIPOPROTEIN CHOLESTEROL

HDL cholesterol was estimated by 2nd generation method using Hitachi-912, Roche diagnostics, Germany, Autoanalyser (Kimberly et al, 1999). (The detailed principle is given in Appendix -VI)

3.3.7. ESTIMATION OF TOTAL PROTEIN

Serum total protein was estimated by Biuret Method using Hitachi-912, Roche diagnostics, Germany, Autoanalyser (Weichselbaum, 1946). (The detailed principle is given in Appendix - VII)

3.3.8. ESTIMATION OF SERUM ALBUMIN

Serum albumin was determined by using bromocresol green (BCG) method in Hitachi-912, Roche diagnostics, Germany, Autoanalyser (Doumas, 1971). (The detailed principle is given in Appendix - VIII)

3.3.9 ESTIMATION OF SERUM L-GAMMA GLUTAMYL TRANSFERASE (GGT) ACTIVITY (EC 2.3.2.2)

Serum GGT activity was estimated by Szasz, (1974) method with Hitachi-912, Roche diagnostics, Germany, Autoanalyser. (The detailed principle is given in Appendix - IX)

3.3.10. ESTIMATION OF SERUM GLUTAMATE PYRUVATE TRANSAMINASE (SGPT) ACTIVITY (EC 2.6.1.2)

Serum GPT activity was estimated by International Federation of Clinical Chemistry (IFCC) derived reference method without pyridoxal phosphate activation in Hitachi-912, Roche diagnostics, Germany, Autoanalyser (Bergmeyer et al, 1985; Greiling and Gressner, 1995). (The detailed principle is given in Appendix-X)

3.3.11. ESTIMATION OF ALKALINE PHOSPHATASE (ALP) ACTIVITY (EC 3.1.3.1)

Serum ALP activity was estimated by 'optimized standard method' assay of the Deutschen Gessell and Chaff Fu Klinische Chemie using Hitachi-912, Roche diagnostics, Germany, Autoanalyser (Deutschen Gesellschaft fur Klinische Chemie, German Society of Clinical Chemistry, 1972). (The detailed principle is given in Appendix – XI)

3.4. HAEMATOLOGICAL PARAMETERS

Complete blood count was estimated by Coulter Method (Coulter, 1956) using Coulter Onyx, USA fully automated blood cell counter. Blood was collected in EDTA vacutainer and the total white blood cells (WBC or leukocyte count), red blood cells (RBC or erythrocyte count), platelet (Plt) (thrombocyte count), haemoglobin (Hgb), packed cell volume (PCV or HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were estimated. The Randox, USA, International Quality Control materials (Level 1, 2 and 3) were used for the standardization of haematobgical parameters. (The detailed principle is given in Appendix - XII)

3.4.1. ESTIMATION OF HAEMOGLOBIN

After WBC count the dilution was lysed, the system passed white light through the WBC aperture path and then through an optical filter. A ratio was calculated between the transmittance of monochromatic light (525 nm wavelength) through a standard path length of haemoglobin solution and the transmittance of such light in the same way through a reagent blank. The system converted this ratio to absorbance which was then converted into haemoglobin values in g/dl using a calibrator factor.

3.4.2. ESTIMATION OF WHITE BLOOD CELL COUNTS

This is the number of leukocytes measured directly and multiplied by the calibration constant and expressed as

$$WBC = n \times 10^3 \text{ cells per } \mu l$$

3.4.3. ESTIMATION OF RED BLOOD CELL COUNTS

This is the number of erythrocyte measured directly and multiplied by the calibration constant and expressed as

$$RBC = n \times 10^6 \text{ cells per } \mu l$$

3.4.4. ESTIMATION OF MEAN CORPUSCULAR VOLUME

MCV is the average volume of individual erythrocyte derived from the RBC histogram. The system multiplies the number of RBCs in each channel by the size of the RBCs in that channel. The products of each channel between 36 fl and 360 fl were added. This sum was divided by the total number of RBCs between 36 fl and 360 fl. The analyser then multiplied the value by a calibrator constant and expressed MCV as femtoliters.

3.4.5. ESTIMATION OF PACKED CELL VOLUME (PCV or HCT)

This is the relative volume of erythrocyte, computed as

$$HCT\ (\%) = \frac{RBC \times MCV}{10}$$

3.4.6. ESTIMATION OF MEAN CORPUSCULAR HAEMOGLOBIN

This is the weight of haemoglobin in the average erythrocyte and is computed as

$$MCH\ (Pg/cells) = \frac{Hgb}{RBC} \times 10$$

3.4.7. ESTIMATION OF MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION

This is the average weight of haemoglobin in a measured dilution and is computed as

$$MCHC\ (g/dl) = \frac{Hgb}{HCT} \times 100$$

3.4.8. ESTIMATION OF PLATELET COUNT

This is the number of thrombocytes derived from the Plt fitted curve, multiplied by the calibrator constant and is expressed as

$$Plt = n \times 10^3 \text{ cells per } \mu l$$

3.4.9. ESTIMATION OF DIFFERENTIAL WBC COUNT

Differential count was performed manually by using Leishman Stain. The blood drops were placed on clean grease-free slide. With a quick movement, the spreader slide was pushed towards the other end of the slide. The blood smear so made was not be too thin or too thick.

The slide was then air dried and stained with Leishman stain. Hundred cells were counted under oil immersion in microscope. Percentage of polymorphs, lymphocytes, eosinophils, monocytes and basophils were calculated (Samuel, 1989). (The detailed principle is given in Appendix - XIII)

3.5. HORMONE ANALYSIS

In the present study, hormones such as Follicle stimulating hormone (FSH), testosterone, prolactin, estradiol and progesterone were measured by electrochemiluminescence immunoassay with Elecsys 1010, Roche diagnostics, Germany a fully automated Immunoassay analyzer (The detailed principle is given in Appendix - XIV). Luteinizing hormone (LH) was estimated by ELISA method in Cobas Core II, Roche diagnostics, Germany fully automated ELISA Analyser.

3.5.2.1. ESTIMATION OF FOLLICLE STIMULATING HORMONE

FSH was estimated by electrochemiluminescence immunoassay (ECLIA) method using Elecsys 1010, Roche diagnostics, Germany. As the molecular weight was more than 5000 Daltons, the sandwich principle was selected for FSH assay (Blackburn et al, 1991; Hoyle, 1994; Kenten et al, 1991; Leland and Powell, 1990; Obeng and Bard, 1991; Xu and Bard, 1994; Reference Guide Elecsys 1010, 1996). (The detailed principle is given in Appendix - XV)

3.5.2.2. ESTIMATION OF LUTEINIZING HORMONE

LH was estimated by ELISA method. Since LH has a molecular weight more than 5000 Daltons, sandwich principle was preferred. ELISA method is the one widely used technique for immunoassay. The antigens or antibodies are labeled with enzymes. The enzymes are alkaline phosphatase and Horse Radish peroxidase. In the present

study the Cobas Core II, Roche diagnostics, Germany, fully automated ELISA analyser was used (Mitchell et al, 1995). (The detailed principle is given in Appendix - XVI)

3.5.2.3. ESTIMATION OF TESTOSTERONE

The androgen testosterone (17β-hydroxyandrosterone) has a molecular weight of 288 Daltons. Testosterone was quantitatively determined by electrochemiluminescence Immunoassay method with Elecsys 1010, Roche diagnostics, Germany. Since Testosterone has a molecular weight less than 5000 Daltons the competitive test principle was preferred using monoclonal antibodies specifically raised against testosterone (Blackburn et al, 1991; Hoyle, 1994; Kenten et al, 1991; Leland and Powell, 1990; Obeng and Bard, 1991; Xu and Bard, 1994; Reference Guide Elecsys 1010, 1996). (The detailed principle is given in Appendix - XVII)

3.5.2.4. ESTIMATION OF PROLACTIN

Prolactin was estimated by electrochemiluminescence Immunoassay (ECLIA) in Elecsys 1010, Roche diagnostics, Germany. As the molecular weight was more than 5000 Daltons, the sandwich principle was selected for prolactin estimation (Blackburn et al, 1991; Hoyle, 1994; Kenten et al, 1991; Leland and Powell, 1990; Obeng and Bard, 1991; Xu and Bard, 1994; Reference Guide Elecsys 1010, 1996). (The detailed principle is given in Appendix - XVIII)

3.5.2.5. ESTIMATION OF ESTRADIOL

17β-estradiol is biologically the most active estrogen. This is a steroid hormone having a molecular weight of 272 Daltons. This hormone was estimated by electrochemiluminescence Immunoassay using Elecsys 1010, Roche diagnostics, Germany. Competitive test principle was employed using a polyclonal antibody (Blackburn et al,

1991; Hoyle, 1994; Kenten et al, 1991; Leland and Powell, 1990; Obeng and Bard, 1991; Xu and Bard, 1994; Reference Guide Elecsys 1010, 1996). (The detailed principle is given in Appendix - XIX)

3.5.2.6. ESTIMATION OF PROGESTERONE

Progesterone was estimated by Electrochemiluminescence Immunoassay using Elecsys 1010, Roche diagnostics, Germany. Since steroid hormone progesterone has a molecular weight of 314.5 Daltons, competitive principle was employed (Blackburn et al, 1991; Hoyle, 1994; Kenten et al, 1991; Leland and Powell, 1990; Obeng and Bard, 1991; Xu and Bard, 1994; Reference Guide Elecsys 1010, 1996). (The detailed principle is given in Appendix - XX)

3.6. SEMEN ANALYSIS

Semen consists of spermatozoa suspended in seminal plasma. Spermatozoa comprise about 5% of the semen volume derived from the testis. Approximately 60% of the semen volume is derived from the seminal vesicles. This viscid, neutral or slightly alkaline fluid is often yellow or even deeply pigmented because of its high flavin content. Prostate contributes 20% of the volume of semen. This milky fluid is slightly acidic, at pH 6.5 largely because of its high content of citric acid. The prostatic secretion rich in proteolytic enzymes is believed to be responsible for the coagulation and liquefaction of semen. Less than 10% to 15% of semen volume is contributed by epididymis, vasa deferentia, bulbourethral and urethral glands (Ramnik Sood, 1987).

The semen samples were analyzed as per the WHO standards. Macroscopic (liquefaction time, appearance, volume, consistency, pH, fructose) and microscopic (sperm concentration, motility, viability and morphology) examinations were carried out.

3.6.1. MACROSCOPIC EXAMINATION

3.6.1.1. LIQUEFACTION

Normal semen sample liquefies within 10 to 30 minutes at room temperature. If complete liquefaction does not occur, this fact has to be recorded. The presence of streaks is a sign of incomplete liquefaction.

3.6.1.2. APPEARANCE

The color of semen is noted by simple inspection at room temperature. The normal appearance of the sample is a homogenous, gray opalescent color and it will appear less opaque when the sperm count is low. When red blood cells are mixed with the sample it would appear brown.

3.6.1.3. VOLUME

This is to be measured either in a graduated test tube or by aspirating the sample with a wide mouth pipette by means of a mechanical device. Normal volume is ≥ 2 and < 6 ml.

3.6.1.4. CONSISTENCY OR VISCOSITY

This is estimated by gentle aspiration into a pipette and then allowing the semen to drop. A normal sample leaves the pipette as discrete drops while in cases of abnormal consistency the drop will form a thread like substance measuring more than 2 cm long.

3.6.1.5. pH

A drop of semen was spread onto a pH paper having a range between 6.0 and 10.0. After 30 seconds the color was compared with the calibration strip to read the pH. The pH was measured within half an hour. Normal pH should be in the range 7.2 to 8.0. If the pH of semen is less than 7.0 it will be suspected for azoospermia, dysgenesis of the

vas deferens and seminal vesicles or epididymis (World Health Organization, 1999).

3.6.1.6. ANALYSIS OF FRUCTOSE IN SEMEN

Semen fructose was analsysed using resorcinol method. 0.5 ml of semen sample was boiled with 5 ml of resorcinol reagent (50 mg of resorcinol mixed with 33 ml of concentrated hydrochloric acid and made upto 100 ml with distilled water). Red colored preciptate formed within 30 seconds indicates the presence of fructose in the semen (Reynolds and Narang, 2003).

3.6.2. MICROSCOPIC INVESTIGATIONS

3.6.2.1. ESTIMATION OF SPERM CONCENTRATION

The concentration of spermatozoa was determined by using the Makler chamber (Makler, 1978). Sperm motility and morphology assessment were also performed on the sample after centrifugation. (Details given in Appendix - XXI)

3.6.2.2. MOTILITY

A fixed volume of well-mixed normal or centrifuged semen was placed on a clean glass slide with a micropipette and covered with a 22mm x 22mm cover slip. The volume of semen and the dimensions of the cover slip were standardized and carried out in a fixed depth of about 20 µM. The depth should allow full expression of the rotation and movement of normal spermatozoa.

The preparation was examined at a magnification of 400x at room temperature. The microscopic field was scanned systematically and the motility of each spermatozoa encountered was graded as a, b, c and d according to the movement of sperm.

3.6.2.2.1. GRADE A- RAPID PROGRESSIVE MOTILITY

3.6.2.2.2 GRADE B- SLOW OR SLUGGISH PROGRESSIVE MOTILITY

3.6.2.2.3. GRADE C- NON PROGRESSIVE MOTILITY

3.6.2.2.4. GRADE D- IMMOTILITY

Four fields were scanned and 200 spermatozoa were observed. From this the percentage of motility and their category was calculated. The motility count was repeated on second drop of semen prepared in the same way. The results of the two counts were then assessed. The values were expressed as percentages.

3.6.2.3. MORPHOLOGICAL CHARACTERISTIC ANALYSIS OF SPERMATOZOA

The eosin-nigrosin stained semen smears were used for observing morphology evaluation. The following categories of defects were scored.

3.6.3.3.1. HEAD DEFECTS

Large, small, tapering, pyriform, amorphous, vacuolated (>20% of the head area) or double heads or any combination of these heads of spermatozoon were included under the category of head defects.

3.6.3.3.2. NECK DEFECTS

Tail absent, non-inserted or bent tail, distended, irregular, bent midpiece, abnormally thin midpiece or any combinations of these were categorized under neck defective spermatozoa.

3.6.3.3.3. TAIL DEFECTS

Short, multiple, hair pin, broken, irregular width, or coiled tails or tails with terminal droplets or combination of these were categorized under tail defects.

3.6.3.3.4. NORMAL FORMS

The spermatozoon free from the above defects were categorized as normal forms. The normal and abnormal form sperms were expressed in percentages (World Health Organization, 1999).

3.6.2.4. VIABILITY

This was done by using the eosin-nigrosin staining technique. This is based on the principle that the live sperm excludes the stain and hence would be white and the dead sperm absorbs the eosin stain and hence stains pink-red.

One drop of semen was mixed with two drops of 1% eosin Y. After 30 seconds three drops of 10% nigrosin solution was added and mixed thoroughly. One drop of the above semen-eosin-nigrosin mixture was placed on a clean microscopic slide, a smear was prepared and air-dried. One hundred spermatozoa were counted under the oil immersion objective and the percentage of live and dead spermatozoa calculated. Vitality staining differentiates spermatozoa that were immotile but alive from those that were dead (World Health Organization, 1999).

3.6.2.5. SEXUAL DRIVE, POTENCY AND FERTILITY STATUS

The sexual drive, potency and fertility status data obtained through questionnaire of controls, alcoholics, smokers and alcoholics with smokers subjects was tabulated and analyzed as per the modified guideline of O'Leary et al, (1995), Rosen et al, (1997) and Kirby (2004).

3.7. STATISTICAL ANALYSIS

Chi-square, Analysis of variance and Scheffe post hoc test methods were used for statistical analysis. This was done using the statistical package “SPSS” for Windows Release 7.5.1, Standard Version (1989 to 1996) General + Clinical Laboratory Statistics. F-test was carried out wherever needed. The calculated F value was referred with appropriate degrees of freedom and the level of significance was accepted at $p < 0.05$. Wherever the F-test was found to be significant the data were further subjected to Scheffe’s post hoc test to analyze the significance of individual variations. The result is that the Scheffés test is often more conservative than other tests which means that a larger difference between means is required for significance. As the sample sizes of different groups were not equal the Scheffe's post hoc test has been used for multiple comparisons (Groebner and Shannon, 1989).

4.0 RESULTS AND DISCUSSIONS

4.1. HEALTH ASSESSMENT OF SUBJECTS UNDER STUDY

The chronic alcoholism and smoking are known to produce chronic diseases like diabetes mellitus, liver diseases, renal diseases, lipid disorders, cardiac diseases, chronic infections, skeletal muscle damage and chronic air way obstructions (Kane and Kumar, 2004; Burns, 2005). In general, the diabetes mellitus, hyperlipidemia, renal failure, liver failure, anemia and infections have frequently been associated with diminished erectile function, impaired libido, decreased sperm count and increased male infertility (Baker, 2001; Lue, 2002; Kirby, 2004). In the present study the controls, alcoholics, smokers and alcoholics with smoking subjects were screened with blood biochemical parameters (glucose, creatinine, lipid profile parameters - cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, VLDL cholesterol, phospholipids and Liver function tests - bilirubin total, protein full, SGPT, GGT and alkaline phophatase in blood) and haematological parameters (hemoglobin, total RBC count, Haematocrit, MCV, MCH, MCHC, Total WBC count, differential WBC count like Lymphocytes, Polymorphs, Eosinophils and platelets) (Tables 1 and 2).

The controls, alcoholics, smokers and alcoholics with smoking whose routine blood biochemical parameters and haematological parameter's results were found to be within the normal limits. These subjects which were statistically insignificant in multiple comparisons between groups were selected for the present study. The physician certified subjects who were all free from the diseases such as diabetes mellitus, hypertension, renal failure, liver failure, anemia, malnutrition and chronic infections were included in the present study. Further, these results suggest that the variables of blood levels of reproductive hormones and the variables of seminal parameters estimated in

TABLE 1.

ANOVA BETWEEN CONTROL, ALCOHOLICS, SMOKERS AND ALCOHOLICS WITH SMOKING

THE RESULTS OF BIOCHEMICAL AND HAEMATOLOGICAL PARAMETERS

S.NO	NAME OF THE PARAMETER	UNITS	'F' VALUE	SIGNIFICANCE
1	GLUCOSE	mg/dl	2.535	0.060 NS
2	CREATININE	mg/dl	0.997	0.397 NS
3	CHOLESTEROL	mg/dl	0.641	0.590 NS
4	TRIGLYCERIDES	mg/dl	0.96	0.414 NS
5	HDL-CHOLESTEROL	mg/dl	1.469	0.226 NS
6	LDL CHOLESTEROL	mg/dl	1.659	0.179 NS
7	VLDL CHOLESTEROL	mg/dl	1.74	0.162 NS
8	PHOSPHOLIPIDS	mg/dl	0.865	0.461 NS
9	BILIRUBIN TOTAL	mg/dl	0.961	0.414 NS
10	PROTEIN TOTAL	g/dl	1.21	0.309 NS
11	ALBUMIN	g/dl	0.493	0.688 NS
12	GLOBULIN	g/dl	0.608	0.616 NS
13	A/G RATIO		0.504	0.680 NS
14	SGPT	U/L	1.59	0.195 NS
15	ALKALINE PHOSPHATASE	U/L	0.304	0.822 NS
16	GGT	U/L	0.956	0.416 NS
17	HAEMOGLOBIN	g/dl	0.782	0.506 NS
18	HCT	%	0.219	0.883 NS
19	TOTAL RBC COUNT	million/ml	0.896	0.446 NS
20	TOTAL WBC COUNT	cells/cumm	0.324	0.808 NS
21	POLYMORPHS	%	1.217	0.307 NS
22	LYMPHOCYTES	%	1.309	0.275 NS
23	EOSINOPHILS	%	1.911	0.131 NS
24	MCV	fl	1.949	0.125 NS
25	MCH	pg	1.495	0.219 NS
26	MCHC	g/dl	0.515	0.673 NS
27	PLATELETS	lakhs/ml	1.528	0.211 NS

NS = NOT SIGNIFIC ANT

TABLE 2.

THE RESULTS OF BIOCHEMICAL HAEMATOLOGICAL PARAMETER: (Mean ± SD)
SCHEFFE'S POST HOC TEST: COMPARISON BETWEEN CONTROL V/S ALCOHOLICS (1 V/S 2), CONTROL V/S SMOKERS (1 V/S 3) AND CONTROL V/S ALCOHOLICS WITH SMOKING (1 V/S 4) GROUPS

S. NO	NAME OF THE PARAMETER	UNIT	CONTROL (1) (n=30)	ALCOHOLICS (2) (n=32) 1 v/s 2	SMOKERS (3) (n=30) 1 v/s 3	ALCOHOLICS WITH SMOKING (4) (n=34) 1 v/s 4
1	GLUCOSE	mg/dl	90.36±15.64	99.37±26.29 NS	85.51±21.85 NS	85.84±18.47 NS
2	CREATININE	mg/dl	0.91±0.16	0.95±0.16 NS	0.85±0.16 NS	0.95±0.10 NS
3	CHOLESTEROL	mg/dl	177.71±30.23	189.66±24.91 NS	184.94±38.05 NS	184.34±40.23 NS
4	TRIGLYCERIDE	mg/dl	135.87±49.75	163.77±68.79 NS	172.29±91.59 NS	151.77±83.29 NS
5	HDL - C	mg/dl	37.93±12.07	43.11±12.65 NS	39.09±11.97 NS	43.46±14.68 NS
6	LDL - C	mg/dl	112.62±29.15	113.77±32.97 NS	111.77±37.46 NS	98.30±29.99 NS
7	VLDL – C	mg/dl	27.13±9.97	32.95±13.81 NS	34.45±18.32 NS	30.13±16.60 NS
8	PHOSPHOLIPID	mg/dl	226.26±29.98	239.33±30.17 NS	232.60±33.86 NS	232.05±35.99 NS
9	BILIRUBIN TOTAL	mg/dl	0.70±0.31	0.86±0.37 NS	0.59±0.29 NS	0.71±0.36 NS
10	PROTEIN TOTAL	g/dl	7.54±0.51	7.44±0.47 NS	7.33±0.48 NS	7.35±0.47 NS
11	ALBUMIN	g/dl	4.51±0.17	4.51±0.17 NS	4.46±0.26 NS	4.44±0.31 NS
12	GLOBULIN	g/dl	3.03±0.46	2.94±0.43 NS	2.99±0.43 NS	2.89±0.44 NS
13	A/G RATIO		1.52±0.24	1.58±0.24 NS	1.49±0.23 NS	1.55±0.32 NS
14	SGPT	U/L	28.81±14.45	35.50±14.51 NS	27.61±18.18 NS	30.95±14.34 NS
15	ALP	U/L	192.74±62.82	203.52±62.82 NS	204.61±61.10 NS	198.59±45.88 NS
16	GGT	U/L	26.39±8.97	31.30±11.12 NS	30.86±20.27 NS	31.12±10.48 NS
17	HAEMOGLOBIN	g/dl	15.48±1.13	15.04±1.49 NS	14.98±1.31 NS	15.18±1.55 NS
18	HCT	%	44.58±3.17	45.36±5.04 NS	44.40±3.73 NS	44.98±7.08 NS
19	TOTAL RBC COUNT	million/ ml	5.00±0.29	4.83±0.51 NS	4.92±0.39 NS	4.86±0.49 NS
20	TOTAL WBC COUNT	cells/ cumm	7460±1466	7740±1816 NS	7897±1475 NS	7791±2253 NS
21	POLYMORPHS	%	64.50±6.28	67.34±7.20 NS	63.77±6.50 NS	65.12±10.30 NS
22	LYMPHOCYTES	%	33.17±6.76	30.03±6.09 NS	33.13±5.31 NS	32.56±9.85 NS
23	EOSINOPHILS	%	2.20±2.02	1.75±1.55 NS	3.13±3.04 NS	2.27±1.90 NS
24	MCV	fl	89.16±2.96	90.83±3.99 NS	90.16±2.80 NS	93.33±12.86 NS
25	MCH	pg	31.05±1.29	31.08±2.14 NS	30.50±0.96 NS	31.40±2.09 NS
26	MCHC	g/dl	32.74±0.07	33.18±0.85 NS	33.72±0.85 NS	33.06±1.59 NS
27	PLATELETS	lakhs/ml	201666±57994	223335±55303 NS	209533±37431 NS	224177±45605 NS

NS = NOT SIGNIFICANT

alcoholics, smokers and alcoholics with smoking subjects are exclusive toxic effect of alcohol or smoke or both alcohol with smoking.

4.2. AGE AND HABITUAL DATA OF SUBJECTS RELATED TO THE STUDY

The present study controls, alcoholics, smokers and alcoholics with smoking subject's age and habitual data obtained through the questionnaire are presented as follows.

The average age of the control group was 35.0 +/- 6.9 years, alcoholics 36.6 +/- 5.7 years, smokers 37.0 +/- 8.9 years, and alcoholics with smoking 35.9 +/- 7.0 years. The individual group subject's age is an important factor in male reproductive function. In the present study, the controls, alcoholics, smokers and alcoholics with smoking group's mean age did not vary significantly between the groups.

The alcoholics consumed alcohol for 6.1 +/- 1.1 days in a week, for an average of 4.5 +/- 2.9 years in a row, and the alcohol volume consumed was 441.1 +/- 323.9 ml per day.

Smokers smoked 26.1 +/- 8.1 cigarettes per day for 10.4 +/- 5.2 years continuously. The alcoholics with smoking consumed 450.1 +/- 345.2 ml of alcohol per day for 5.7 +/- 1.5 days in a week for 5.0 +/- 4.7 years without interruption and they smoked 25.6 +/- 6.0 cigarettes per day for 5.0 +/- 4.7 years continuously.

The present study has shown the following variations in male reproductive hormones, semen quality and sexual functions, of alcoholics, smokers and alcoholics with smoking.

4.3 EFFECT OF ALCOHOL AND SMOKING ON REPRODUCTIVE HORMONES

Hormone levels of the four study groups compared with one-way analysis of variance (ANOVA) showed significant differences. ANOVA of hormones FSH ($p<0.002$), LH ($p<0.001$), testosterone ($p<0.001$), estradiol ($p<0.003$) and progesterone ($p<0.001$) showed highly significant between groups. Prolactin ($p<0.564$) did not show any significant variation between the study groups (Table 3). The above significant parameters were further compared between the two groups by Scheffe's post hoc method.

4.3.1 EFFECT OF ALCOHOL AND SMOKING ON FSH

The average mean FSH levels of control group, alcoholics, smokers and alcoholics with smoking are presented in Table 4 and Figure 11.

The average mean FSH level of control is 4.60+/-1.94 mIU/ml, of alcoholics is 7.41+/-3.18 mIU/ml, of smokers is 5.10+/-3.65 mIU/ml and of alcoholics with smoking is 7.41+/-4.95 mIU/ml.

It is evident from the study that the average mean FSH level is statistically elevated at 5 % level in alcoholics and alcoholics with smoking when compared to the control group. No significant elevation is noted in smokers. No significant change has been noted when mean FSH level of alcoholics is compared with mean FSH level of smokers and alcoholics with smoking and also when smokers group is compared with alcoholics with smoking group.

The increased levels of FSH in alcoholics and alcoholics with smoking shown in the present study is due to the toxic effect of alcohol on the testicular seminiferous tubules and Sertoli cells, which lead to

TABLE 3.

HORMONES

ANOVA BETWEEN

CONTROL (n=30), ALCOHOLICS (n=32), SMOKERS (n=30)

AND ALCOHOLICS WITH SMOKING (n=34) GROUPS

S.NO	NAME OF THE HORMONE	UNITS	'F' – VALUE	LEVEL OF SIGNIFICANCE
1	FSH	mIU/ml	5.26	0.002 **
2	LH	mIU/ml	9.17	0.001 ***
3	PROLACTIN	ng/ml	0.68	0.564 NS
4	TESTOSTERONE	ng/ml	13.96	0.001 ***
5	ESTRADIOL	pg/ml	5.50	0.003 **
6	PROGESTERONE	ng/ml	9.85	0.001 ***

** = $p<0.01$ = SIGNIFICANCE AT 1% LEVEL
*** = $p<0.001$ = SIGNIFICANCE AT 0.1% LEVEL
NS = NOT SIGNIFICANT

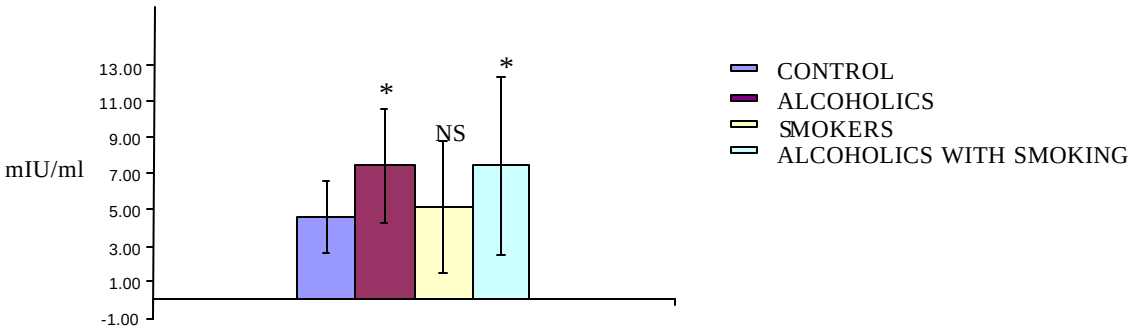
TABLE 4. SCHEFFE'S POST HOC MULTIPLE COMPARISON

BETWEEN GROUPS

FOLLICLE STIMULATING HORMONE in mIU/ml

GROUP OF SUBJECTS	Mean ± SD	GROUPS COMPARED	P- VALUE
CONTROL - A (n = 30)	4.60±1.94	**A VS B**	0.030 *
		A VS C	0.963 NS
		A VS D	0.027 *
ALCOHOLICS - B (n = 32)	7.41±3.18	*B VS C*	0.107 NS
		B VS D	1.000 NS
SMOKERS - C (n = 30)	5.10±3.65	C VS D	0.100 NS
ALCOHOLICS WITH SMOKING – D (n = 34)	7.41±4.95		

Fig 11. **FOLLICLE STIMULATING HORMONE in mIU/ml**



*= p<0.05 = SIGNIFICANT AT 5% LEVEL
NS = NOT SIGNIFICANT

diminished testicular function including spermatogenesis. The toxicity of alcohol might have suppressed the secretion of paracrine functional proteins in Sertoli cells, which includes the inhibin peptide, which suppresses the synthesis of FSH at pituitary. The decreased inhibin lead to the loss of negative feed back at pituitary and as a compensatory mechanism the FSH might be increased. The present study shows no significant change in blood level of FSH in smokers and this demonstrates that the smoking has not produced any change in the inhibin synthesis of the Sertoli cells. The increased FSH in alcoholics and no significant change in FSH in smokers suggest that the alcohol is having more toxic effect on male reproduction than the smoking.

Gumus et al, (1998) who studied the effect of long-term alcohol abuse on male sexual function and found higher levels of serum FSH in chronic alcoholic men reflecting a deleterious effect of alcohol on spermatogenesis which confirmed the present study.

Emanuele et al, (1992) have reported a significant fall in blood FSH levels after acute ethanol exposure and have suggested that the alcohol-induced increased degradation of FSH in pituitary gonadotropic cells might be possible explanation for the marked FSH fall. This result is contradicting the present study of increased FSH levels in alcoholics. It is suggested that in the initial stage of alcohol consumption the toxicity of alcohol suppressed the pituitary gonadotropic cell function and in chronic stage the alcohol toxicity has suppressed the testicular seminiferous tubular function and as a compensatory mechanism the FSH was found to be elevated in blood.

The present study has shown no significant change in FSH levels of smokers. This finding is supported by the study of Sofikitis et al, (1995) which found that smoking did not produce any significant change in the FSH levels. On the contrary, Shaarawy and Mahmoud, (1982)

who studied endocrine profile and semen characteristics in male smokers and reported that the blood level of FSH in smokers was significantly higher than those of nonsmokers.

4.3.2 EFFECT OF ALCOHOL AND SMOKING ON LH

The average mean LH levels of control group, alcoholics, smokers and alcoholics with smoking are presented in Table 5 and Figure 12.

The average mean LH level of control is 4.69+/-2.44 mIU/ml, of alcoholics is 6.74+/-2.43 mIU/ml, of smokers is 4.78+/-2.29 mIU/ml and of alcoholics with smoking is 7.67+/-3.54 mIU/ml.

The average mean LH level of control is compared with alcoholics and alcoholics with smoking and is found that there is an increase which is statistically significant at 5% level and at 0.1% level respectively. Control is compared with smokers and found that there is no significant change. When alcoholics are compared with smokers and alcoholics with smoking, it is found that there is no statistical significant change. But when smokers are compared with alcoholics with smoking, it is found that the increase, which is statistically significant at 0.1% level.

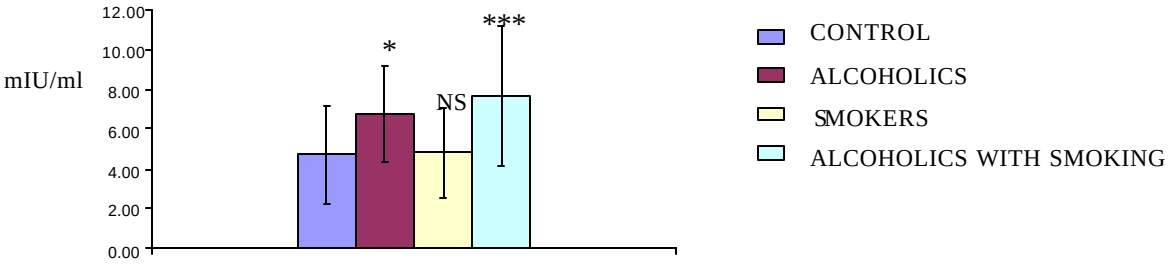
The increased blood levels of LH in alcoholics and alcoholics with smoking is due to decreased levels of blood testosterone. The decreased blood level of testosterone leads to the loss of negative feed back at pituitary and as a compensatory mechanism the pituitary secretes more amount of LH in alcoholics and alcoholics with smoking. The present study has shown no significant change in blood levels of LH in smokers and demonstrated that smoking alone does not produce any decrease in blood testosterone levels.

In the present study, increased LH levels in chronic alcoholics and alcoholics with smoking have demonstrated that alcohol directly

TABLE 5. SCHEFFE'S POST HOC MULTIPLE COMPARISON BETWEEN GROUPS LUTEINIZING HORMONE in mIU/ml

GROUP OF SUBJECTS	Mean ± SD	GROUPS COMPARED	P- VALUE
CONTROL - A (n = 30)	4.69±2.44	A VS B	0.040 *
		A VS C	0.999 NS
		A VS D	0.001 ***
ALCOHOLICS - B (n = 32)	6.74±2.43	B VS C	0.055 NS
		B VS D	0.596 NS
SMOKERS - C (n = 30)	4.78±2.29	C VS D	0.001 ***
ALCOHOLICS WITH SMOKING – D (n = 34)	7.67±3.54		

Fig 12. **LUTEINIZING HORMONE in mIU/ml**



***= p<0.001 = SIGNIFICANT AT 0.1% LEVEL
*= p<0.05 = SIGNIFICANT AT 5% LEVEL
NS = NOT SIGNIFICANT

enters into the testis and decreases the testosterone synthesis and spermatogenesis.

Similar to the present study on increased blood levels of LH, Iranmanesh et al, (1988) have reported that acute alcohol ingestion has increased the pulsatile secretion of LH in men.

The decreased testosterone and increased LH findings in alcoholics, and alcoholics with smoking suggest that the major effect of alcohol on plasma testosterone in humans is exerted at a peripheral (testis) rather than at the central (hypothalamus-pituitary) site. This study is supported by the finding of Ida et al, (1992) who reported that alcohol administration to normal subjects produced not only decreased plasma testosterone levels, but also increased plasma LH levels. Decreased plasma testosterone level is associated with the toxic effects of alcohol on the peripheral mechanism, which regulates the biosynthesis and biotransformation of testosterone.

Trummer et al, (2002) have studied the impact of cigarette smoking on male reproductive hormones and reported an increased blood levels of LH, testosterone and free testosterone which is contradicted the present study of no significant change in LH and testosterone in smokers. They further suggested that the significantly elevated LH in smokers reflects a central activation of Leydig cells which explains elevated testosterone and free testosterone levels.

Smoking does not produce any significant change in LH in the present study. This finding is supported by Shaarawy and Mahmoud, (1982) and Sofikitis et al, (1995) who found that the serum LH did not vary significantly in smokers, compared to the non-smokers group.

4.3.3 EFFECT OF ALCOHOL AND SMOKING ON TESTOSTERONE

The average mean testosterone levels of control group, alcoholics, smokers and alcoholics with smoking are presented in Table 6 and Figure 13.

The average mean testosterone level of control is 5.89+/-2.07 ng/ml, of alcoholics is 4.49+/-1.19 ng/ml, of smokers is 6.47+/-1.95 ng/ml and of alcoholics with smoking is 4.33+/-0.90 ng/ml.

The average mean testosterone levels of alcoholics and alcoholics with smoking and smokers are compared with control and is found that the decrease is statistically significant at 1% level except in smokers. When alcoholics are compared with smokers, it is found that the decrease of testosterone is statistically significant at 0.1% level. When alcoholics with smoking are compared with smokers, it is found that the decrease is statistically significant at 0.1% level.

The decreased blood levels of testosterone in alcoholics and alcoholics with smoking suggest that the toxic effect of alcohol acts directly on testicular Leydig cells, affects the autocrine and paracrine functions and it decreases the synthesis of testosterone and accelerate the metabolic clearance of testosterone.

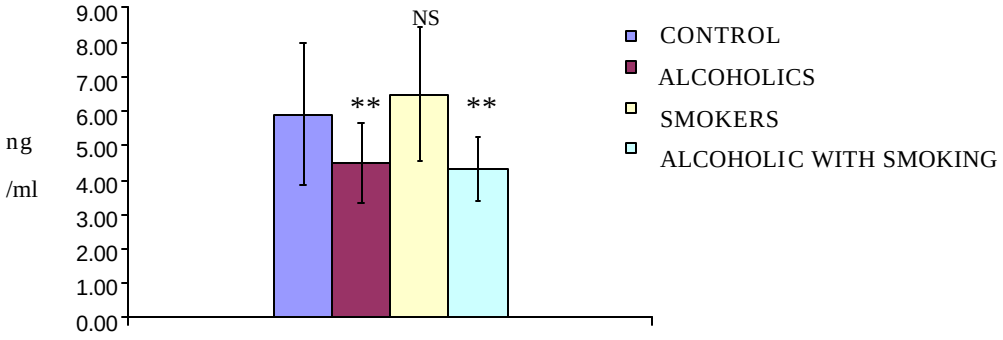
The insignificant change in the blood levels of testosterone and LH in smoking and decreased blood levels of testosterone and increased blood levels of LH in alcoholics and alcoholics with smoking makes it evident that the alcohol is having more toxic effect than the smoking. The same results suggest that the toxic effect of alcohol is only at the testicular site and not at pituitary. Further, the toxicity is found to increase in testicular Leydig cells in alcohol together with smoking.

TABLE 6. SCHEFFE'S POST HOC MULTIPLE COMPARISON

BETWEEN GROUPS
TESTOSTERONE in ng/ml

GROUP OF SUBJECTS	Mean ± SD	GROUPS COMPARED	P- VALUE
CONTROL - A (n = 30)	5.89±2.07	A VS B	0.009 **
		A VS C	0.568 NS
		A VS D	0.002 **
ALCOHOLICS - B (n = 32)	4.49±1.16	B VS C	0.001 ***
		B VS D	0.981 NS
SMOKERS - C (n = 30)	6.47±1.95	C VS D	0.001 ***
ALCOHOLICS WITH SMOKING – D (n = 34)	4.33±0.90		

Fig 13. **TESTOSTERONE in ng/ml**



***= p<0.001 = SIGNIFICANT AT 0.1% LEVEL
**= p<0.01 = SIGNIFICANT AT 1% LEVEL
NS = NOT SIGNIFICANT

The decreased blood testosterone levels of alcoholics and alcoholics with smoking is supported by the reports of Adams and Cicero, (1991) who reported that the alcohol has stimulated the secretion of the EOP (â-endorphin) into Testicular Interstitial Fluid (TIF) which in turn suppressed the secretion of testosterone. Similar study was carried out by Adams et al, (1991, 1997) which showed that alcohol stimulated the secretion of testicular EOP, which in turn inhibited the testosterone secretion. It has been suggested that EOP exert inhibitory autocrine or paracrine effects on testicular steroidogenesis. Similarly, Frias et al, (2000) studied the effect of acute alcohol intoxication in human adolescents and reported that alcohol produced a high increase in plasma and testicular β-endorphin could decrease testicular testosterone production via their autocrine and paracrine effects.

In addition to the above, the decreased blood levels of testosterone in alcoholics and alcoholics with smoking also suggests that the decrease in testicular Leydig cell steroid acute regulatory protein (StAR protein) may result in the decreased blood level of testosterone. This StAR protein is synthesized in the testicular Leydig cells and it functions as steroid hormone synthesis rate limiting step regulatory protein (West et al, 2001; Schlegel and Hardy, 2002). Supporting this study, Kim et al, (2003) have stated the effects of alcohol in testicular Leydig cells and reported that chronic administration of ethanol inhibited the gene expression of StAR protein in the Leydig cells and decreased the testosterone level in blood. This diminution of testosterone secretion could lead to impaired spermatogenesis, accessory duct failure and contribute to decreased male fertility.

In support of present study, Akane et al, (1988) studied the effects of alcohol on testicular steroidogenesis and reported that the ethanol decreased the blood testosterone by the inhibition of pregnenolone-to-progesterone step, which supported the decreased

testosterone in the present study alcoholics. Further, supporting to the present study, alcohol-induced decreased blood level of testosterone was reported by Johnson et al, (1981). They have suggested that the inhibitory action of ethanol decreased the ratio of androstenedione/17-hydroxyprogesterone, which leads to decreased blood level of testosterone.

The decreased blood level of testosterone in alcoholics and alcoholics with smoking is supported by the study of Kalla et al, (1980) which suggested that the increased plasma levels of estradiol acts directly at testicular function and decreases the synthesis of testosterone in alcoholics.

The decreased testosterone levels of alcoholics and alcoholics with smoking is supported by the reports of Feek et al, (1989) and Adams et al, (1991) who found that alcohol stimulated corticosterone release from the adrenals and corticosteroids appeared to inhibit testosterone secretion through testicular glucocorticoid receptors.

Smoking did not produce any significant change in the levels of testosterone. Number of similar studies have been reported that there is no significant change in the levels of total or free testosterone in smokers (Barrett-Connor and Khaw, 1987; Klaiber and Broverman, 1988; Sofikitis et al, 1995).

Trummer et al, (2002) have studied the impact of cigarette smoking on human semen parameters and hormones and reported a decreased blood level of testosterone. They have suggested that the smoking leads to a degeneration of Leydig cells, which in turn reduces testosterone production, which is contradicting the present study of no significant change in blood level of testosterone in smokers.

Field et al, (1994) and English et al, (2001) have studied the effects of smoking on levels of testosterone and found that the levels of total testosterone have increased in male smokers, which is contradicting to the present study of no significant increase in blood levels of testosterone in smoking. Similar findings had been made by Vogt et al, (1986) which had stated that the testosterone level was significantly elevated in smoking. Significant elevation of LH in smoking suggested a central activation of Leydig cells and subsequent elevation of testosterone and free testosterone.

4.3.4 EFFECT OF ALCOHOL AND SMOKING ON PROLACTIN

The average mean prolactin levels of controls, alcoholics, smokers and alcoholics with smoking are presented in Table 7 and Fig 14.

The average mean prolactin level of control is 19.33+/-5.03 ng/ml, of alcoholics is 20.06+/-14.85 ng/ml, of smokers is 17.55+/-9.75 ng/ml and of alcoholics with smoking is 16.79+/-9.51 ng/ml.

The average mean prolactin level of alcoholics, smokers and alcoholics with smoking are compared with control and are found to have no significant changes in its level. Alcoholics compared with smokers and alcoholics with smoking have shown statistically no significant changes. Smokers compared with alcoholics with smoking have also shown no significant changes.

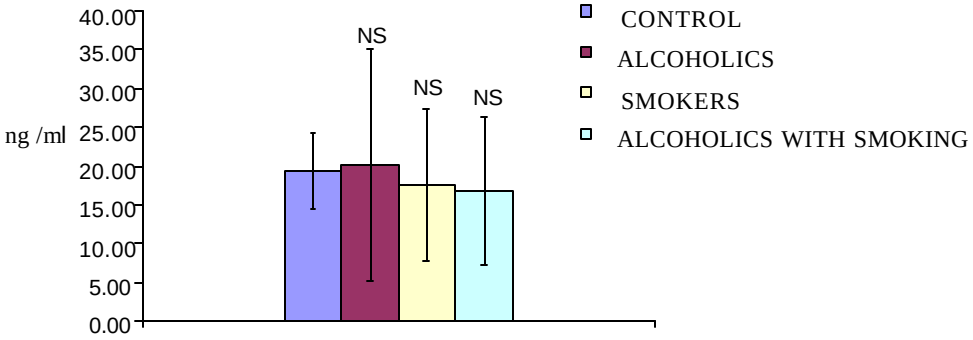
In the present study there is no significant change in the blood levels of pituitary hormone prolactin in alcoholics, smokers, alcoholics with smoking. It suggested that the toxicity of alcohol and smoke did not affect the pituitary lactotrophic cell functions.

TABLE 7. SCHEFFE'S POST HOC MULTIPLE COMPARISON

BETWEEN GROUPS
PROLACTIN in ng/ml

GROUP OF SUBJECTS	Mean ± SD	GROUPS COMPARED	P- VALUE
CONTROL - A (n = 30)	19.33±5.03	A VS B	0.995 NS
		A VS C	0.932 NS
		A VS D	0.816 NS
ALCOHOLICS - B (n = 32)	20.06±14.85	B VS C	0.827 NS
		B VS D	0.658 NS
SMOKERS - C (n = 30)	17.55±9.75	C VS D	0.994 NS
ALCOHOLICS WITH SMOKING – D (n = 34)	16.79±9.51		

Fig 14. **PROLACTIN in ng/ml**



NS = NOT SIGNIFICANT

Ching and Lin, (1994) studied the effect of EtOH on hypothalamus and pituitary and reported that EtOH could increase Dopamine (DA) stores in the median eminence which decrease the synthesis of prolactin. Their findings are contradicted to the findings of the present study.

Esquifino et al, (1989) and Fermoso et al, (1988) studied the effect of ethanol on hypothalamic –pituitary and testicular function and reported that the toxicity of ethanol acted directly on central nervous system and increased the plasma prolactin and lowered the plasma LH levels which contradicted the present study as well as the study of Ching and Lin, (1994).

The insignificant change in blood prolactin levels in smokers is substantiated by the study of Pickworth and Fant, (1998) who reported that there was no significant change in the plasma prolactin level of smokers.

Decreased prolactin levels secondary to the down regulation of prolactin gene expression have been reported in male smokers reported by Trummer et al, (2002) also contradicted the present study in which it is reported as no significant change in blood prolactin level in smokers.

4.3.5 EFFECT OF ALCOHOL AND SMOKING ON ESTRADIOL

The average mean estradiol levels of control group, alcoholics, smokers and alcoholics with smoking are presented in Table 8 and Fig 15.

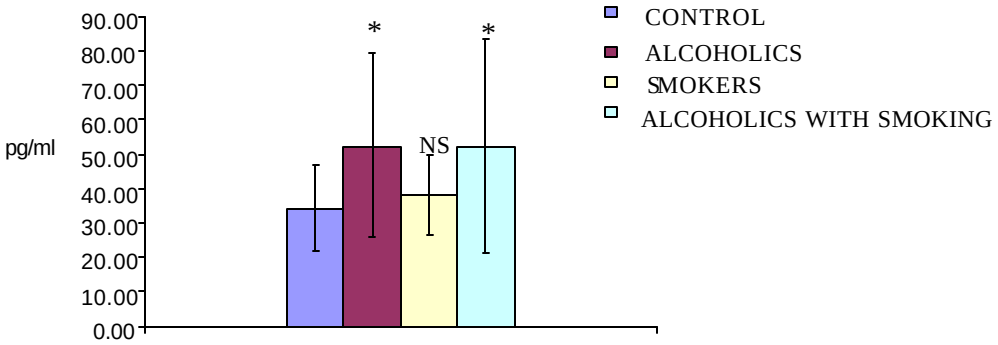
The average mean estradiol level of control is 34.28+/-12.39 pg/ml, of alcoholics is 52.44+/-26.70 pg/ml, of smokers is 38.17+/-11.76 pg/ml and of alcoholics with smoking is 52.25+/-30.89 pg/ml.

TABLE 8. SCHEFFE'S POST HOC MULTIPLE COMPARISON

BETWEEN GROUPS
ESTRADIOL in pg/ml

GROUP OF SUBJECTS	Mean ± SD	GROUPS COMPARED	P- VALUE
CONTROL - A (n = 30)	34.28±12.39	A VS B	0.021 *
		A VS C	0.930 NS
		A VS D	0.021 *
ALCOHOLICS - B (n = 32)	52.44±26.70	B VS C	0.108 NS
		B VS D	1.000 NS
SMOKERS - C (n = 30)	38.17±11.76	C VS D	0.108 NS
ALCOHOLICS WITH SMOKING – D (n = 34)	52.25±30.89		

Fig 15. **ESTRADIOL in pg/ml**



*= p<0.05 = SIGNIFICANT AT 5% LEVEL
NS = NOT SIGNIFICANT

The average mean estradiol level of control group is compared with the alcoholics group and the alcoholics with smoking group. It is found that the increase in estradiol level in both the group is statistically significant at 5% level. No significant change has been noted in estradiol in smokers when compared to the control, alcoholics compared with smokers and alcoholics with smoking.

The increased blood levels of estradiol in alcoholics and alcoholics with smoking suggested that the metabolic clearance of testosterone is accelerated by alcohol toxicity by the stimulation of aromatization the steroidogeneic pathway in the testicular Leydig cells. In the steroidogenesis pathway the aromatase enzyme converts the testosterone into estradiol. The decreased blood levels of testosterone and increased blood levels of estradiol in alcoholics and alcoholics with smoking also suggest that the toxic effect of alcohol accelerate the aromatase enzyme and increases the blood level of estradiol. The increased estradiol in alcoholics and alcoholics with smoking in the present study also suggested that the toxicity of alcohol decreased the metabolic clearance of estradiol. Smoke alone did not produce any change in the blood levels of estradiol, but smoke with alcohol increases the estradiol level in blood.

Emanuele et al, (1999a) studied the effect of ethanol on the reproductive axis of male and reported increased blood level of estradiol which supported the elevated blood levels of estradiol in alcoholics and alcoholics with smoking in this study. They suggested that the increased metabolic clearance of testosterone via the stimulation of aromatase, the key enzyme in the conversion of testosterone to estradiol and that leads to decrease in the testosterone and an increase in the estradiol values. Similarly, Esquifino et al, (1989) studied the effect of alcohol on testicular function and reported that the ethanol increased the blood

level of estradiol by decreasing the enzymatic metabolic clearance of estradiol in liver.

The present study stated increased blood level of estradiol in alcoholics and alcoholics with smoking which is contradicting to the study of Frias et al, (2002) who stated that the effects of acute alcohol intoxication on pituitary – gonadal axis and found no significant change in estradiol levels in blood of acute alcohol intoxication in men.

In the present study, there is no statistically significant elevation of estradiol in smokers compared to control groups. This statement is supported by English et al, (2001) who found that there was no significant elevation in 17β-estradiol levels in healthy smokers.

4.3.6 EFFECT OF ALCOHOL AND SMOKING ON PROGESTERONE

The average mean progesterone levels of control group, alcoholics, smokers and alcoholics with smoking are presented in Table 9 and Fig 16.

The average mean progesterone level of control is 0.71+/-0.25 ng/ml, of alcoholics is 0.53+/-0.21 ng/ml, of smokers is 0.77+/-0.30 ng/ml and of alcoholics with smoking is 0.50+/-0.18 ng/ml.

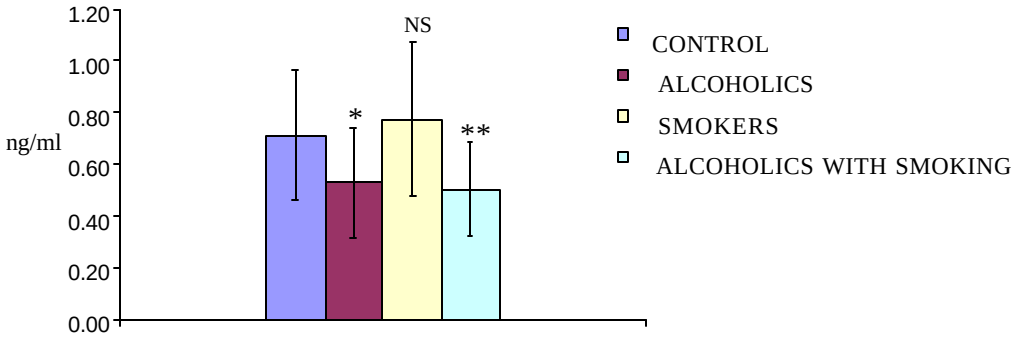
The average mean progesterone level of control group is compared with alcoholics group and alcoholics with smoking group. It is found that the decrease in progesterone level in both the group is statistically significant at 5% level. No significant decrease in progesterone level is noted in smokers. It is evident from this study that smoking is not altering the levels of progesterone. When the average mean progesterone level in smoking group is compared with alcoholics

TABLE 9. SCHEFFE'S POST HOC MULTIPLE COMPARISON

BETWEEN GROUPS
PROGESTERONE in ng/ml

GROUP OF SUBJECTS	Mean ± SD	GROUPS COMPARED	P- VALUE
CONTROL - A (n = 30)	0.71±0.25	A VS B	0.032 *
		A VS C	0.802 NS
		A VS D	0.009 **
ALCOHOLICS - B (n = 32)	0.53±0.21	B VS C	0.002 **
		B VS D	0.981 NS
SMOKERS - C (n = 30)	0.77±0.30	C VS D	0.001 ***
ALCOHOLICS WITH SMOKING – D (n = 34)	0.50±0.18		

Fig 16. **PROGESTERONE in ng/ml**



***= p<0.001 = SIGNIFICANT AT 0.1% LEVEL
**= p<0.01 = SIGNIFICANT AT 1% LEVEL
*= p<0.05 = SIGNIFICANT AT 5% LEVEL
NS = NOT SIGNIFICANT

group and alcoholics with smoking, it is noted that the decrease in progesterone levels are statistically significant at 5% level and 0.1 % level respectively.

The decreased blood level of progesterone in alcoholics and alcoholics with smoking suggests that both the synthesis and metabolic clearance of progesterone are being altered by the toxic effect of alcohol in testicular Leydig cells. Smoking alone does not produce any change in the blood levels of progesterone. The decreased blood level of progesterone in alcoholics and no change in progesterone blood levels in smoking suggested that alcohol is having more toxic effect than smoking. Alcohol together with smoking decreases the blood progesterone further down than alcoholics. This finding suggested that alcohol together with smoking increases the toxicity on testis.

The present study is supported by the study of Orpana et al, (1990b) who studied the role of ethanol metabolism in the inhibition of testosterone biosynthesis and reported a decreased progesterone and testosterone levels in blood. It was suggested that the ethanol-induced inhibition in the steroidogenic pathway observed in vitro are the reactions from pregnenolone to progesterone by the NAD- dependent 3β-hydroxysteroid dehydrogenase/oxysteroid isomerase and from androstenedione to testosterone by the NADPH-dependent 17-ketosteroid reductase.

Frias et al, (2002) contradicted the statement of decreased level of progesterone in alcoholics in this study. They studied the effects of acute alcohol intoxication on pituitary – gonadal axis and found significant increase in progesterone levels in blood of acute alcoholism.

Decreased progesterone levels of alcoholics and alcoholics with smoking confirm that the enzymes involved in the conversion of

pregnenolone to testosterone synthesis is either inactivated or the synthesis itself may be reduced by the toxic effect.

4.4 EFFECT OF ALCOHOL AND SMOKING ON SEMINAL PARAMETERS

Semen volume ($p<0.009$), sperm count ($p<0.001$), rapid progressive motile sperms ('A' – grade) ($p<0.001$), slow progressive motile sperms ('B' – grade) ($p<0.002$), non progressive motile sperms ('C' – grade) ($p<0.001$), immotile sperms ('D' – grade) ($p<0.001$), live sperms ($p<0.001$), dead sperms ($p<0.001$), morphologically normal sperms ($p<0.001$), head defective sperms ($p<0.006$) and neck defective sperms ($p<0.001$) of four experimental groups showed statistical significance in ANOVA. The tail defective sperms ($p<0.124$) did not show any statistical significance among the four experimental groups (Table 10). Further the Scheffe's post hoc analysis between the two groups was carried out for individual group statistical significances. The Chi-square values of semen color, appearance, pH, viscosity, liquefaction, fructose of study groups controls, alcoholics, smokers, and alcoholics with smoking did not show any statistical significances.

4.4.1 EFFECT OF ALCOHOL AND SMOKING ON SEMEN VOLUME

The average mean semen volume of control group, alcoholics, smokers and alcoholics with smoking are presented in Table 11 and Fig 17.

The average mean semen volume is 2.17+/-0.71 ml in controls, 1.58+/-0.73 ml in alcoholics, 1.77+/-0.87 ml in smokers and 1.54+/-0.85 ml in alcoholics with smoking.

The average mean semen volume in ml of control is compared with alcoholics and alcoholics with smoking and it is found that there is statistically significant decrease at 5% level in volume of semen. No

TABLE 10.

SEMEN ANALYSIS

ANOVA BETWEEN

CONTROL (n=30), ALCOHOLICS (n=32), SMOKERS (n=30) AND ALCOHOLICS WITH SMOKING (n=34) GROUPS

S.NO	NAME OF THE PARAMETER	UNITS	'F' – VALUE	LEVEL OF SIGNIFICANCE
1	SEMEN VOLUME	ML	4.057	0.009 **
2	SPERM COUNT	Million /ml	12.767	0.001 ***
3	RAPID PROGRESSIVE MOTILE SPERMS ('A' – GRADE)	%	27.11	0.001 ***
4	SLOW PROGRESSIVE MOTILE SPERMS ('B' – GRADE)	%	5.22	0.002 **
5	NON PROGRESSIVE MOTILE SPERMS ('C' – GRADE)	%	7.64	0.001 ***
6	IMMOTILE SPERMS ('D' – GRADE)	%	14.46	0.001 ***
7	ALIVE SPERMS	%	16.18	0.001 ***
8	DEAD SPERMS	%	16.65	0.001 ***
9	NORMAL FORM SPERMS – MORPHOLOGICAL	%	8.42	0.001 ***
10	HEAD DEFECTIVE SPERMS	%	4.356	0.006 **
11	NECK DEFECTIVE SPERMS	%	7.63	0.001 ***
12	TAIL DEFECTIVE SPERMS	%	1.96	0.124 NS

** = $p<0.01$ = SIGNIFICANCE AT 1% LEVEL

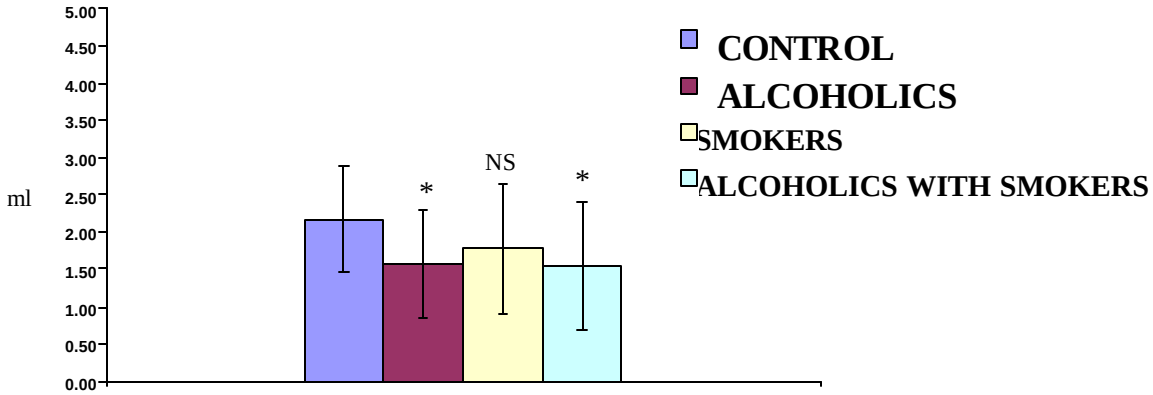
*** = $p<0.001$ = SIGNIFICANCE AT 0.1% LEVEL

NS = NO SIGNIFICANCE

TABLE 11. SCHEFFE'S POST HOC MULTIPLE COMPARISON BETWEEN GROUPS SEMEN VOLUME in ml

GROUP OF SUBJECTS	Mean ± SD	GROUPS COMPARED	P- VALUE
CONTROL - A (n = 30)	2.17±0.71	A VS B	0.040 *
		A VS C	0.304 NS
		A VS D	0.023 *
ALCOHOLICS - B (n = 32)	1.58±0.73	B VS C	0.810 NS
		B VS D	0.999 NS
SMOKERS - C (n = 30)	1.77±0.87	C VS D	0.715 NS
ALCOHOLICS WITH SMOKING – D (n = 34)	1.54±0.85		

Fig 17. **SEMEN VOLUME in ml**



*= p<0.05 = SIGNIFICANT AT 5% LEVEL
NS = NOT SIGNIFICANT

significant decrease in volume of semen is noted in smokers. When alcoholics are compared with smokers and alcoholics with smoking group, it is found that there are no statistical significant changes. Smokers compared with alcoholics with smoking have also shown no statistical significant change in volume of semen.

In the present study in alcoholics and alcoholics with smoking reveals that the toxic effect of alcohol decreases the secretory capacity of testicular seminiferous tubule and accessory sex organs leading to decreased semen volume. No significant change in ejaculatory semen volume in smokers suggests that smoking alone does not produce any toxic effect in the secretory capacity of testis and accessory sex organs. The decreased semen volume of alcoholics and insignificant change in semen volume of smokers suggest that alcohol is having more toxic effect than smoking in testis and accessory sex organs.

The decreased volume of semen in alcoholics and alcoholics with smoking in the present study correlated with the study of Martini et al, (2004) who reported that the effect of alcohol and cigarette smoking reduced semen volume. Brzek, (1987) studied the effect of alcohol consumption on semen quality and found that the consumption of alcohol decreases the volume of semen. It was further suggested that the toxicity induced suppression of secretory capacity of testis and accessory sex organs may be the reason for the decreased semen volume in alcoholics.

Celis et al, (2000), Kunzle et al, (2003), Trummer et el, (2002) and Zavos, et al, (1998a) have studied the effect of cigarette smoking on semen parameters and reported that smoking did not produce any alteration in the semen volume of smokers. Their study supported the present study of no significant change in semen volume of smokers.

The present study of insignificant change in semen volume of smokers is contradicted by the studies of Zhang et al, (2000), Zenzes et al, (1999), Rubes et al, (1998), and Chia et al, (2000) who reported that smoking decreased the semen volume. These authors suggested that smoking decreases the secretory function of accessory sex organs, which may be the reason for the decreased semen volume in smokers.

4.4.2 EFFECT OF ALCOHOL AND SMOKING ON SPERM COUNT

The average mean semen sperm count of control group, alcoholics, smokers and alcoholics with smoking are presented in Table 12 and Fig 18.

The average mean semen sperm count of control is 132.97+/-89.02 million sperms/ml, alcoholics is 53.41+/-51.55 million sperms/ml, smokers is 88.33+/-52.68 million sperms/ml and alcoholics with smoking is 50.65+/-37.92 million sperms/ml.

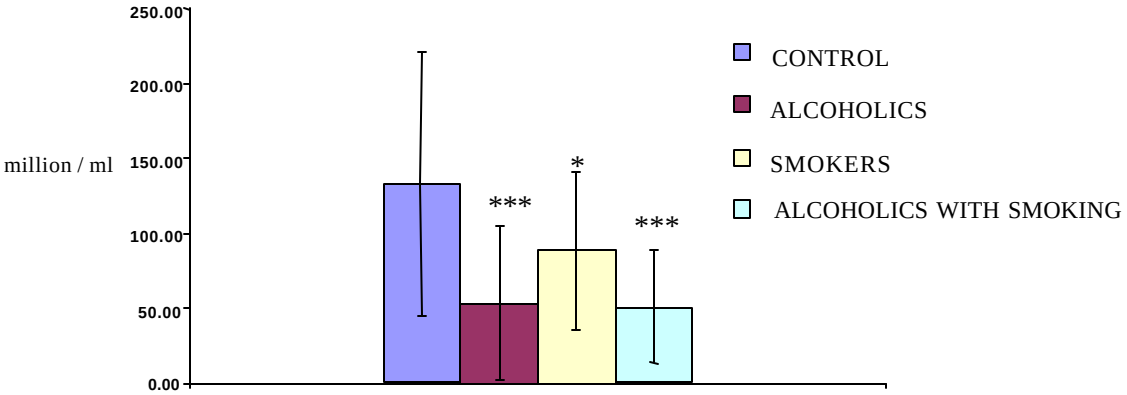
The average mean semen sperm count in control is compared with alcoholics, smokers and alcoholics with smoking. It is found that the decrease in sperm count is statistically significant at 0.1% level in alcoholics and alcoholics with smoking. Also the decrease in smokers is found to be statistically significant at 5% level. No significant decrease in sperm count is noted when alcoholics group is compared with smokers and alcoholics with smoking groups. When smokers are compared with alcoholics with smoking group, no statistically significant decrease is noted.

The decreased sperm count in alcoholics and alcoholics with smoking suggests that the alcohol is having direct toxic effect at testicular seminiferous tubule, Sertoli cells and germ cells. The decreased sperm count in alcoholics and alcoholics with smoking may be due to two reasons: 1.The toxicity of alcohol might have directly

TABLE 12. SCHEFFE'S POST HOC MULTIPLE COMPARISON BETWEEN GROUPS SEMEN SPERM COUNT in million sperm/ml

GROUP OF SUBJECTS	Mean ± SD	GROUPS COMPARED	P- VALUE
CONTROL - A (n = 30)	132.97±89.02	A VS B	0.001 ***
		A VS C	0.045 *
		A VS D	0.001 ***
ALCOHOLICS - B (n = 32)	53.41±51.55	B VS C	0.161 NS
		B VS D	0.998 NS
SMOKERS - C (n = 30)	88.33±52.68	C VS D	0.105 NS
ALCOHOLICS WITH SMOKING – D (n = 34)	50.65±37.92		

Fig 18. **SEMEN SPERM COUNT in million sperm/ml**



*= p<0.05 = SIGNIFICANT AT 5% LEVEL
***= p<0.001 = SIGNIFICANT AT 0.1% LEVEL
NS = NOT SIGNIFICANT

affected the proliferation of the developing germ cells and spermatogonia. 2.The decreased blood level of testosterone in the present study on alcoholics and alcoholics with smoking may decrease the synthesis of transport proteins transferrin, ceruloplasmin and other nutritional components of Sertoli cells essential for germ cell proliferation and spermatogenesis. Both the above toxic effects may decrease the germ cell proliferation in seminiferous tubules and lead to decreased semen sperm count. In the present study, the increased blood level of FSH in alcoholics and alcoholics with smoking suggests that the alcohol induce decrease in Sertoli cell function.

In this study, semen total sperm count in smokers also decreased significantly, but the toxicity has not altered the blood levels of FSH. This result suggests that the smoking is not altering the normal function of Sertoli cells and the toxic effect of smoking is mainly at developing germ cells. Further the above result indicates that the germ cells are more sensitive to toxic substances like alcohol and smoke than the Sertoli cells. Alcohol together with smoking decreases the semen sperm count further. The decreased testosterone and increased FSH in alcoholics with smoking suggest that in the alcohol with smoking, the toxic effect is severe on both Sertoli cell and germ cell functions. The present study shows that the alcohol is having more toxic effect than smoking on sperm count and alcohol together with smoking increases the toxic effect further more.

The decreased sperm count in the present study in alcoholics and alcoholics with smoking is supported by the study of Pajarinen and Karhunen, (1994) who reported that alcohol toxicity decreases the seminiferous tubular fluid secretion in Sertoli cells and induces the spermatogenic arrest in the germ cells.

Martini et al, (2004) studied the effect of alcohol and cigarette consumption on human seminal quality and reported that both the habits decreased the semen volume, sperm concentration and motility, but unlike in the present study the sperm morphology was not significantly modified, but the reason for the decreased sperm count was not stated.

The decreased sperm count in smokers in the present study is supported by the study of Vine et al, (1996) who stated that there was a reduction of 13% to 17 % in sperm counts in smokers. Also the findings of the present study is confirmed by Shaarawy and Mahmoud, (1982) and Handelsman et al, (1984) that there was a marked reduction in sperm count and motility in smokers. In addition to nicotine and tars, cigarette smoke contains carbon monoxide and radioactive particles, which might have detrimental effects on the testis.

Rantala and Koskimies, (1987), Chia et al, (1994) and Hermann et al, (1986) have studied the effect of cigarette smoking and reported a decreased total sperm count in smokers, which supports the findings of the present study. Smokers had significant higher cadmium levels in blood and seminal plasma compared to the nonsmokers. Cadmium in cigarette is a possible cause for the reduction in sperm count.

The decreased total sperm count in smokers in the present study is not correlated with the study of Goverde et al, (1995), Wong et al, (2000) and Trummer et al, (2002) who stated that the cigarette smoking did not produce any significant decrease in the sperm count.

4.4.3 EFFECT OF ALCOHOL AND SMOKING ON MOTILITY OF SPERM

The average mean 'A' grade or rapid progressive motile sperm of control group, alcoholics, smokers and alcoholics with smoking are presented in Table 13 and Fig 19.

The average mean rapid progressive motile sperm count of control is 56.10+/-8.72 %, of alcoholics is 31.41+/-17.65 %, of smokers is 45.17+/-11.33 % and of alcoholics with smoking is 29.41+/-14.08 %.

The average mean rapid progressive motile sperm count of control is compared with the average mean rapid progressive motile sperm of alcoholics, smokers and alcoholics with smoking and it is found that the decrease is statistically significant at 0.1% level, 5% level and 0.1 % level respectively. No significant decrease in average mean rapid progressive motile sperm is noted when alcoholics group is compared with alcoholics with smoking groups. When the smoking group is compared with alcoholics group and alcoholics with smoking group, the decrease is found to be statistically significant at 1% level and 0.1% level respectively.

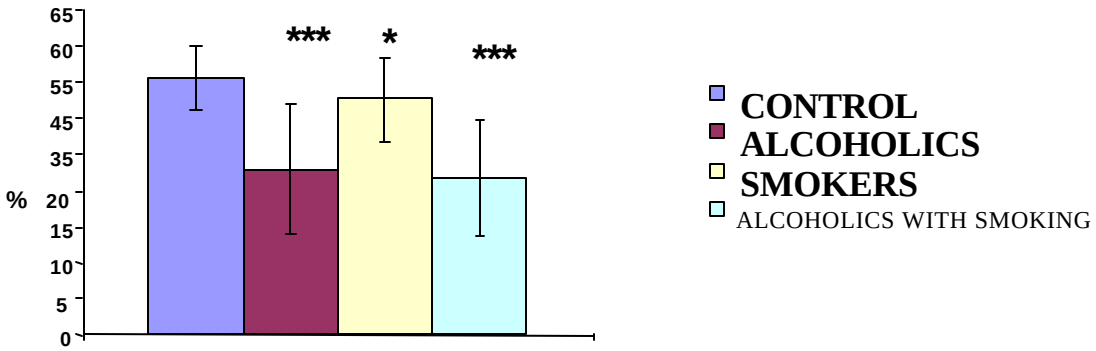
The average mean "B" grade sperm or slow or sluggish progressive motile sperm count of the control group, alcoholics, smokers and alcoholics with smoking are presented in Table 14 and Fig 20.

The average mean slow or sluggish progressive motile sperm count of control is 21.48+/-2.76 %, of alcoholics is 29.08+/-8.48 %, of smokers is 26.63+/-12.26 % and of alcoholics with smoking is 28.96+/-8.38 %.

TABLE 13. SCHEFFE'S POST HOC MULTIPLE COMPARISON BETWEEN GROUPS 'A' GRADE or RAPID PROGRESSIVE MOTILE SPERM in %

GROUP OF SUBJECTS	Mean ± SD	GROUPS COMPARED	P- VALUE
CONTROL - A (n = 30)	56.10±8.72	A VS B	0.001 ***
		A VS C	0.023 *
		A VS D	0.001 ***
ALCOHOLICS - B (n = 32)	31.41±17.65	B VS C	0.002 **
		B VS D	0.948 NS
SMOKERS - C (n = 30)	45.17±11.33	C VS D	0.001 ***
ALCOHOLICS WITH SMOKING – D (n = 34)	29.41±14.08		

Fig 19. **'A' GRADE or RAPID PROGRESSIVE MOTILE SPERM in %**

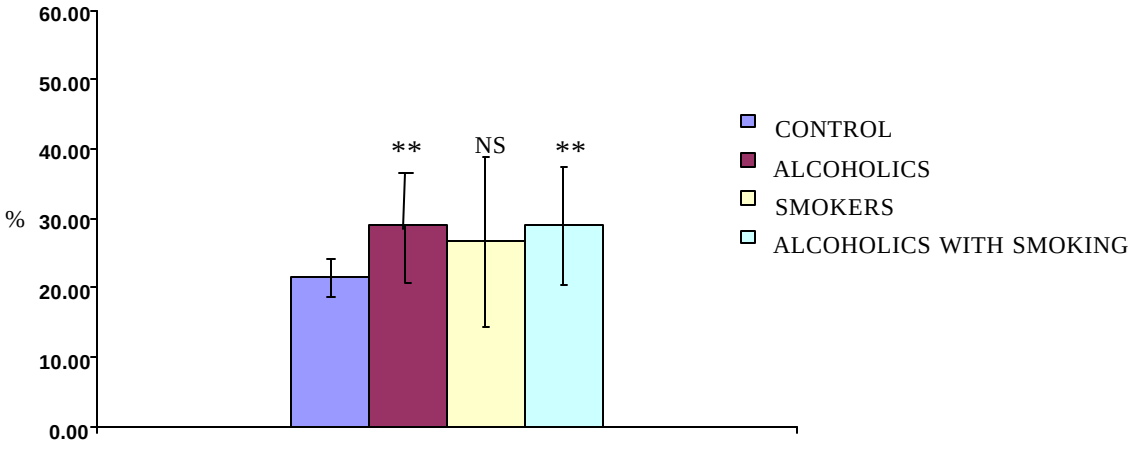


*= p<0.05 = SIGNIFICANT AT 5% LEVEL
**= p<0.01 = SIGNIFICANT AT 1% LEVEL
***= p<0.001 = SIGNIFICANT AT 0.1% LEVEL
NS = NOT SIGNIFICANT

TABLE 14. SCHEFFE'S POST HOC MULTIPLE COMPARISON BETWEEN GROUPS "B"GRADE SPERM or SLOW or SLUGGISH PROGRESSIVE MOTILE in %

GROUP OF SUBJECTS	Mean ± SD	GROUPS COMPARED	P- VALUE
CONTROL - A (n = 30)	21.48±2.76	A VS B	0.010 **
		A VS C	0.156 NS
		A VS D	0.010 **
ALCOHOLICS - B (n = 32)	29.08±8.48	B VS C	0.744 NS
		B VS D	1.000 NS
SMOKERS - C (n = 30)	26.63±12.26	C VS D	0.765 NS
ALCOHOLICS WITH SMOKING – D (n = 34)	28.96±8.38		

Fig 20. **"B"GRADE SPERM or SLOW OR SLUGGISH PROGRESSIVE MOTILE in %**



**= p<0.01 = SIGNIFICANT AT 1% LEVEL
NS = NOT SIGNIFICANT

The average mean sluggish progressive motile sperm count of control is compared with alcoholics and alcoholics with smoking and it is found to have a statistical significant increase at 5% level. No significant change has been noted in smokers when compared to the controls. When alcoholics compared with smokers and alcoholics with smoking, there is statistically no significant change. Similarly when smokers compared with alcoholics with smoking, there is statistically no significant alteration.

The average mean "C" grade sperm or non progressive motile sperm count of control group, alcoholics, smokers and alcoholics with smoking are presented in Table 15 and Fig 21.

The average mean non progressive motile sperm count of control is 2.50+/-1.07 %, of alcoholics is 4.05+/-1.77 %, of smokers is 2.70+/-1.02 % and of alcoholics with smoking is 4.15+/-2.58 %.

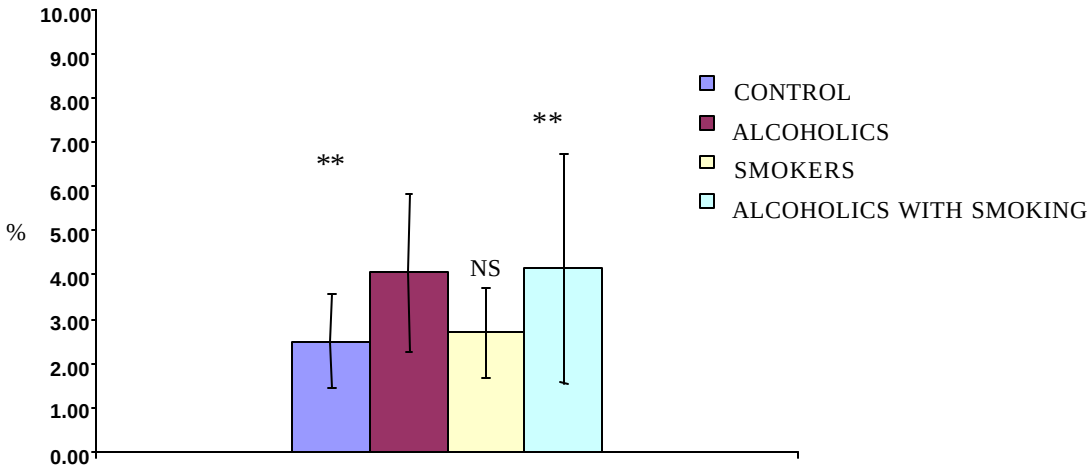
The average mean non progressive motile sperm count of control is compared with the average mean non progressive motile sperm of alcoholics and alcoholics with smoking and it is found that the increase is statistically significant at 1% level. No significant increase in average mean non progressive motile sperm is noted when control is compared with smokers group and alcoholics group is compared with alcoholics with smoking group. The average mean non progressive motile sperm in smokers is compared with alcoholics and alcoholics with smoking group and it is found that the increase in non progressive motile sperm is statistically significant at 5% level in alcoholics and alcoholics with smokers.

The average mean "D" grade or immotile sperm count of control group, alcoholics, smokers and alcoholics with smoking are presented in Table 16 and Fig 22.

TABLE 15. SCHEFFE'S POST HOC MULTIPLE COMPARISON BETWEEN GROUPS "C" GRADE SPERM or NON PROGRESSIVE MOTILE in %

GROUP OF SUBJECTS	Mean ± SD	GROUPS COMPARED	P- VALUE
CONTROL - A (n = 30)	2.50±1.07	A VS B	0.010 **
		A VS C	0.979 NS
		A VS D	0.004 **
ALCOHOLICS - B (n = 32)	4.05±1.77	B VS C	0.033 *
		B VS D	0.997 NS
SMOKERS - C (n = 30)	2.70±1.02	C VS D	0.016 *
ALCOHOLICS WITH SMOKING – D (n = 34)	4.15±2.58		

Fig 21. **"C" GRADE SPERM or NON PROGRESSIVE MOTILE in %**

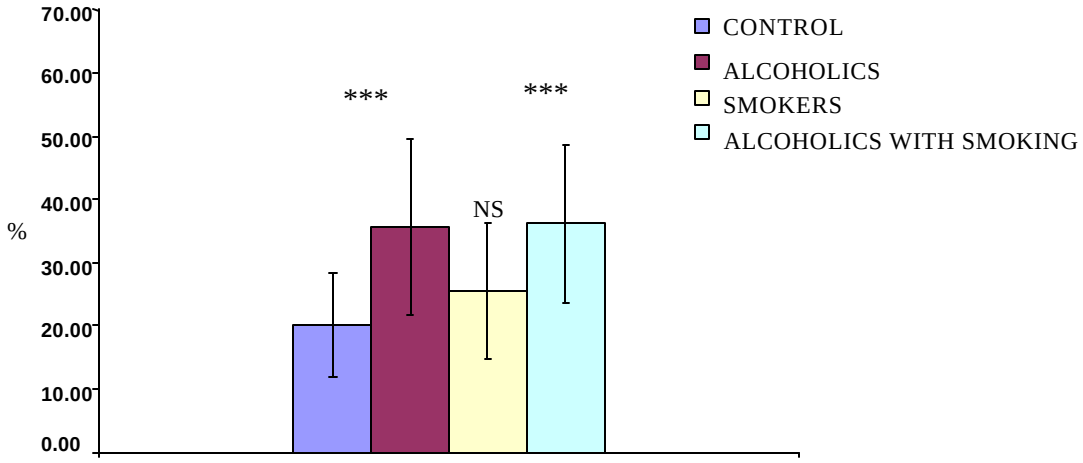


*= p<0.05 = SIGNIFICANT AT 5% LEVEL
**= p<0.01 = SIGNIFICANT AT 1% LEVEL
NS = NOT SIGNIFICANT

TABLE 16. SCHEFFE'S POST HOC MULTIPLE COMPARISON BETWEEN GROUPS "D" GRADE or IMMOTILE SPERM in %

GROUP OF SUBJECTS	Mean ± SD	GROUPS COMPARED	P- VALUE
CONTROL - A (n = 30)	20.17±8.25	A VS B	0.001 ***
		A VS C	0.368 NS
		A VS D	0.001 ***
ALCOHOLICS - B (n = 32)	35.63±13.84	B VS C	0.011 *
		B VS D	0.998 NS
SMOKERS - C (n = 30)	25.50±10.78	C VS D	0.005 **
ALCOHOLICS WITH SMOKING – D (n = 34)	36.18±12.37		

Fig 22. **"D" GRADE or IMMOTILE SPERM in %**



*= p<0.05 = SIGNIFICANT AT 5% LEVEL
**= p<0.01 = SIGNIFICANT AT 1% LEVEL
***= p<0.001 = SIGNIFICANT AT 0.1% LEVEL
NS = NOT SIGNIFICANT

The average mean immotile sperm count of control is 20.17+/-8.25 %, of alcoholics is 35.63+/-13.84 %, of smokers is 25.50+/-10.78 % and of alcoholics with smoking is 36.18+/-12.37 %.

The average mean immotile sperm count of control group is compared with alcoholics group and alcoholics with smoking group. It is found that the increase in immotile sperm in both the groups is statistically significant at 0.1% level. No significant increase in immotile sperm is noted in smokers compared to the control group. When the average mean immotile sperm in smoking group is compared with alcoholics group and alcoholics with smoking group, it is noted that the increase in immotile sperm are statistically significant at 5% level and 1 % level respectively.

In the present study, decreased percentage of rapid progressive motile sperm in alcoholics, smokers and alcoholics with smoking suggests two possible ways of effects 1). the toxicity of alcohol and smoke may reach the seminiferous tubule and alter the paracrine regulations of Sertoli cells and germ cells, which leads into a decrease of the nutritional component supply to developing germ cells and decreases the sperm motility. The decreased blood level of testosterone and increased blood level of FSH in alcoholics and alcoholics with smoking suggest that the alcohol toxicity affects Sertoli cells, Leydig cells and germ cells functions and decrease the sperm motility. Absence of significant change in blood levels of testosterone and FSH in smokers suggests that smoking affects only the functions of germ cells and decreases the sperm motility and the functions of Leydig and Sertoli cells are not affected by smoking. 2). the toxicity of alcohol may affect the sperm maturation, transport and storage, accessory sex organs such as, seminal vesicle and prostate and their secretory products resulting in decreased sperm motility.

The decreased semen sperm motility, decreased testosterone and increased FSH levels in blood of alcoholics and alcoholics with smoking and only the decreased sperm motility in smokers suggests that the alcohol is having more toxic effect than the smokers in testicular physiology. In the same way alcohol together with the smoking decreases the sperm motility further down than the alcoholics. The increased percentage of sluggish motile, non progressively motile and immotile sperms in the semen of alcoholics and alcoholics with smoking also suggests the toxic effect of alcohol at the place of accessory sex organs like reti testis, epididymis, vas deferens, seminal vesicle and prostate.

The decreased motility in alcoholics and alcoholics with smoking in the present study is supported by the study of Gomathi et al, (1993) and Goverde et al, (1995) who stated that there is a decrease in sperm motility in alcoholics, but the biological mechanism involved in the decrease was not stated.

Decreased rapid progressive motile sperm of smokers group in the present study is supported by the study of Vine et al, (1994) also who have reported an average 20% reduction in sperm motility among smokers. Similar to the present study on decreased sperm motility in smokers, Pacifici et al, (1993) have observed a significant decrease in motility of smokers and reported that seminal plasma cotinine and hydroxycotinine are negatively correlated with sperm motility.

The decreased sperm motility and count in smokers in the present study is supported by the study of Yamamoto et al, (1998) that smoking reduced the caudal epididymal sperm count and motility secondary to the secretory disturbances of Leydig and Sertoli cells.

The decreased sperm motility in smokers is supported by Gandini et al, (1997) who studied the in-vitro effects of nicotine and cotinine on human semen quality and reported that the detrimental effects of smoking on the spermatozoal cytoskeleton, deriving from disturbances during spermatogenesis and the epididymal sperm maturation process which lead to decreased sperm motility. Similarly, Zavos et al, (1998b) studied the effect of axonemal ultrastructure in human spermatozoa from male smokers and reported that smoking lowered sperm motility by completely disappearing the one or more of the nine fibre doublets and one or more of the central fibres.

The present study is supported by Kunzle et al, (2003), Kulikauskas et al, (1984) and Rantala and Koskimies, (1987) who have observed a decrease in progressive motile sperm count in cigarette smokers and they suggested that the cigarette smoke nicotine and its water-soluble metabolite cotinine are detectable in the seminal plasma, the harmful components of tobacco smoke pass through the blood-testis barrier and decreases the spermatogenesis.

The decreased semen quality in the smokers is correlated with the study of Zhang et al, (2000) who studied the effect of smoking in the semen quality in men and reported that smoking decreased Zinc, copper levels and the activity of super oxide dismutase activity. The decreased superoxide dismutase decreases the sperm motility in smokers.

The decreased sperm motility in the present study is contradicted by the study of Trummer et al, (2002) who have found that there is no change in sperm motility of smokers.

4.4.4 EFFECT OF ALCOHOL AND SMOKE ON VIABILITY OF SPERM

The average mean alive sperm count of control group (Plate 1), alcoholics, smokers and alcoholics with smoking are presented in Table 17 and Fig 23.

The average mean alive sperm count of control is 80.80+/-6.29 %, of alcoholics is 60.84+/-14.77 %, of smokers is 70.60+/-11.61 % and of alcoholics with smoking is 59.59+/-18.38 %.

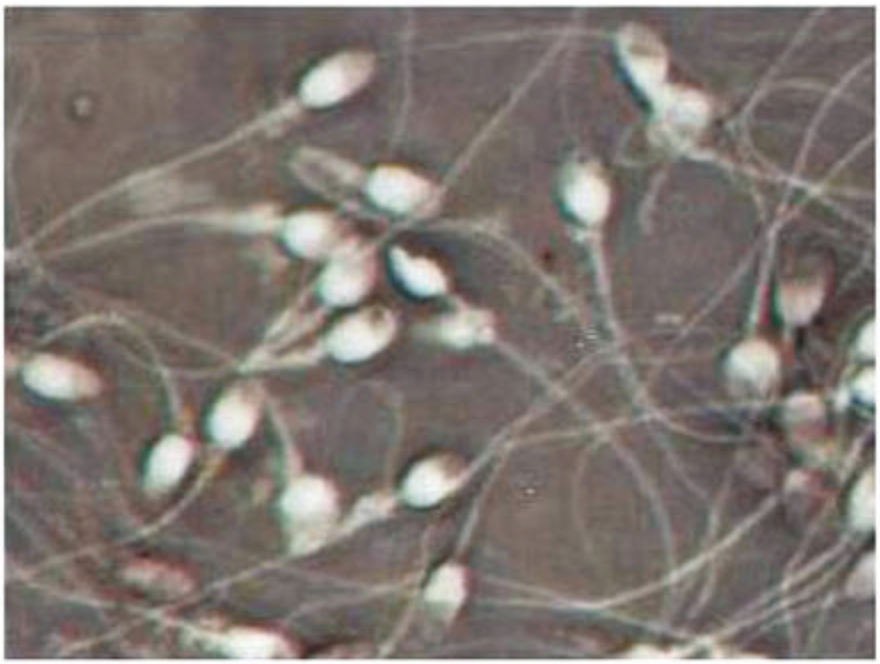
The average mean alive sperm count in percentage of control is compared with alcoholics, smokers and alcoholics with smoking. It is found that the decrease in alive sperm is statistically significant at 0.1% level in alcoholics and alcoholics with smoking and in smokers statistically significant at 5% level. No significant decrease in alive sperm is found when alcoholics group is compared with smokers and alcoholics with smoking groups. When smokers are compared with alcoholics with smoking group, it is found that the decrease in alive sperm is statistically significant at 5% level.

The average mean dead sperm count of control group, alcoholics, smokers and alcoholics with smoking are presented in Table 18 and Fig 24 Plate 2,3 and 4.

The average mean dead sperm count of control is 19.20+/-6.29 %, of alcoholics is 39.16+/-14.77 %, of smokers is 28.40+/-11.24 % and of alcoholics with smoking is 40.35+/-18.39%.

The average mean dead sperm count in percentage of control is compared with the average mean percentage of dead sperm of alcoholics, smokers and alcoholics with smoking and it is found that the increase is statistically significant at 0.1% level, 5% level and 0.1 %

PLATE 1. SPERMS OF CONTROL



NORMAL MORPHOLOGY
ALIVE SPERMS

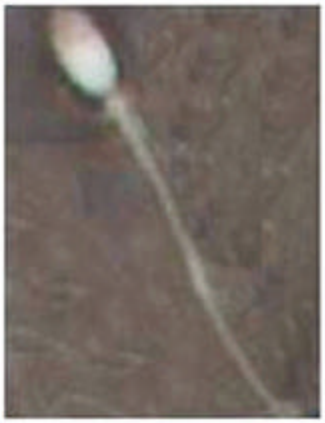
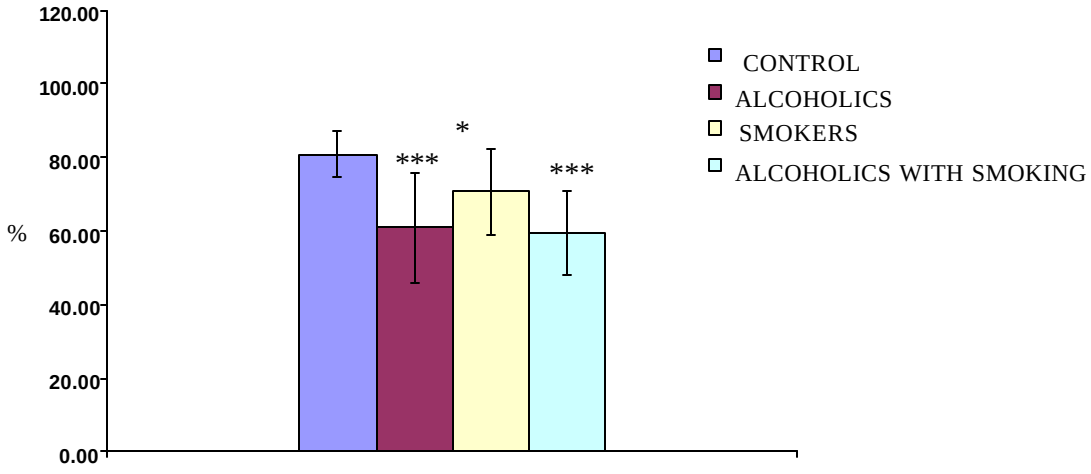


TABLE 17. SCHEFFE'S POST HOC MULTIPLE COMPARISON

BETWEEN GROUPS
ALIVE SPERM in %

GROUP OF SUBJECTS	Mean ± SD	GROUPS COMPARED	P- VALUE
CONTROL - A (n = 30)	80.80±6.29	A VS B	0.001 ***
		A VS C	0.045 *
		A VS D	0.001 ***
ALCOHOLICS - B (n = 32)	60.84±14.77	B VS C	0.055 NS
		B VS D	0.987 NS
SMOKERS - C (n = 30)	70.60±11.61	C VS D	0.019 *
ALCOHOLICS WITH SMOKING – D (n = 34)	59.59±18.38		

Fig 23. **ALIVE SPERM in %**



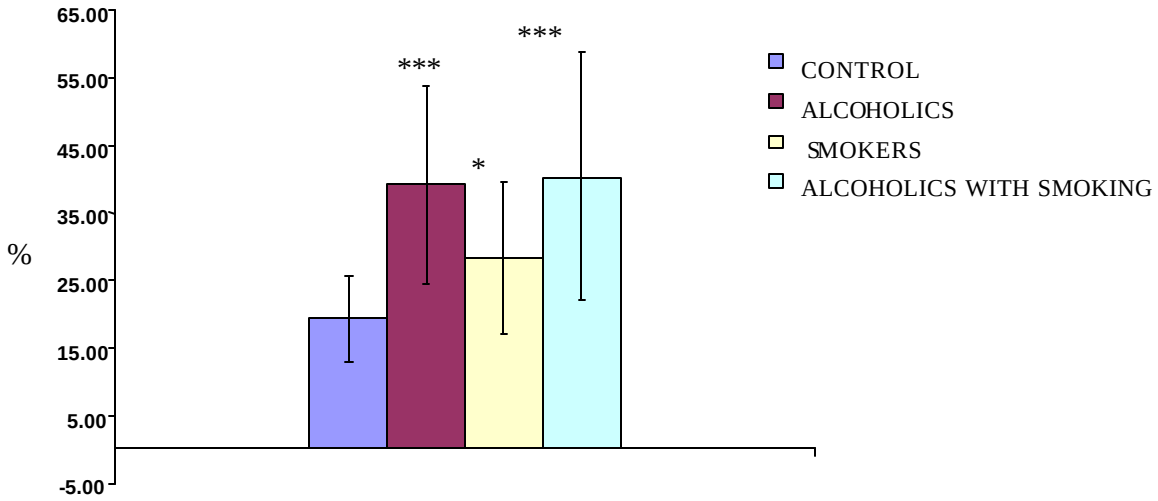
*= p<0.05 = SIGNIFICANT AT 5% LEVEL
***= p<0.001 = SIGNIFICANT AT 0.1% LEVEL
NS = NOT SIGNIFICANT

TABLE 18. SCHEFFE'S POST HOC MULTIPLE COMPARISON

BETWEEN GROUPS
DEAD SPERM in %

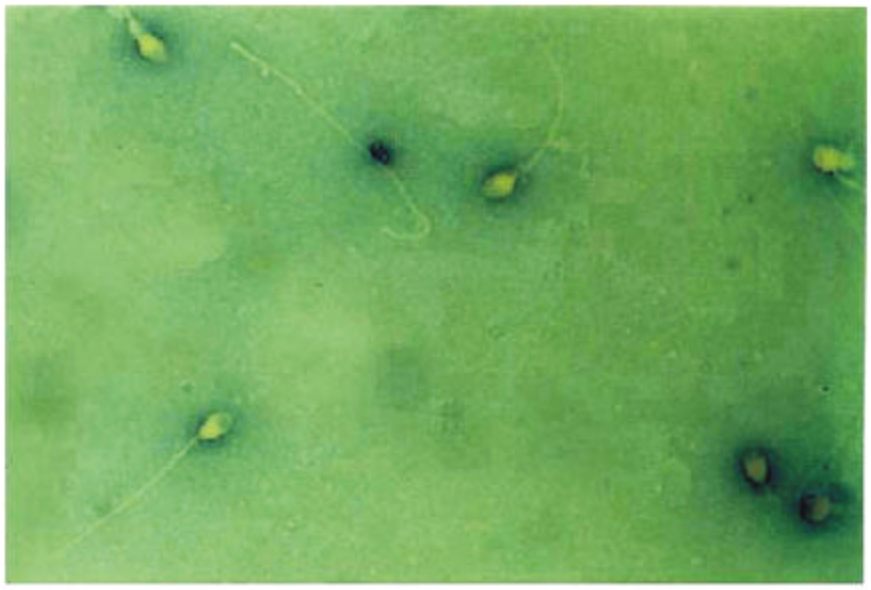
GROUP OF SUBJECTS	Mean ± SD	GROUPS COMPARED	P- VALUE
CONTROL - A (n = 30)	19.20±6.29	A VS B	0.001 ***
		A VS C	0.049 *
		A VS D	0.001 ***
ALCOHOLICS - B (n = 32)	39.16±14.77	B VS C	0.026 *
		B VS D	0.988 NS
SMOKERS - C (n = 30)	28.40±11.24	C VS D	0.009 **
ALCOHOLICS WITH SMOKING – D (n = 34)	40.35±18.39		

Fig 24. **DEAD SPERM in %**



*= p<0.05 = SIGNIFICANT AT 5% LEVEL
**= p<0.01 = SIGNIFICANT AT 1% LEVEL
***= p<0.001 = SIGNIFICANT AT 0.1% LEVEL
NS = NOT SIGNIFICANT

PLATE 2. SPERMS OF ALCOHOLICS



DEAD SPERM

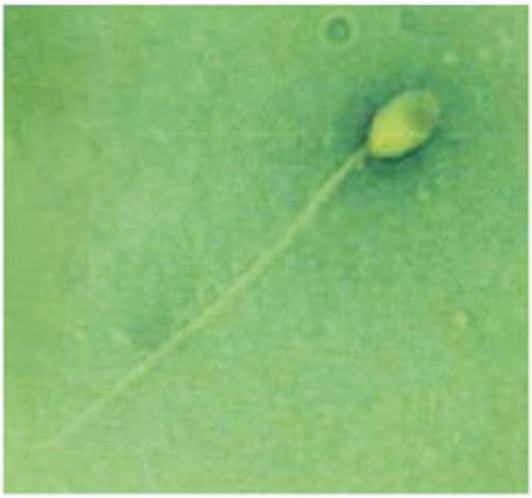
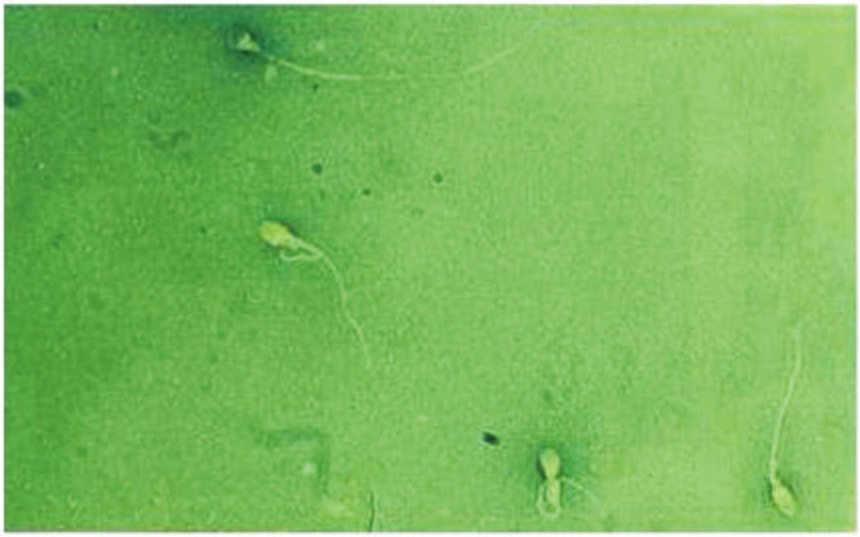
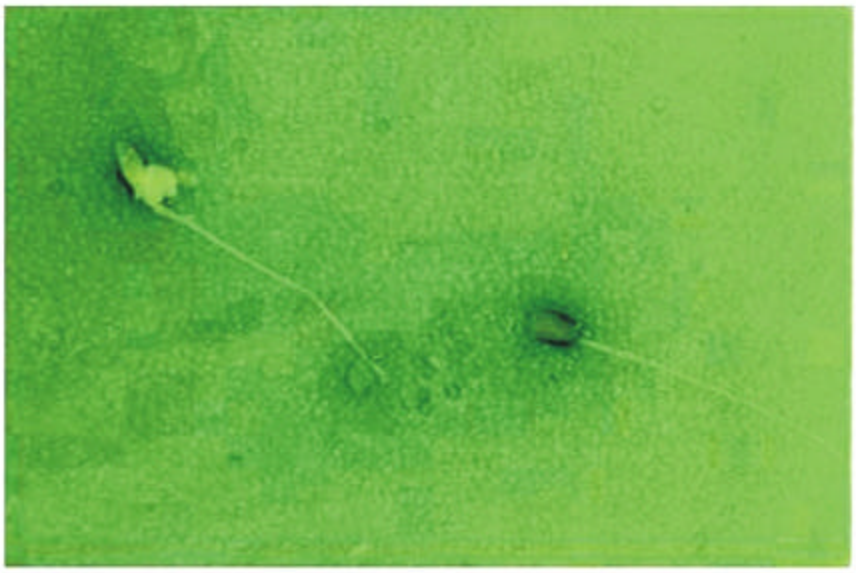


PLATE 3. SPERMS OF SMOKERS



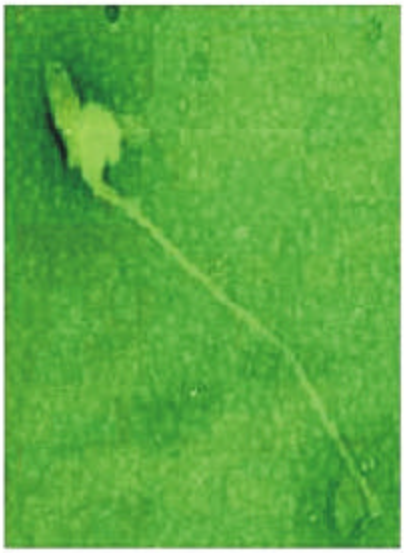
DEAD SPERM

PLATE 4. SPERMS OF ALCOHOLICS WITH SMOKING



DEAD SPERM

HEAD DEFECTIVE SPERM



level respectively. No significant increase in average mean dead sperm percentage is noted when alcoholics group is compared with alcoholics with smoking groups. When the smoking group is compared with alcoholics group and alcoholics with smoking group, the decrease is statistically significant at 5 % level and 1% level respectively.

The decreased percentage of alive sperm in the alcoholics, smokers, alcoholics with smoking in the present study suggest that the toxicity of alcohol and smoke reaches the testicular seminiferous tubules and accessory sex organs, epididymis, vas deferens, seminal vesicle, prostate the places where the spermatogenesis, spermiogenesis (sperm maturation) and sperm transport are taking place. At the time of spermiogenesis and transport of sperm in accessory sex organs the toxicity of alcohol and smoke may alter the paracrine regulatory proteins, nutritional component supply to the sperm and also the toxicity of alcohol and smoke directly act on the sperm cells and kill the sperms which leads into a decreased number of viable sperm. The increased percentage of dead sperm in the present study alcoholics, smoking and alcoholics with smoking also suggest the toxicity of alcohol and smoke affects the seminiferous tubules and accessory sex organs. The present study on decreased semen alive sperm percentages and increased dead sperm percentages of alcoholics is higher when compared with smoking suggest that the alcohol is having more toxic effect than smoking on the sperm viability. In alcohol together with smoking, the added toxicity further severely decreases the alive sperms and increases the dead sperm numbers in semen.

Goverde et al, (1995) and Brzek, (1987) have observed a significant decrease in the viability of the spermatozoa in alcoholics, which has been correlated in the present study on decreased sperm

viability of alcoholics and alcoholics with smoking, but the biological mechanism involved in the decrease was not stated by them.

Decreased sperm viability in smokers in the present study was contradicted by the study of Kunzle et al, (2003) who studied the effect of smoking on semen quality and reported that the smoking did not produce any change in the number of viable sperm in smokers.

The decreased sperm viability in smokers is supported by the studies of Zavos et al, (1998a) and Merino et al, (1998) who found that smoking decreased the viability of sperms. Similarly, Zhang et al, (2000) studied the effect of smoking on semen quality of infertile men and reported a decreased sperm viability in smokers and suggested that smoking decreased the zinc and copper levels and increased superoxide dismutase activity, which adversely affected the sperms viability.

4.4.5 EFFECT OF ALCOHOL AND SMOKING ON MORPHOLOGY OF SPERM

The average mean normal morphology sperms count of control group, (Plate 1) alcoholics, smokers and alcoholics with smoking are presented in Table 19 and Fig 25.

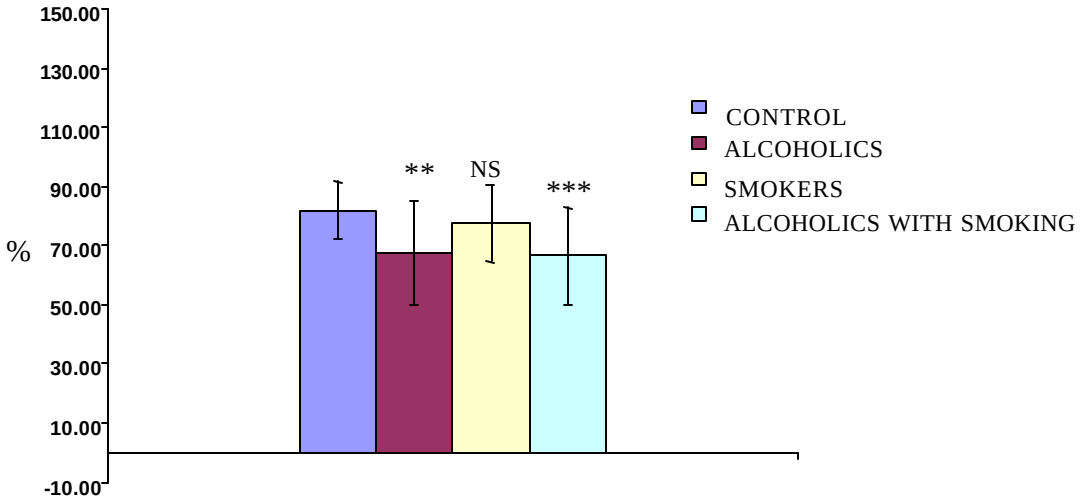
The average mean normal morphology sperm count of control is 82.00+/-9.76 %, of alcoholics is 67.75+/-17.54 %, of smokers is 77.83+/-13.26 % and of alcoholics with smoking is 66.62+/-16.18 %.

The average mean normal morphology of sperm in percentage of control is compared with alcoholics and alcoholics with smoking and it is found that the decrease in normal morphology is statistically significant at 1% level, and 0.1% respectively. No significant change has been noted when the percentage normal morphology is compared with

TABLE 19. SCHEFFE'S POST HOC MULTIPLE COMPARISON BETWEEN GROUPS NORMAL SPERM MORPHOLOGY in %

GROUP OF SUBJECTS	Mean ± SD	GROUPS COMPARED	P- VALUE
CONTROL - A (n = 30)	82.00±9.76	A VS B	0.003 **
		A VS C	0.748 NS
		A VS D	0.001 ***
ALCOHOLICS - B (n = 32)	67.75±17.54	B VS C	0.066 NS
		B VS D	0.992 NS
SMOKERS - C (n = 30)	77.83±13.26	C VS D	0.028 *
ALCOHOLICS WITH SMOKING – D (n = 34)	66.62±16.18		

Fig 25. **NORMAL SPERM MORPHOLOGY in %**



*= p<0.05 = SIGNIFICANT AT 5% LEVEL
**= p<0.01 = SIGNIFICANT AT 1% LEVEL
***= p<0.001 = SIGNIFICANT AT 0.1% LEVEL
NS = NOT SIGNIFICANT

smokers. Similarly when alcoholics are compared with smokers and alcoholics with smoking, it is found that statistically there is no significant change. But when smokers are compared with alcoholics with smoking, it is found that there is a decrease, which is statistically significant at 5 % level.

The average mean head defective sperm count of control group, alcoholics, smokers and alcoholics with smoking are presented in Table 20 and Fig 26 Plate 4,5,6 and 7.

The average mean head defective sperm count of control is 11.30+/-7.81 %, of alcoholics is 18.56+/-11.15 %, of smokers is 13.73+/-10.26 % and of alcoholics with smoking is 18.00+/-7.64 %.

The average mean head defective sperm count in percentage of control is compared with alcoholics and alcoholics with smoking and it has been found that there is an increase which is statistically significant at 5% level. No significant increase has been noted in smokers when compared to control group. When alcoholics compared with smokers and alcoholics with smoking, it is found that statistically there is no significant change. When smokers are compared with alcoholics with smoking, it is found that there is no statistically significant change.

The average mean neck defective sperms count of control group, alcoholics, smokers and alcoholics with smoking are presented in Table 21 and Fig 27 Plate 6,7,8,9,10 and 11.

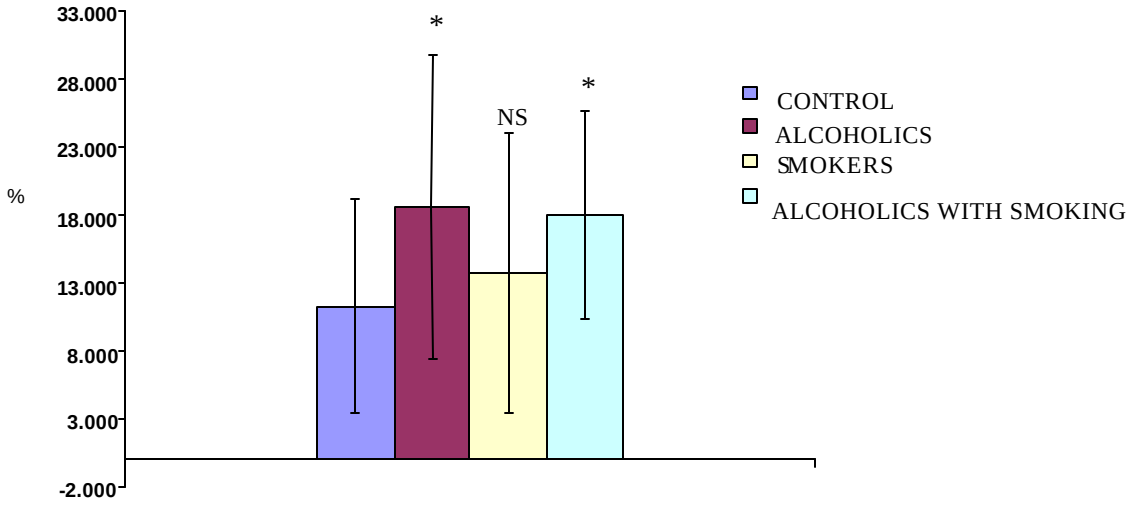
The average mean neck defective sperm count of control is 4.27+/-2.70 %, of alcoholics is 9.03+/-6.33 %, of smokers is 5.80+/-4.02 % and of alcoholics with smoking is 9.06+/-5.40 %.

The average mean neck defective sperm count in percentage of control is compared with alcoholics and alcoholics with smoking and

TABLE 20. SCHEFFE'S POST HOC MULTIPLE COMPARISON BETWEEN GROUPS HEAD DEFECTIVE SPERM in %

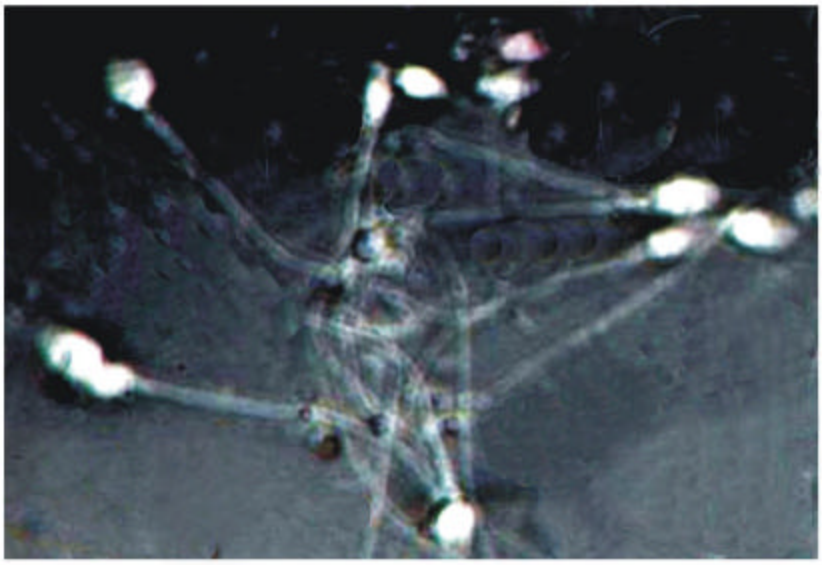
GROUP OF SUBJECTS	Mean ± SD	GROUPS COMPARED	P- VALUE
CONTROL - A (n = 30)	11.30±7.81	A VS B	0.028 *
		A VS C	0.796 NS
		A VS D	0.046 *
ALCOHOLICS - B (n = 32)	18.56±11.15	B VS C	0.251 NS
		B VS D	0.996 NS
SMOKERS - C (n = 30)	13.73±10.26	C VS D	0.347 NS
ALCOHOLICS WITH SMOKING – D (n = 34)	18.00±7.64		

Fig 26. **HEAD DEFECTIVE SPERM in %**



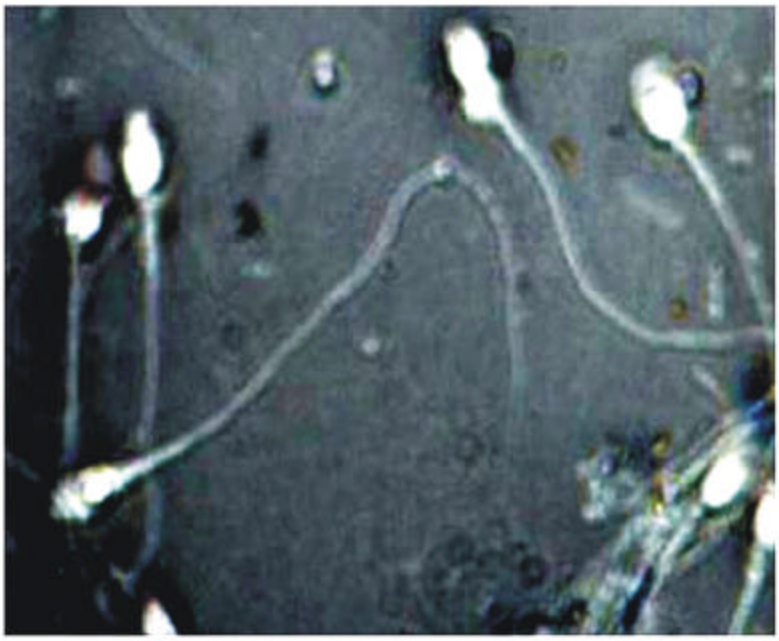
*= p<0.05 = SIGNIFICANT AT 5% LEVEL
NS = NOT SIGNIFICANT

PLATE 5. SPERMS OF SMOKERS

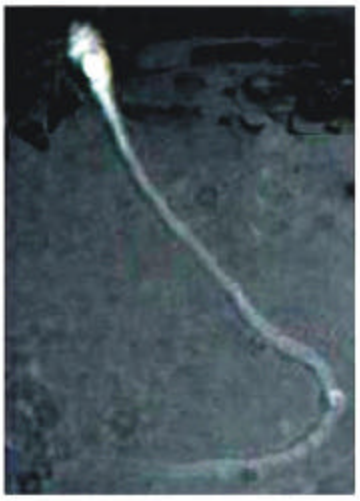


HEAD DEFECTIVE SPERM

PLATE 6. SPERMS OF SMOKERS



HEAD DEFECTIVE SPERM NECK DEFECTIVE SPERM



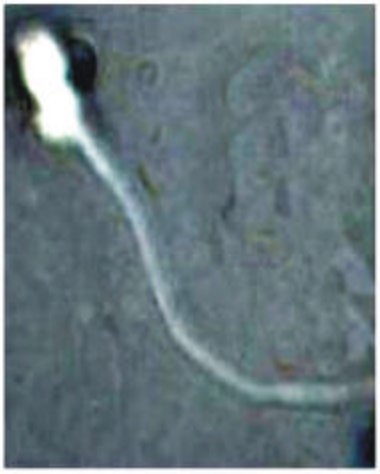
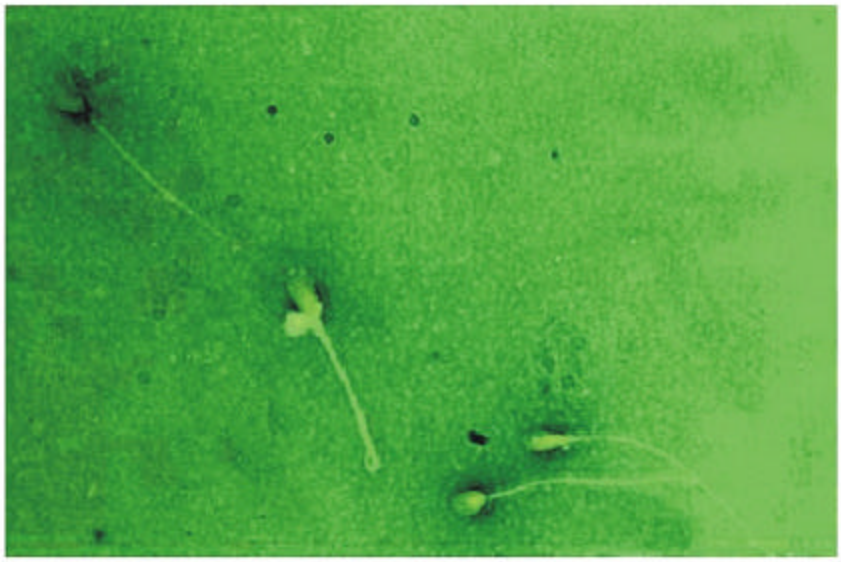
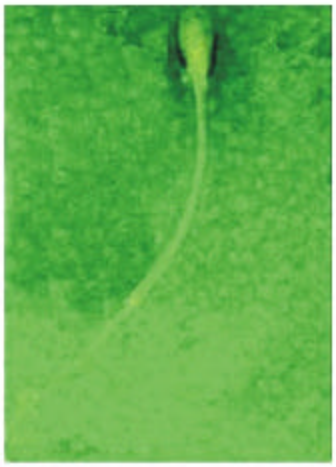


PLATE 7. SPERMS OF ALCOHOLICS



HEAD DEFECTIVE SPERM NECK DEFECTIVE SPERM



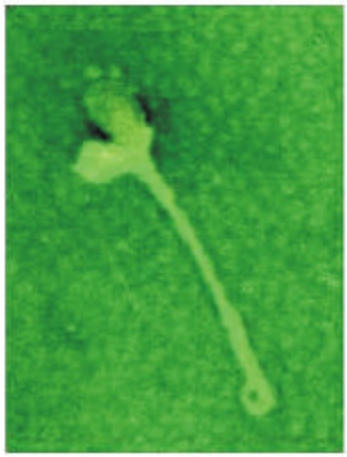
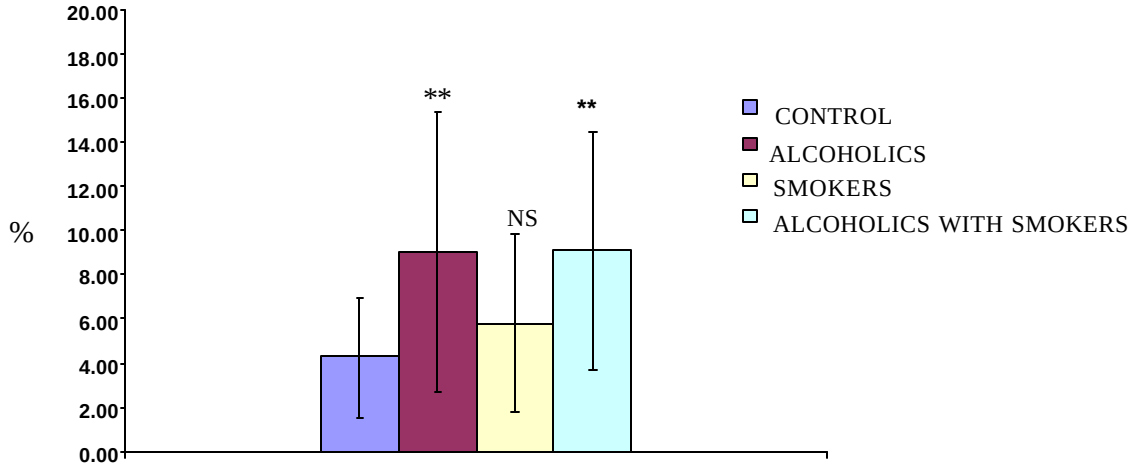


TABLE 21. SCHEFFE'S POST HOC MULTIPLE COMPARISON BETWEEN GROUPS NECK DEFECTIVE SPERM in %

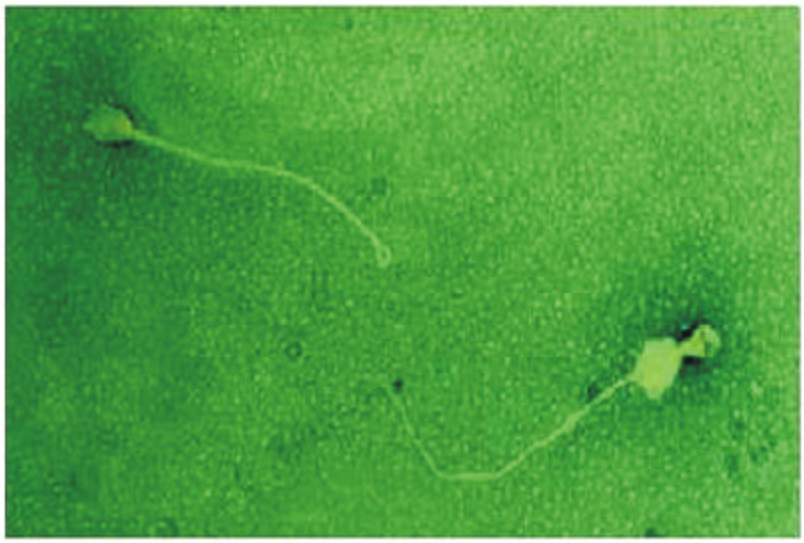
GROUP OF SUBJECTS	Mean ± SD	GROUPS COMPARED	P- VALUE
CONTROL - A (n = 30)	4.27±2.70	A VS B	0.003 **
		A VS C	0.685 NS
		A VS D	0.002 **
ALCOHOLICS - B (n = 32)	9.03±6.33	B VS C	0.083 NS
		B VS D	1.000 NS
SMOKERS - C (n = 30)	5.80±4.02	C VS D	0.072 NS
ALCOHOLICS WITH SMOKING – D (n = 34)	9.06±5.40		

Fig 27. **NECK DEFECTIVE SPERM in %**



**= p<0.01 = SIGNIFICANT AT 1% LEVEL
NS = NOT SIGNIFICANT

PLATE 8. SPERMS OF ALCOHOLICS



NECK DEFECTIVE SPERM

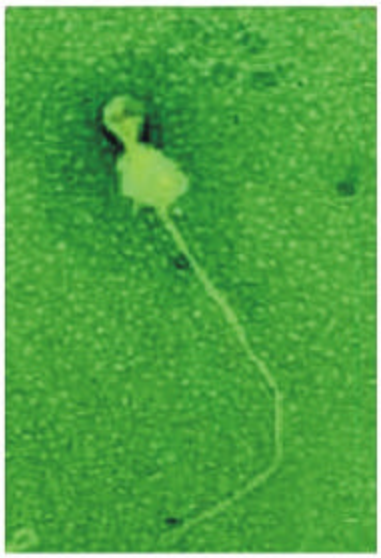
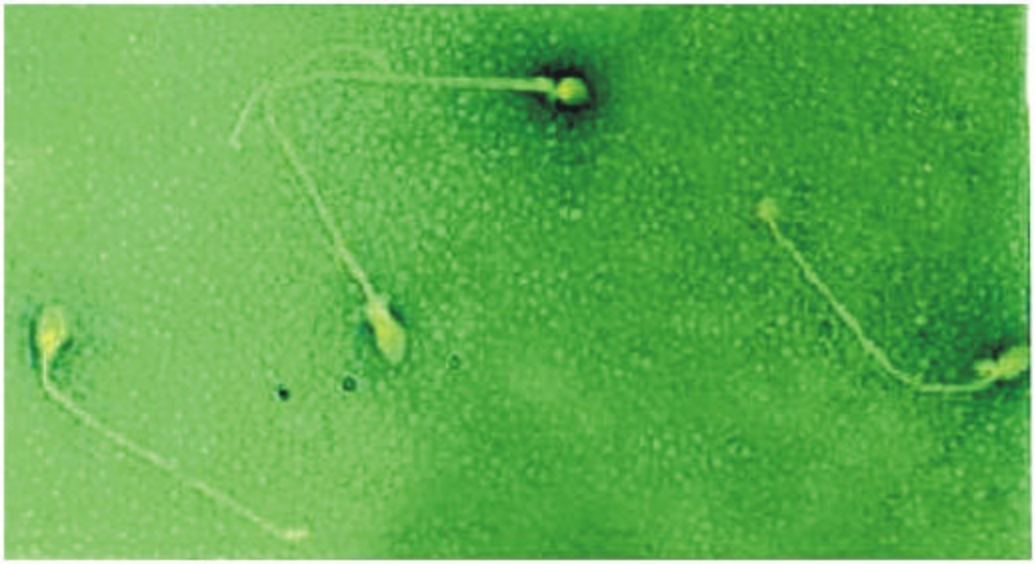


PLATE 9. SPERMS OF ALCOHOLICS



NECK DEFECTIVE SPERM

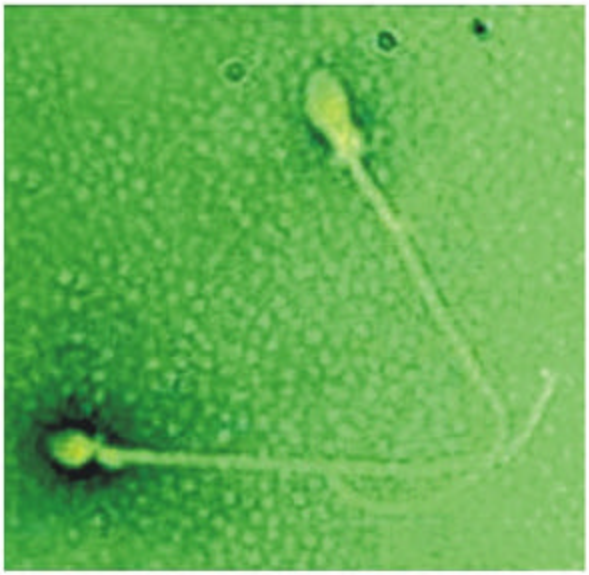
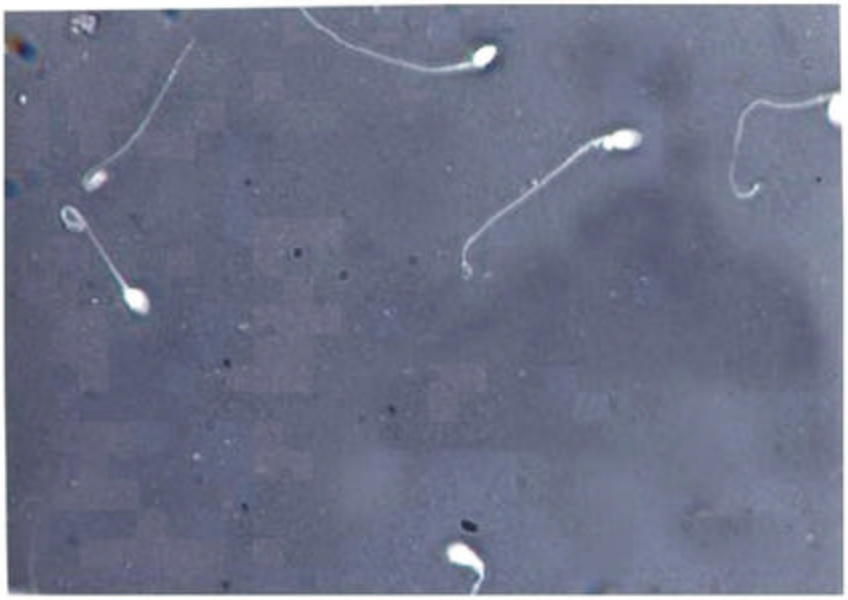


PLATE 10. SPERMS OF SMOKERS



NECK DEFECTIVE SPERM

PLATE 11. SPERMS OF ALCOHOLICS WITH SMOKING

DEAD SPERM

NECK DEFECTIVE SPERM

TAIL DEFECTIVE SPERM

it is found that the increase is statistically significant at 1% level. No significant change has been noted in smokers when compared to control. When alcoholics are compared with smokers and alcoholics with smoking, it is found that statistically there is no significant change. When smokers are compared with alcoholics with smoking, it is found that statistically there is no significant change.

The average mean tail defective sperms levels of control group, alcoholics, smokers and alcoholics with smoking are presented in Table 22 and Fig 28, Plate 11,12 and 13.

The average mean tail defective count levels of control is 2.43+/-1.68 %, of alcoholics is 4.56+/-4.27 %, of smokers is 2.63+/-1.30 % and of alcoholics with smoking is 6.50+/-6.13 %.

The average mean tail defective sperm count in percentage of control is compared with alcoholics, smokers and alcoholics with smoking and it is found that there is no statistical significant change. Alcoholics are compared with smokers and alcoholics with smoking and statistically there is no significance. Smokers compared with alcoholics and alcoholics with smoking are found to have statistically no significant changes.

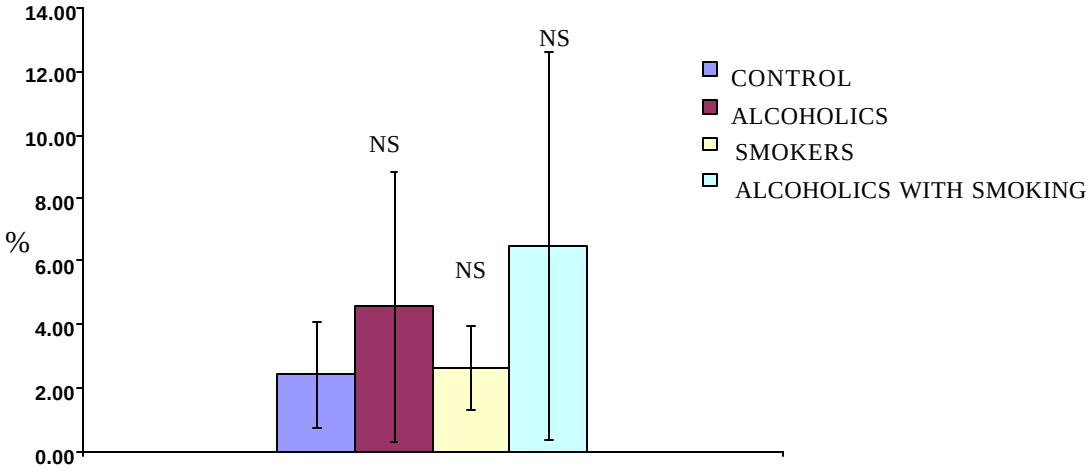
The decreased percentages of normal morphological sperm and increased percentages of head defective sperm and neck defective sperm in the semen of the alcoholics and alcoholics with smoking suggests that the toxic effect of alcohol and smoking reaches the testicular seminiferous tubules and accessory sex organs reti testis, epididymis, vas deferens, seminal vesicle, prostate. At the time of spermiogenesis and transportation of sperms through these organs either the toxic substances of alcohol and smoke act directly on sperm cells or by the toxicity induced secretory alterations in the accessory sex

TABLE 22. SCHEFFE'S POST HOC MULTIPLE COMPARISON

BETWEEN GROUPS
TAIL DEFECTIVE SPERM in %

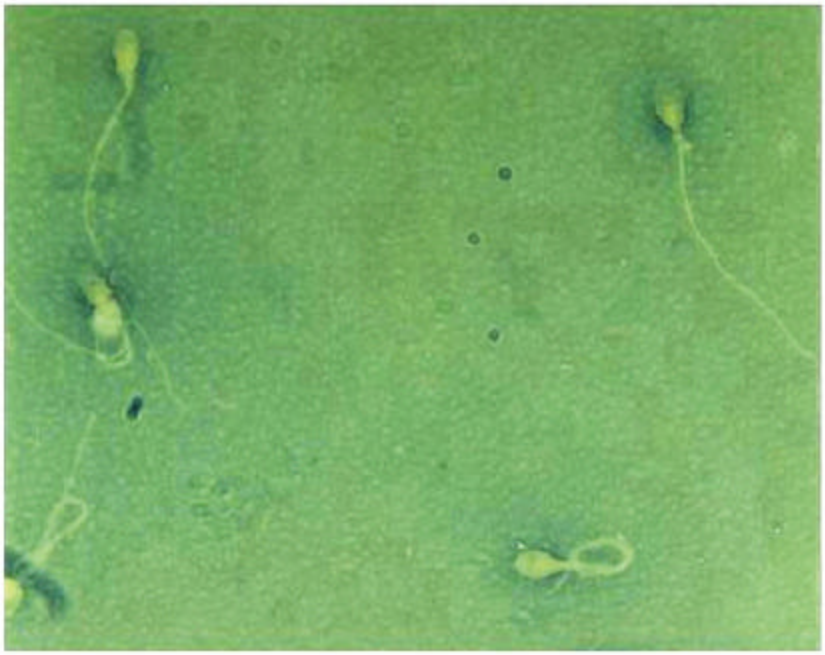
GROUP OF SUBJECTS	Mean ± SD	GROUPS COMPARED	P- VALUE
CONTROL - A (n = 30)	2.43±1.68	A VS B	0.759 NS
		A VS C	1.000 NS
		A VS D	0.226 NS
ALCOHOLICS - B (n = 32)	4.56±4.27	B VS C	0.810 NS
		B VS D	0.792 NS
SMOKERS - C (n = 30)	2.63±1.30	C VS D	0.268 NS
ALCOHOLICS WITH SMOKING – D (n = 34)	6.50±6.13		

Fig 28. **TAIL DEFECTIVE SPERM in %**



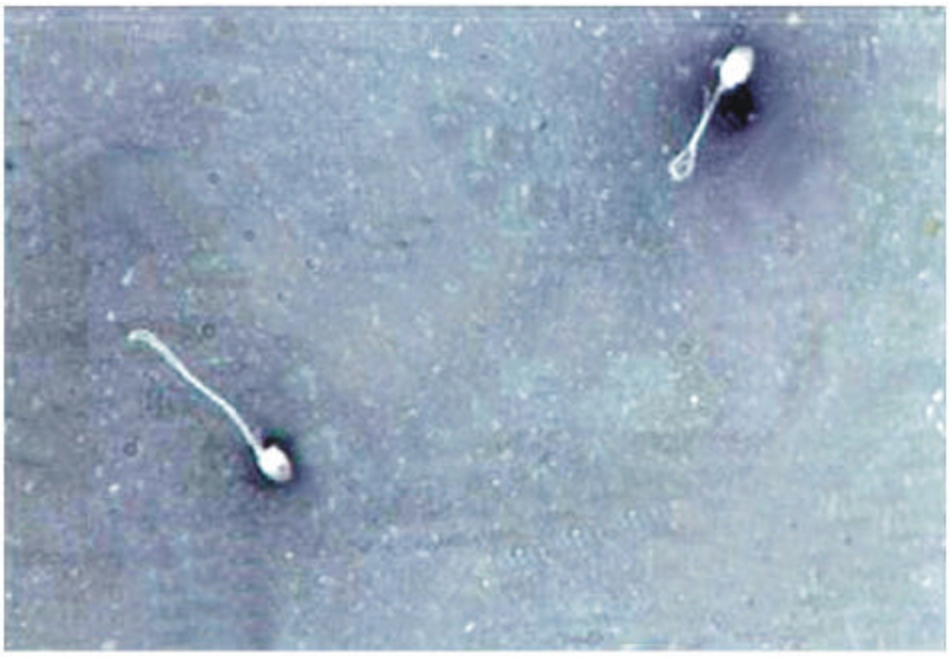
NS = NOT SIGNIFICANT

PLATE 12 . SPERMS OF ALCOHOLICS

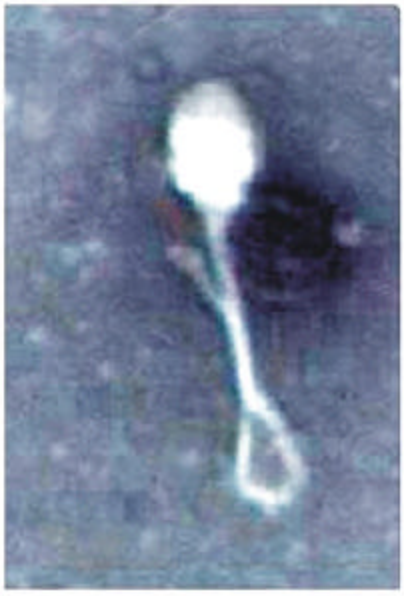


TAIL DEFECTIVE SPERM

PLATE 13. SPERMS OF SMOKERS



TAIL DEFECTIVE SPERMS



organs damage the normal morphology of sperm into head, neck defective forms. The present comparative study results of sperm morphology of alcoholics and smokers suggest that the alcohol is having more toxic effect than the smoking on sperm morphology. In alcohol together with the smoking the toxicity gets increased on the sperm morphology further decreases the normal morphological sperms and increases the head, neck defective morphological sperm numbers. The insignificant change in the semen tail defective sperm percentage in the present study alcoholics, smokers and alcoholics with smoking suggests that the toxicity of alcohol and smoke does not produce any damage on the tail part of the sperm.

The decreased percentage of normal morphological sperm in alcoholics and alcoholics with smoking in this study is supported by the studies of Donnelly et al, (1999), Gomathi et al, (1993) and Nagy et al, (1986) who have found that the alcohol consumption decrease the number of spermatozoa with normal morphology and increase the number of spermatozoa with morphological abnormalities. In addition, Farghali et al, (1993) studied the effect of alcohol on the Sertoli cells in vitro and reported that the toxic effect of ethanol deplete ATP reserves in Sertoli cells and alter intracellular ca^{++} homeostasis and both mechanisms could account for the gonadal toxicity. So the alcohol induced decrease in ATP and ca^{++} homeostasis in Sertoli cells may be the reason for decreased number of normal morphological sperm.

The decrease in the number of sperm with normal morphology of the alcoholics and alcoholics with smoking in the present study is supported by the study of Benoff et al, (2003) who studied the effect of seminal plasma lead levels in the fertility potential of sperm and have reported that cigarette smoking and alcohol consumption increase the blood lead levels in seminal plasma and it may decrease human male

fertility by premature sperm acrosome breakdown and decrease the normal morphological sperm.

The decreased percentages of normal morphological sperm in alcoholics with smoking is also supported by the report of Telisman et al, (2000) who studied the effects of Lead, Cadmium, Zinc and Copper in semen quality of men, and have stated that the cadmium in cigarette smoking and alcohol consumption can increase the number of sperms with abnormal morphology and decrease the sperm motility.

The insignificant change in the percentage of normal morphological sperm in smoking in the present study is supported by the studies of Marshburn et al, (1989), Rantala and Koskimies, (1987), Holzki et al, (1991), Dikshit et al, (1987) and Vogt et al, (1986) who found that smoking did not affect the normal sperm morphology.

The decreased semen quality in smokers and alcoholics with smoking in the present study is supported by Kunzle et al, (2003) who studied the semen quality of male smokers and non smokers and reported that the harmful components of tobacco smoke nicotine and its water soluble metabolite cotinine would pass through the blood-testis barrier and damage the normal sperm in the smokers.

The decreased semen quality in smokers and alcoholics with smoking in the present study is supported by the study of Mak et al, (2000) that the effect of smoking associated with retention of cytoplasm by human spermatozoa and reported that cigarette smoking is associated with the impaired disposal of residual sperm cytoplasm by the testis and/or epididymis of smokers. Possible mechanisms include 1). the direct or indirect effect of cigarette smoke on testicular microcirculation and oxidative balance in the testis and 2). the detection of sperm cytoplasmic droplets increases the semen reactive oxygen

species (ROS) levels and in turn these ROS levels correlate negatively with sperm quality and the fertilizing capacity of sperm.

The decreased semen quality in alcoholics, smokers and alcoholics with smoking may be because of increased levels of reactive oxygen species (ROS) in the semen. In semen the ROS is produced by both the white blood cells (pus cells) and sperm cells (Sigman and Jarow, 2002). Since the present study on white blood cells in semen is normal in all study subject groups (data not shown), the sperm cells themselves may produce more amounts of ROS in the semen of alcoholics and smokers. The ROS is known to induce peroxidative damage to sperm lipid membranes and produce detrimental effect on sperm metabolism, morphology, motility and fertilizing capacity. Supportive to this statement, Saleh et al, (2002) and Trummer et al, (2002) studied the effect of cigarette smoking on semen of men and had reported that there is an increase in semen levels of ROS and sperm DNA damage in infertile cigarette smoking men. So in the present study, the ROS synthesis may be increased in the semen of alcoholics, smokers and alcoholics with smoking. The increased semen ROS levels may damage the sperm DNA and this may be the reason for the present study decreased sperm count, motility, viability, male fertility in alcoholics, smokers and alcoholics with smoking subjects.

Schlorff et al, (1999) studied the effect of ethanol on antioxidant system enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione in rat testis and reported that the ethanol decreases the entire antioxidant system, eliminate the free radicals out of the body and increases the ROS levels in semen. Based on the present study, it is further suggested that alcohol and smoke may decrease the antioxidant system in semen, the decreased antioxidant system may increase the ROS levels in semen and the increased ROS may damage the sperm

and decrease the sperm count, motility, viability and morphology in alcoholics, smokers and alcoholics with smoking.

4.5. EFFECT OF ALCOHOL CONSUMPTION AND SMOKING ON SEXUAL FUNCTION

The control groups, alcoholics, smokers and alcoholics with smoking subject's sexual drive, potency and fertility status obtained in questionnaire were compared between groups with Chi-square statistical test and the following significant changes were noticed.

4.5.1. EFFECT OF ALCOHOL CONSUMPTION AND SMOKING ON MALE SEXUAL DRIVE (LIBIDO)

Normal sexual drive is approximately 6 and more days (times) for a period of 30 days (Broderick and Lue, 2002; Kirby, 2004). In the present study, the subjects who reported decreased sexual drive for less than 5 days in the last 30 days (less than 5 times in 30 days) were listed as decreased sexual drive (libido) subjects. In the present study, almost all the subjects (except 1 out of 30) in the control group reported normal sexual drive (libido) and 47% alcoholics (15 subjects), 10% smokers (3 subjects) and 50% of alcoholics with smoking (17 subjects) reported decreased sexual drive (Table. 23).

The sexual drive status of alcoholics and alcoholics with smoking and smokers are compared with control and is found that the decrease in sexual drive is statistically significant at 1% level except in smokers. When alcoholics are compared with smokers, it is found that the decrease in sexual drive is statistically significant at 1% level. When alcoholics with smoking are compared with smokers, it is found that the decrease in sexual drive is statistically significant at 1% level.

TABLE: 23. SEXUAL DRIVE (LIBIDO) STATUS - MULTIPLE COMPARISON BETWEEN GROUPS (CHI-SQUARE - TEST)

GROUP	NUMBERS OF DECREASED SEXUAL DRIVES (OUT OF)	SEXUAL DRIVE DECREASE in %	CHI-SQUARE VALUE		TESTOSTERONE OF DECREASED SEXUAL DRIVE'S. MEAN±SD (OVERALL GROUP MEAN±SD) in ng/ml
			BETWEEN GROUP	p - VALUE	
CONTROL (A)	1 (30)	3	-	-	3.84 (5.89 ± 2.07)
ALCOHOLICS (B)	15 (32)	47	A vs B	0.01 **	3.55 ± 0.85 (4.49 ± 1.19)
SMOKERS (C)	3 (30)	10	A vs C	NS	3.61 ± 0.55 (6.47 ± 1.95)
			B vs C	0.01 **	
ALCOHOLICS WITH SMOKING (D)	17 (34)	50	A vs D	0.01 **	3.28 ± 0.38 (4.33 ± 0.90)
			B vs D	NS	
			C vs D	0.01 **	

**=p = < 0.01 = Significant at 1 % level

NS = Not Significant

TABLE: 24. ERECTILE STATUS - MULTIPLE COMPARISON BETWEEN GROUPS (CHI-SQUARE - TEST)

GROUP	NUMBERS OF DECREASED POTENTS (OUT OF)	POTENCY DECREASE in %	CHI-SQUARE VALUE		TESTOSTERONE OF DECREASED POTENT'S. MEAN±SD (OVERALL GROUP MEAN±SD) in ng/ml
			BETWEEN GROUP	p - VALUE	
CONTROL (A)	2 (30)	7	-	-	4.09 ± 0.70 (5.89 ± 2.07)
ALCOHOLICS (B)	23 (32)	72	A vs B	0.01 **	4.07 ± 1.02 (4.49 ± 1.19)
SMOKERS (C)	6 (30)	20	A vs C	NS	4.00 ± 0.73 (6.47 ± 1.95)
			B vs C	0.01 **	
ALCOHOLICS WITH SMOKING (D)	28 (34)	82	A vs D	0.01 **	4.04 ± 0.86 (4.33 ± 0.90)
			B vs D	NS	
			C vs D	0.01 **	

**=p = < 0.01 = Significant at 1 % level

NS = Not Significant

The mean testosterone levels in blood in the decreased libido control was 3.84 ng/ml, alcoholics was 3.55 ± 0.85 ng/ml, smokers 3.28 ± 0.38 ng/ml and alcoholics with smoking 3.61 ± 0.55 ng/ml. Compared with controls, alcoholics, smokers and alcoholics with smoking individual group mean blood levels of testosterone, the testosterone of the decreased libido subjects were found to be further decreased (Table. 23).

4.5.2. EFFECT OF ALCOHOL CONSUMPTION AND SMOKING ON ERECTILE (POTENCY) FUNCTION

The normal sexual potency is the maintenance of adequate penis erection at the time of intercourse or masturbation. The normal potency varies from 3 and more minutes (Broderick and Lue, 2002; Kirby, 2004). In the present study, the subjects who reported penile erection for less than two minutes at the time of intercourse are treated and listed as decreased potency subjects.

In the present study, 7% controls (2 subjects) are reported to have decreased sexual potency, and 72% alcoholics (23 subjects), 20% smokers (6 subjects) and 82% alcoholics with smoking (28 subjects) were reported to have decreased sexual potency (Table. 24).

The sexual potency of control group is compared with alcoholics group and alcoholics with smoking group. It is found that the decrease in sexual potency in both the group is statistically significant at 1% level. No significant decrease in sexual potency is noted in smokers when compared with control group. When the sexual potency in smoking group is compared with alcoholics group and alcoholics with smoking, it is noted that the decrease in sexual potency in both alcoholics group and alcoholics with smoking are statistically significant at 1% level.

The mean testosterone in blood of decreased potent control was 4.09 ± 0.70 ng/ml, alcoholics 4.07 ± 1.02 ng/ml, smokers 4.00 ± 0.73 ng/ml and alcoholics with smoking 4.04 ± 0.86 ng/ml. The decreased potent alcoholics, smokers and alcoholics with smoking subjects blood level of testosterone is further decreased when compare to their group means (Table. 24).

The decreased libido and potency of the alcoholics, smokers and alcoholics with smoking in the present study may be due to decreased blood testosterone levels. Alcohol has more association than the smoking with potency. Alcohol together with smoking makes the toxic effect increasing on libido and potency.

Sexual disorders have been frequently reported in chronic alcoholics. The present study is supported by the studies of Mulligan et al, (1988), Rosen, (1991) and Gumus et al, (1998) which showed that high levels of blood alcohol cause reduced sexual stimulation, inability to enjoy orgasm and retarded ejaculation. Also it is supported by the study of Masters and Johnson, (1970) who noted that in 35 men out of 213 men with secondary impotence, the erectile disorder occurred as a direct result of alcohol intake. Whalley, (1978) reported that 54% of hospitalized alcoholic men and 24 % of healthy controls had erectile impotence. Similar to the present study on the decreased sexual drive in alcoholics and alcoholics with smoking, Jensen, (1984) reported that 63% of married alcoholic men and 10 % of controls had sexual dysfunctions due to lack of sexual drive.

The decreased sexual drive and decreased potency in alcoholics and alcoholics with smoking in the present study was supported by, Ida et al, (1992) that they have studied the effects of repeated alcohol ingestion on hypothalamic-pituitary-gonadal (HPG) functioning in normal males and reported that the excessive alcohol consumption over a long

period may produce more toxic effects on the functioning of the HPG system. So the impairment in gonadal function of the alcoholics and alcoholics with smoking may be the reason for the impaired potency and libido in the alcoholics, and alcoholics with smoking. Ruusa and Bergman, (1996) studied the effect of alcohol withdrawal on sex hormones and reported that the chronic alcohol intake is known to cause hypogonadism including gynecomastia, impotence and testicular atrophy and found that the underlying mechanism involved in these disturbance is alcohol induced suppression of testicular production of testosterone. So the decreased testosterone level in blood may be the reason for the decreased potency in alcoholics and alcoholics with smoking. Similarly, Gonzalez-Reimers et al, (1994) reported the chronic direct effect of ethanol itself on the testis impairing testosterone synthesis and spermatogenesis.

No significant change in the prevalence of potency in smokers in the present study is contradicted by Yardimci et al, (1997), who studied the long-term effect of cigarette-smoke exposure on plasma reproductive hormones and reported an increased prevalence of impotence in smokers. Similarly, Kane and Kumar, (2004) and Schuckit, (2005) reported that the male chronic cigarette smokers have developed a decreased potency.

4.5.3. EFFECT OF ALCOHOL CONSUMPTION AND SMOKING ON MALE FERTILITY

In the present study, all the control's female partners conceived within 2 years of marriage. Twenty-eight percentages of female partners of the alcoholics (9 subjects), 13% of smokers (4 subjects) and 47% (16 subjects) of alcoholics with smoking did not conceive even after 2 years of marriage (Table. 25).

TABLE: 25. FERTILITY STATUS - MULTIPLE COMPARISON BETWEEN GROUPS (CHI-SQUARE - TEST)

GROUP	NUMBERS OF FEMALE PARTNERS NOT CONCEIVED AFTER 2 YEARS OF MARRIAGE (OUT OF)	MALE INFERTILITY in %	CHI-SQUARE VALUE		SEMEN SPERM COUNT OF INFERTILE MALE. MEAN ± SD (OVERALL GROUP MEAN ± SD) in million/ml
			BETWEEN GROUP	p-VALUE	
CONTROL (A)	0 (30)	0	-	-	-
ALCOHOLICS (B)	9 (32)	28	A vs B	0.01 **	17.53 ± 14.09 (53.41 ± 51.55)
SMOKERS (C)	4 (30)	13	A vs C	0.05 *	19.75 ± 18.50 (88.33 ± 52.68)
			B vs C	0.05 *	
ALCOHOLICS WITH SMOKING (D)	16 (34)	47	A vs D	0.01 **	10.56 ± 6.74 (50.65 ± 37.92)
			B vs D	0.01 **	
			C vs D	0.01 **	

**=p = < 0.01 = Significant at 1 % level

**=p = < 0.05 = Significant at 5 % level

NS = Not Significant

The fertility status of control is compared with alcoholics, smokers and alcoholics with smoking. It is found that the decrease in fertility status is statistically significant at 1% level in alcoholics and alcoholics with smoking and in smokers statistically significant at 5% level. No significant decrease in fertility status is found when alcoholics group is compared with smokers. When alcoholics with smoking group is compared with smokers group and alcoholics group, it is found that the decrease in fertility status in alcoholics with smoking is statistically significant at 1% level.

The mean semen sperm count of infertile alcoholics is 17.53 ± 14.09 millions per milliliter, of smokers is 19.75 ± 18.50 millions per milliliter and of alcoholics with smoking is 10.56 ± 6.74 millions per milliliter. The infertile male alcoholics, smokers and alcoholics with smoking group mean semen sperm count is decreased when compared to their group mean sperm counts. According to WHO reference, the sperm count less than 20 million/ml has more chances of male infertility (Farah and Blackwell, 2001). In the present study, it is evident that, alcohol, smoke and alcohol with smoking decreases the sperm count and increases the risk of male infertility. Chronic alcoholism is having more risk than smoking in the male fertility. Chronic alcoholism together with smoking further increases the risk of male infertility.

Similarly, Farah and Blackwell, (2001) and Sigman and Jarow, (2002) have reported that the heavy alcohol consumption (abuse) decreases sperm counts and testosterone levels in men. In addition, decreased sperm concentration as well as abnormal morphology and decreased numbers of motile sperms has been reported in men who smoke heavily. It is evident from the results of the present study and from literature that the chronic alcohol consumption and smoking decrease the sperm count and increases the risk of male infertility.

The decreased male fertility in smokers, alcoholics with smoking may be because of reduced sperm concentration, motility, morphology and function. This statement is supported by the report of Practice Committee of the ASRM (American Society of Reproductive Medicine), (2004), that the zona free hamster egg penetration test reflects the reduced ability of sperm to successfully fertilize a human oocyte, the available evidence suggests that smoking may have adverse effects on sperm function and male fertility.

The present study was supported by the study of Villalta et al, (1997) that they have studied the testicular function in asymptomatic alcoholics and reported that the alcohol exerts toxic effect on testicular function, decreases the sperm count, motility, morphology and a primary testicular insufficiency and compensatory increase of FSH and LH secretion and suggested that alcohol misusers may develop a situation of primary hypogonadism.

The decreased testosterone and increased LH and FSH levels in blood and decreased sperm count, motility, viability and morphology in the alcoholics and alcoholics with smoking were observed in the present study. The above results suggest that alcohol toxicity induces the primary hypogonadism. The alcohol toxicity induces alterations in the Leydig cell steroidogenesis pathway. The results and the literature reveal that alcohol decreases the blood levels of progesterone and testosterone. The decrease may be due to impaired activities of 3β-hydroxysteroid dehydrogenase, 17-hydroxylase, 17,20-desmolase and 17-ketoreductase enzymes, the Leydig cell StAR protein, testicular EOP and other autocrine and paracrine regulatory proteins. Further, alcohol might enhance the metabolic clearance of testosterone by way of activating aromatase enzyme, which converts testosterone into estradiol. The decreased testosterone as a feedback regulation increases the blood LH levels in alcoholics. The decreased testosterone

along with the alcohol toxicity alters the Sertoli cell and germ cell autocrine and paracrine functions. The increased FSH level in blood demonstrates the suppression of Sertoli cell and germ cell function, decreased spermatogenesis and spermiogenesis in testis. As a result, the alcohol toxicity decreases the semen volume, sperm count, motility, viability and morphology.

Smoking does not alter the blood levels of testosterone, LH, FSH and estradiol. It reveals that the smoke toxicity does not affect the functions of Leydig cells and Sertoli cells, but the smoke toxicity acts directly on the seminiferous tubule germ cells and decreases the seminal sperm count, motility and viability.

Along with decreased blood testosterone level, alcohol and smoke toxicity might have decreased the ATP, Ca^{++} homeostasis in Sertoli cells, decreased antioxidant levels in semen and increased cadmium, lead, ROS levels in semen. The above biological alterations might have decreased the semen quality in alcoholics, smokers and alcoholics with smoking.

The alcoholic's and smoker's blood levels of testosterone, LH, FSH, progesterone, prolactin and estradiol and semen parameters, semen volume, sperm count, motility, viability and morphology results reveal that alcohol is having more toxic effect than smoking, alcohol together with smoking further increases the toxicity in male reproductive function. The low toxic smoke itself decreases the semen quality quite considerably and this suggests that the germ cells are highly sensitive to the toxic substances like smoke and alcohol than any others cells in the testis.

5.0 SUMMARY AND CONCLUSIONS

The effect of chronic alcoholism, chronic smoking and chronic alcoholism with smoking on pituitary, testicular hormones and semen pertaining to male reproductive functions were studied in human beings. The male population who were included in the study were categorized into four groups: Group I. Control (non alcoholics and non smokers), Group II. Alcoholics, Group III. Smokers and Group IV. Alcoholics with smoking.

The Questionnaire specially prepared for this study were carefully analyzed. Subjects falling under the age group of 20 to 50 years, married, who are free from chronic diseases were selected. Based on the questionnaire, the inclusion and exclusion criteria were fixed and the eligible subjects were included in the present study. All the investigations planned in the study were done before alcohol de-addiction treatment.

Alcohol and smoking are two different pleasure-seeking habits. Initially people start these for fun but slowly most of the people become addicted to these habits.

Controls and smokers were selected from 6000 people registered in Health registry. About 1700 de-addiction center alcoholic persons were screened and selected for alcoholics and alcoholics with smoking groups.

In all the subjects, haematological parameters such as haemoglobin, total WBC count, differential WBC count, platelet count, total RBC count, MCV, MCH, MCHC, PCV were estimated. Biochemical parameters such as glucose, creatinine, total bilirubin, total protein, albumin, globulin, albumin / globulin ratio, SGPT,

alkaline phosphatase, gamma GT, cholesterol, triglycerides, HDL-cholesterol, LDL – cholesterol, VLDL-cholesterol and phospholipids were estimated in blood.

Functions of liver and kidneys, lipid profile, glucose and haematological parameters were found statistically normal. Along with above results clinical examination was carried out by a physician showed that all the subjects were free from diseases.

The inclusion criteria such as age limit, volume of alcohol consumption, duration of alcohol consumed in a week, number of years of consumption of alcohol in a row and number of cigarettes smoked in a day, libido, potency and fertility status data were tabulated and statistically analyzed.

Fertility hormones FSH, LH, testosterone, progesterone, estradiol and prolactin were estimated in blood.

Volume of semen, sperm count, sperm motility (sperm rapid progressive motility, slow or sluggish motility, non progressive motility and immotility), viability (alive sperm and dead sperm) and sperm morphology (normal morphology, head defective morphology, neck defective morphology and tail defective morphology) were analysed. The semen viscosity, pH, color, appearance and fructose were also analysed.

The testosterone and progesterone levels in the blood were found to be significantly decreased in alcoholics and alcoholics with smoking. The levels of FSH, LH, and estrodiol in the blood were significantly increased in alcoholics and alcoholics with smoking. Prolactin level in the blood was not significantly altered in alcoholics

and alcoholics with smoking. Smoking alone did not alter the levels of the above hormones in blood.

The volume of semen, sperm count, motility, viability and morphology were decreased in alcoholics and alcoholics with smoking. In smokers, the volume of semen and the morphology of sperm were not affected, but the sperm count, motility and viability were decreased. Seminal pH, viscosity, appearance, color and fructose were found to be normal in all the groups studied.

In the present study, on alcoholics, the testicular hormonal and seminal impairments were observed. Alcohol affected both testicular Leydig cells and seminiferous tubule functions. In Leydig cells, alcohol decreased the synthesis of testosterone, progesterone hormones and increased the synthesis of estradiol, which might be by way of altering the functions of steroidogenesis enzymes. Because of the suppression of testosterone, the pituitary feedback regulation was affected and by the compensatory mechanism, the pituitary LH was increased.

At seminiferous tubular level, the toxicity of alcohol might acted on Sertoli cells, germ cells, accessory sex organs and decreased the process of spermatogenesis and spermiogenesis, which might have led to decreased semen volume, sperm count, motility, viability and morphology. This variation was due to decreased testosterone level in blood and the entry of alcohol directly to seminiferous tubules. Due to the loss of seminiferous tubular function, the pituitary FSH level was increased. This elevated FSH levels in alcoholics demonstrated that the alcohol was having direct toxic effects at seminiferous tubule Sertoli cells and germ cells. The increased level of estradiol revealed that the metabolic clearance (aromatization) rate of testosterone was

accelerated in alcoholics. Along with low sperm count, decreased testosterone level and increased estradiol level in blood, alcohol decreased the libido, potency and led to male infertility.

In the present study, chronic cigarette smoking, did not produce any alteration in the levels of testosterone, progesterone, estradiol, FSH, LH and prolactin in the blood. Smoking reduced the semen quality to a certain extent.

Smoking might have acted on germ cells and accessory sex organs directly and decreased the process of spermatogenesis, spermiogenesis and led to decreased total sperm concentration, viability and motility. The semen volume and morphology of the sperm were not affected by smoking.

An unaltered levels of testosterone in smokers suggested that the smoking acted only at seminiferous tubules and it was not at Leydig cells. Chronic smoking was having a definite toxic effect on male reproduction by altering the sperm count, motility, and viability. Because of this, male infertility was possible to a certain limit. Sexual functions, libido and potency were not affected much by smoking alone because testosterone, the male reproductive hormone was not affected.

In this study, it is found that chronic alcoholism together with chronic smoking increased the severity of the reproductive illnesses compared to alcoholism or smoking alone. Due to the toxicity of alcohol together with smoke on the steroidogenesis pathway, the synthesis of testosterone and progesterone decreased and estradiol was found to be elevated significantly. The toxicity of alcohol together with smoking acted at the level of testicular Leydig cell steroidogenic

enzymes, StAR protein, autocrine and paracrine regulations and decreased the progesterone and testosterone levels in blood. Elevated levels of FSH and LH were noted in alcoholics with smoking. These elevations were due to the decreased negative feedback regulations on pituitary.

The toxicity of alcohol together with smoking acted at seminiferous tubular Sertoli cells, germ cells and accessory sex organs, decreased the semen quality severely along with decreased testosterone. Semen sperm count was significantly suppressed, the progressive motile sperms were significantly reduced, sluggish motile, non-progressive motile and immotile sperms were significantly increased in alcoholics with smoking. In the viability study, alcoholics with smoking have a significant negative effect on alive sperms. Dead sperms were found to be significantly increased. Morphology of sperms was significantly altered by alcohol with smoking. Percentage of normal sperm morphology decreased and abnormal forms like defective head, neck and tail were increased in alcoholics with smoking.

In the libido and potency study, smoking did not alter the libido and potency significantly. Alcohol and alcohol with smoking decreased the libido and potency significantly. This may be due to the decreased level of testosterone in blood. In the fertility status, smoking had less effect, followed by alcohol, which was having more effect and alcohol together with smoking the effect was very severe. Decreased blood testosterone and seminal parameters, decreased libido and potency might be the reason for the decreased fertility in alcoholics and alcoholics with smoking. In smokers the decreased sperm quality alone decreased the male fertility to a certain extent.

It is concluded that cigarette smoking decreased the male reproductive function in unique direction by acting at testicular germ cells, accessory sex organs and decreased the semen quality alone. Alcohol has decreased the reproductive function bidirectionally, one by acting at testicular Leydig cells, decreasing the male reproductive hormone testosterone and the other by acting at seminiferous tubular Sertoli cells, germ cells and accessory sex organs and decreasing the semen quality. Consumption of alcohol and smoking together have severely suppressed the male reproductive function by further decreasing testosterone and semen quality.

Therefore, by reviewing the effects of alcohol, smoking and alcohol with smoking, it is concluded that smoking has less toxic effect by impairing only the semen quality and alcohol abuse is having more toxic effect than smoking is evidenced by decrease in blood testosterone level and semen quality. Alcohol together with smoking the toxicity on male reproductive function is increased further. All the possible toxic actions of alcohol and smoke are at testicular level than at the central pituitary level.

Further to know about the molecular mechanisms involved in the present study of decreased levels of testosterone, progesterone, increased levels of LH, FSH, estradiol in blood, decreased semen quality in alcoholics and alcoholics with smoking and the decreased semen quality alone in smokers, additional molecular level study needs to be carried out. To know the status of restoration of testosterone, progesterone, estradiol, LH, FSH levels in blood and the quality of semen in alcoholics, smokers and alcoholics with smoking, a further study on the withdrawal of the habits of chronic alcoholism, chronic smoking and chronic alcoholism with smoking can be carried out.

This research has proved beyond doubt that chronic alcohol consumption and chronic smoking have a detrimental effect on the male reproductive hormones and also on the quality of semen, which, in turn, will make people impotent and sterile.

Hence, men are advised to refrain from these addictions if they want to procreate and also to lead a normal sexual life.

6. BIBLIOGRAPHY

Adams M L, Cicero T J, (1991). Effects of alcohol on beta-endorphin and reproductive hormones in the male rat. Alcohol Clin Exp Res. 15:685-692.

Adams M L, Little P J, Bell R D, Cicero T J, (1991). Alcohol affects rat testicular interstitial fluid volume and testicular secretion of testosterone and beta-endorphin. J Pharmacol Exp Ther. 258:1008-1014.

Adams M L, Meyer E R, Cicero T J, (1997). Interactions between alcohol- and opioid-induced suppression of rat testicular steroidogenesis in vivo. Alcohol Clin Exp Res. 21:684-690.

Adams M L, Meyer E R, Sewing B N, Cicero T J, (1994). Effect of Nitric-Oxide related agents on alcohol narcosis. Alcohol Clin Exp Res. 18:969-975.

Adler R A, (1992). Clinically important effects of alcohol on endocrine function. Clinical review 33. J Clin Endocrinol Metab. 74:957-960.

Akane A, Fukushima S, Shiono H, Fukui Y, (1988). Effects of ethanol on testicular steroidogenesis in the rat. Alcohol Alcohol. 23:203-209.

Amann J M, Howards S S, (1980). Daily spermatozoal production and epididymal spermatozoal reserves of the human male. J Urol. 124:211-215.

Anakwe O O, Murphy P R, Moyer W H, (1985). Characterization of beta - adrenergic binding sites on rodent Leydig cells. Biol Reprod. 33:815-826.

Anderson R A, Berryman S H, Philips J E, Feathergill K A, Zaneweld J L, Russel L D, (1989). Biochemical and structural evidence for ethanol-induced impairment of testicular development:Apparent lack of Leydig-cell involvement. Toxicology and Applied Pharmacology. 100:62-85.

Anderson R A, Willis B R, Oswald C, Zaneweld J L, (1983). Ethanol-induced male infertility:Impairment of spermatozoa. J Pharmacol Exp Ther. 225:479-486.

Anderson A N, Semczuk M, Tabor A, (1984). Prolactin and pituitary-gonadal function in cigarette smoking infertile patients. Andrologia. 16:391-396.

Armstrong B G, McDonald A D, Sloan B A, (1992). Cigarette, alcohol, and coffee consumption and spontaneous abortion. Am J Public Health. 82:85-87.

Aron D C, Findling J W, Blake Tyrrell J, (2001). Hypothalamus and pituitary. In: Basic and Clinical Endocrinology. Greenspan F S, Gardner D G (Edrs), 6th Ed, New York, Lange Medical Publications, pp100-162

Arora M, Aghi M, Reddy K S, (2001). Report on the Results of the Global Youth Tobacco Survey in New Delhi India - 2001. Undertaken by: HRIDAY (Health Related Information Dissemination Amongst Youth), New Delhi, India, Supported by: CDC- USA, WHO- Geneva, TIFR-Mumbai, 1-9.

ASRM (The Practice Committee of the American Society of Reproductive Medicine) (2004). Smoking and infertility. Fertil Steril. 81:1181-1186.

Bahnsen M, Gluud C, Johnsen S G, Bennett P, Svenstrup S, Micic S, Dietrichson O, Svendsen L B, Brodthagen U A, (1981). Pituitary-testicular function in patients with alcoholic cirrhosis of the liver. Eur J Clin Invest. 11:473-479.

Baker H W G, (2001). Male Infertility. In: Endocrinology. DeGroot L J, Jameson J L, (Edrs), 4th Ed, Vol.3, Philadelphia, W B Saunders, pp2308-2328.

Bannister P, Losowsky M S, (1987). Ethanol and hypogonadism. Alcohol Alcohol. 22:213-217.

Bardin C W, (1986). Endocrine regulation of the reproductive system. In: Pituitary-testicular axis. Yen S S C, Jaffe R B (Edrs), 2nd Ed, Philadelphia, W B Saunders, pp177-199.

Barrett-Connor E, Khaw K T, (1987). Cigarette smoking and increased endogenous estrogen levels in men. Am J Epidemiol. 129:187-192.

Benoff S, Centola G M, Millan C, Napolitano B, Marmar J L, Hurley I R, (2003). Increased seminal plasma lead levels adversely affect the fertility potential of sperm in IVF. Hum Reprod. 18:374-383.

Benoff S, Cooper G W, Hurley I, (1994). The effect of calcium ion channel blockers on sperm fertilization potential. Fertil Steril. 62:606-617.

Benoff S, Jacob A, Hurley I R, (2000). Environmental metals and male infertility. Hum Reprod Update. 6:107-121.

Benvold E, Gottlieb C, Bygdeman M, (1991). Depressed semen quality in Swedish men from barren coulples:a study over three decades. Arch Androl. 26:189-194.

Bergmeyer H U, Horder M, Rej R, (1985). Approved recommendation (1958) on IFCC methods for the measurement of catalytic concentration of enzymes. Part 3. IFCC Method for alanine aminotransferase. J Clin Chem Clin Biochem. 24:481-489.

Bertello P, Agrimonti F, Gurioli L, Frairia R, Fornaro D, Angeli A, (1982). Circadian patterns of plasma cortisol and testosterone in chronic male alcoholics. Alcohol Clin Exp Res. 6:475-481.

Blackburn G F, Shah H P, Kenten J H, Leland J, Kamin R A, Link J, (1991). Electrochemiluminescence detection for development of immunoassays and DNA probe assays for clinical diagnostics. Clin Chem. 37:1534-1539.

Braun R E, (2001). Packing paternal chromosomes with protamine. Nature Genet. 28:10-12.

Braunstein G D, (2001). Testes. In: Basic and Clinical Endocrinology. Greenspan F S, Gardner D G (Edrs), 6th Ed, New York, Lange Medical Publications, pp422-452.

Briggs M H, (1973). Cigarette smoking and infertility in men. Med J Aust. 1:616-617.

Broderick G A, Lue T F, (2002). Evaluation and nonsurgical management of erectile dysfunction and priapism. In: Campbell's Urology. Walsh P C, Retik A B, Vaughan E D, Wein A J (Edrs), 8th Ed, Vol.2, Philadelphia, W B Saunders, pp1619-1671.

Brzek A, (1987). Alcohol and male fertility. Andrologia. 19:32-36.

Burger H, (2001). Male reproduction. In: Endocrinology. DeGroot L J, Jameson J L (Edrs), 4th Ed, Vol.3, Philadelphia, W B Saunders, pp2209-2243.

Burns G, Almeida O F X, Passarelli F, Herz A, (1989). A two-step mechanism by which corticotropin-releasing hormone releases hypothalamic beta-endorphIn: The role of vasopressin and G-proteins. Endocrinology. 125:1365-1372.

Burns M D, (2005). Nicotine addiction. In: Harrison's Principles of Internal Medicine. Kasper D L, Fauci A S, Longo D L, Braunwald E, Hauser S L, Jameson J L (Edrs), 16th Ed, Vol.2, New York, McGraw-Hill International, pp2573- 2576.

Cailleau J, Vermeire S, Verhoeven C, (1990). Independent control of the production of insulin-like growth factor-1 and its binding protein by cultured testicular cells. Mol Cell Endocrinol. 69:79-85.

Carlsen E, Giwereman A, Keiding M, Skakkebaek N E, (1992). Evidence for decreasing quality of semen during the past 50 years. BMJ. 305:609-613.

Castilla-Garcia A, Santolaria-Fernandez F J, Gonzalez-Reimers C E, Batista-Lopez N, Gonzalez-Garcia C, Jorge-Hernandez J A, Hernandez-Nieto L, (1987). Alcohol-induced hypogonadism:Reversal after ethanol withdrawal. Drug Alcohol Depend. 20:255-260.

Catt K J, Dufau M L, (1991). Gonadotropic Hormones:Biosynthesis, Secretion, Receptors and Actions. In: Reproductive Endocrinology. Yen S S C, Jaffe R B (Edrs), 3rd Ed, Philadelphia, W B Saunders, pp105-151.

Celis R D, Feria-Velasco A, Gonzalez-Unzaga M, Torres-Calleja J, Pedron-Nuevo N, (2000). Semen quality of workers occupationally exposed to hydrocarbons. Fertil Steril. 73:221-228.

Chatterjea M N, Shinde R (2002). Hormones-Chemistry, Mechanism of Action and Metabolic Role. In: Text Book of Medical Biochemistry. Chatterjea M N, Shinde R (Edrs), New Delhi, Jaypee Brothers, pp482-525.

Chemes H, Dym M, Fawcett D W, Jaradpour N, Sherins R J, (1977). Pathophysiological observations of Sertoli cells in patients with germinal aplasia or severe germ cell depletion. Ultrastructural findings and hormone levels. Biol Reprod. 17:108-123.

Chia S E, Lim S T, Tay S K, (2000). Factors associated with male infertility:A case-control study of 218 infertile and 240 fertile men. Br J Obstet Gynecol. 107:55-61.

Chia S E, Ong C N, Tsakok F M H, (1994). Effects of cigarette smoking on human semen quality. Arch Androl. 33:163-168.

Chiao Y B, Van Thiel D H, (1983). Biochemical mechanisms that contribute to alcohol-induced hypogonadism in the male. Alcohol Clin Exp Res. 7:131-134.

Ching M, Lin C Y, (1994). Ethanol Acutely Reduces LH and Prolactin Secretion: Possible Involvement by Dopamine. Alcohol 11:105-112.

Cicero T J, (1980). Effect of exogenous and endogenous opiates on the hypothalamic pituitary-gonadal axis in the male rat. Fed Proc. 39:2551-1554.

Cicero T J, (1981). Neuroendocrinological effects of alcohol. Ann Rev Med. 32:123-142.

Cicero T J, (1982). Pathogenesis of alcohol-induced endocrine abnormalities. Adv Alcohol Substance Abuse. 1:87-112.

Cicero T J, Badger T M, (1976). Effects of alcohol on the hypothalamic-pituitary-gonadal axis. J Pharmacol Exp Ther. 210:427-433.

Cicero T J, Meyer E R, Bell R D, (1979). Effects of ethanol on the hypothalamic-pituitary-luteinizing hormone axis and testicular steroidogenesis. J Pharmacol Exp Ther. 208:210-215.

Clemmesen J, (1997). Is smoking during pregnancy a cause of testicular cancer. Ugeskr Laeger. 159:6815-6819.

Clifton D K, Bremner W J, (1983). The effects of testicular X- irradiation on spermatogenesis in man. A comparison with the mouse. J Androl. 4:387-395.

Close C E, Roberts P L, Berger R E, (1990). Cigarette, alcohol and marijuana are related to pyospermia in infertile men. J Urol. 144:900-903.

Cobas Core Method Manual, (2000). Cobas Core Method Manual. In: Version 1.0. Germany, Roche Diagnostics GMBH, 68298 Mannheim, Germany, pp11-21.

Coulter W H, (1956). High speed automatic blood cell counter and cell size analyzer. Paper presented at National Electronic Conference, Chicago, IL, pp1034-1042.

Creasy D M, Foster J R, Foster P M D, (1983). The morphological development of di-n-pentyl phtalate induced testicular atrophy in the rat. J Pathol. 139:309-321.

Curtis K M, Savitz D A, Arbuckle T E, (1997). Effect of cigarette smoking, caffeine consumption and alcohol intake on fecundability. Am J Epidemiol. 146:32-41.

Damber J E, Janson P O, (1978). The effects of LH, adrenaline and noradrenaline on testicular blood flow and plasma testosterone concentrations in anaesthetized rats. Acta Endocr. 88:390-396.

de Kretser D M, Burger H G, Fortune D, Hudson B, Long A R, Paulsen C A, Taft H P, (1972). Hormonal, histological and chromosomal studies in adult males with testicular disorders. J Clin Endocrinol Metab. 35:392-99.

de Kretser D M, Meinhardt A, Meehan T, (2000). The roles of inhibin and related peptides in gonadal function. Mol Cell Endocrinol. 161:43-46.

de Kretser D M, Robertson D M, (1989). The isolation and physiology of inhibin and related proteins. Biol Reprod. 40:33-39.

Deutschen Gesellschaft fur Klinische Chemie, (German Society of Clinical Chemistry), (1972). Recommendations of the Deutschen Gesellschaft fur Klinische Chemie (German Society of Clinical Chemistry). Standard-Methode zur Bestimmung der Aktivitat der alkalischen Phosphatase. Z Klin Chem U Klin Biochem. 10:191-199.

DiFranza J R, Guerrera M P, (1990). Alcoholism and smoking. J Stud Alcohol. 51:130-135.

Dikshit R K, Buch J G, Mansuri S M, (1987). Effects of tobacco consumption on semen quality of a population of male infertility. Fertil Steril. 48:334-336.

DiZerga G S, Sherins R J, (1981). Endocrine control of adult testicular function. In: The Testis. Burger B, DeKretser D (Edrs), New York, Raven, pp127-160.

Donnelly G P, McClure N, Kennedy M S, Lewis S E, (1999). Direct effect of alcohol on the motility and morphology of human spermatozoa. Andrologia. 31:43-47.

Douglass J, Cox B, Quinn B, Civelli O, Herbert E, (1987). Expression of the prodynorphin gene in male and female mammalian reproductive tissues. Endocrinology. 120:707-713.

Doumas B T, (1971). Albumin standards and the measurement of serum albumin with bromocresol green. Clin Chem Acta. 31:87-96.

Dufau M L, (1988). Endocrine regulation and communicating functions of the Leydig cell. Ann Rev Physiol. 50:483-95.

Dunphy B C, Barratt C L, Cooke I D, (1991). Male alcohol consumption and fecundity in couples attending an infertility clinic. Andrologia. 23:219-221.

Eckhoff R F, (1967). An experimental indication of the volume proportional response of the Coulter Counter for irregularly shaped particles. J Sci Inst. 44:648-649.

Ellingboe J, (1987). Acute effects of ethanol on sex hormones in non-alcoholic men and women. Alcohol Alcohol Suppl. 1:109-116.

Emanuele M A, Tentler J, Emanuele N V, Kelley M R, (1991). In vivo effects of acute EtOH on rat alpha and beta luteinizing hormone gene expression. Alcohol. 8:345-348.

Emanuele M A, Tentler J J, Halloran M M, Emanuele N V, Wallock L, Kelley M R, (1992). The Effect of Acute in vivo Ethanol Exposure on Follicle Stimulating Hormone Transcription and Translation. Alcohol Clin Exp Res. 16:776-780.

Emanuele M A, Tentler J, Reda D, Kirsteins L, Emanuele N V, Lawrence A M, (1989). In-vivo effect of ethanol on release of LH-releasing hormone and LH in rats. J Endocrinol. 121:37-41.

Emanuele N V, LaPaglia N, Steiner J, Kirsteins L, Emanuele M A, (1999b). Reversal of Chronic ethanol-induced testosterone suppression in peripubertal male rats by opiate blockade. Alcohol Clin Exp Res. 23:60-66.

Emanuele N V, LaPaglia N, Vogi W, Steiner J, Kirsteins L, (1999a). Impact and reversibility of Chronic Ethanol Feeding on the Reproductive Axis in the Peripubertal Male Rat. Endocrine. 11:277-284.

English K M, Pugh P J, Parry H, Scutt N E, Channer K S, Jones T H, (2001). Effects of cigarette smoking on levels of bioavailable testosterone in healthy men. Clinical Science. 100:661-665.

Eriksson C J P, Widenius T V, Leinonen P, Harkonen M, Ylikahri R H, (1983). Inhibition of testosterone biosynthesis by ethanol. Biochemical Journal. 210:29-36.

Esquifino A I, Mateos A, Agrasal C, Martin I, Canovas J M, Fermoso J, (1989). Time-dependent effects of alcohol on the hypothalamic-hypophyseal-testicular function in the rat. Alcohol Clin Exp Res.13:219-223.

Evans H J, Fletcher J, Torrance M, Hargreave T B, (1981). Sperm abnormalities and cigarette smoking. Lancet. 21:627-629.

Farah L A, Blackwell R E, (2001). Female Infertility:Evaluation and treatment. In: Endocrinology. DeGroot L J, Jameson J L (Edrs), 4th Ed, Vol.3, Philadelphia, W B Saunders, pp2209-2243.

Farghali H, Williams D S, Caraceni P, Borle A B, Gasbarrini A, Gavaler J S, Rilo H L, Ho C, Van Thiel D H, (1993). Effect of ethanol on energy status and intracellular calcium of Sertoli cells; A study using immobilized perfused cells. Endocrinology. 133:2749-2755.

Feek C M, Tuzi N L, Edwards C R W, (1989). The adrenal gland and progesterone stimulates testicular steroidogenesis in the rat in vivo. J Steroid Biochem. 32:573-579.

Fermoso J, Esquifino A I, Mateos A, Agrasal C, Martin R, (1988). Possible role of prolactin in the induction of hypogonadism by chronic alcohol treatment in male rat. Pharmacol Biochem Behrv. 29:489-493.

Fewcett D W, (1975). Observation on the organisation of the interstitial tissue of testis and on the occluding cell functions in the seminiferous epithelium. In: Advances in the biosciences. Raspeg G, Bernhard S (Edrs), Vol.10, New York, Schering Symposium Contraception, Pergamon, pp83-99.

Field A E, Colditz G A, Willett W C, Longcope C, MckinlyJ B, (1994). The relation of smoking, age, relative weight and dietary intake to serum adrenal steroids, sex hormones and sex hormone binding globulin in middle-aged men. J Clin Endocrinol Metab. 79:1310-6.

Fiore M C, (1992). Trends in Cigarette smoking in the United States:The Epidemiology of Tobacco use. In: The Medical Clinics of North America:Cigarette Smoking. A Clinical Guide to Assessment and Treatment. Fiore M C (Edr), Philadephia, W B Saunders, 76:289-330.

Fisher E B, (1990). State of the Art: Smoking and smoking cessation. Am Rev Respir Dis. 142:702-720.

Fossa S D, Ass N, Kaalhus O, (1989). Long term morbidity after intradiaphragmatic radiotherapy in young man with testicular cancer. Cancer. 64:404-408.

Foster-Swanson A, Swartzentruber M, Roberts P, (1994). Reference Intervel Studies of the Rate-Blanked Creatinine/Jaffe Method on BM/Hitachi Systems in Six US Laboratories. Clin Chem. Abstract. No.361.

Free M J, Jaffe R A, (1972). Dynamics of circulation in the testis of the concious rat. Am J Physiol. 223:241-248.

Frias J, Torres J M, Miranda M T, Ruiz E, Ortega E, (2002). Effects of acute alcohol intoxication on pituitary-gonadal axis hormones, pituitary-adrenal axis hormones, beta-endorphin and prolactin in human adults of both sexes. Alcohol Alcohol. 37:169-73.

Frias J, Rodriguez R, Torres J M, Ruiz E, Ortega E, (2000). Effects of acute alcohol intoxication on pituitary gonadal axis hormones, pituitary adrenal axis hormones, beta-endorphin and prolactin in human adolescents of both sexes. Life Sci. 67:1081-1086.

Gandini L, Lombardo F, Lenzi A, Culasso F, Pacifici R, Zuccaro P, Dondero F, (1997). The in-vitro effects of nicotine and cotinine on sperm motility. Hum Reprod. 12:727-733.

Gerson L W, Preston D A, (1979). Alcohol consumption and the incidence of violent crime. J Stud Alcohol. 40:307-312.

Gianoulakis C, (1990). Characterization of the effects of acute ethanol administration on the release of beta-endorphin peptides by the rat hypothalamus. Eur J Pharmacol. 180:21-20.

Glaxo Welcome, (2000). Zyban 150 mg prolonged-release tablets (buproion hydrochloride). Summary of product characteristics. June, 1-6.

Glezerman M, Bartoov B, (1993). Semen Analysis. In: Infertility:Male and Female. Insler V, Lunenfeld B (Edrs), 2nd Ed, Edinburgh, Churchill Livingstone, pp285-316.

Gluud C, (1988). Testosterone and alcoholic cirrhosis. Epidemiologic, pathophysiologic and therapeutic studies in men. 35:564-574.

Gomathi C, Balasubramanian K, Bhanu N V, Srikanth V, Govindarajulu P, (1993). Effect of chronic alcoholism on semen-studies on lipid profiles. Int J Androl. 16:175-181.

Gonzalez-Reimers E, Martinez-Riera A, Santolaria-Fernandez F, Conde-martel A, Arguelles H A, Herrera C S, Moreno F R, (1994). Relative and Combined Effects of Ethanol and protein Deficiency on Gonadal Function and Histology. Alcohol 11:355-360.

Gordon G G, Altman K, Southern A L, Rubin E, Lieber C S, (1976). Effect of alcohol (ethanol) administration on sex-hormone metabolism in normal men. N Engl J Med. 295:793 - 797.

Goverde H J, Dekker H S, Janssen H J, Bastiaans B A, Rolland R, Zielhuis G A, (1995). Semen quality and frequency of smoking and alcohol consumption. An explorative study. Int J Fertil Menopausal Stud. 40:135-138.

Greiling H, Gressner A M, (1995). Lehrbuch der Klinischen Chemie und Pathobiochemie. 3rd Ed, Stuttgart/New York, Schattauer Verlag, p36.

Griffin J E, Wilson J D, (1983). Harrison's Principles of Internal medicine. Petersdorf R G, Adams R D, Braunwald E, Isselbacher K J, Martin J B, Wilson J D (Edrs), Auckland, McGraw-Hill International, pp680-700.

Groebner D F, Shannon P W, (1989). Tests of Variances and Introduction to ANOVA. In: Business Statistics A Decision Making approach. Groebner D F, Shannon P W (Edrs), 3rd Ed, New York, Merrill, Macmillan, pp320-325.

Gumus B, Yigitoglu M R, Lekili M, Uyanik B S, Muezzinoglu T, Buyuksu C, (1998). Effect of Long-term Alcohol Abuse on Male Sexual Function and Serum Gonadal Hormone Levels. Int Urol and Nephrol. 30:755-759.

Gupta P C, Saxena S, Pednekar S M, Maulik P K, (2003). Alcohol consumption among middle-aged and elderly men. A community study from Western India. Alcohol Alcohol. 38:327-331.

Hahn E W, Feingold S M, Simpson L, (1982). Recovery from aspermia indused by low-dose radiation in seminoma. Cancer. 50:337-340.

Hammond G L, (1978). Endogenous steroid levels in the human prostate from birth to old age:A comparison normal and diseased states. J Endocrinol. 78:7-19.

Handelsman D J, Conway A J, Boylan L M, Turtle J R, (1984). Testicular function in potential sperm donors:normal ranges and the effects of smoking and varicocele. Int J Androl. 7:369-382.

Harkonen K, Viitanen T, Larsen S B, Bonde J P, Lahdetie J, (1999). Aneuploidy in sperm and exposure to fungicides and lifestyle factors. ASCLEPIOS. A European concerted action on occupational hazards to male reproductive capability. Environmental Molecular Mutagen. 34:39-46.

Heindel J J, Treinen K A, (1989). Physiology of male reproductive system:endocrine, paracrine and autocrine regulation. Toxicol Pathol. 17:411-445.

Hembree W C, Zeidenbert P, Nahas G, (1976). Marijuana:Chemistry Biochemistry and Cellular Effects. In: Marijuana effects on human gonadal function. Nahas G, Poton W D M (Edrs), Indanpaan-Heitilia J, New York, Springer-Verlag, pp521-527.

Hermann J V, Wolf-D H, Seigfried B, (1986). Sperm quality of healthy smokers, ex-smokers and never-smokers. Fertil Steril. 45:106-110.

Holzki G, Gall H, Hermann J, (1991). Cigarette smoking and sperm quality. Andrologia. 23:141-144.

Hoyle N R, (1994). The application of electrochemiluminescence to immunoassay-based analyte measurement. J Biolumin Chemilumin. 9:289-296.

Ida Y, Tsujimaru S, Nakamaura K, Shiro I, Mukasa H, Egami H, Nakazawa Y, (1992). Effects of acute and repeated alcohol ingestion on hypothalamic-pituitary-gonadal and hypothalamic-pituitary-adrenal functioning in normal males. Drug Alcohol Depend. 31:57-64.

Inder W J, Joyce P R, Wells J E, Evans M J, Ellis M J, Mattioli L, Donald R A, (1995). The acute effects of oral ethanol on the hypothalamic-pituitary-adrenal axis in normal human subjects. Clin Endocrinol. 42:65-71.

Insler V, Lunenfeld B, (1993). Infertility:the dimension of the problem. In: Infertility: Male and Female. Insler V, Lunenfeld B (Edrs), 2nd Ed, London, Churchill Livingstone, pp3-7.

Iranmanesh A, Veldhuis J D, Samojlik E, Rogol A D, Johnson M L, Lizarralde G, (1988). Alterations in the Pulsatile Properties of Gonadotropin Secretion in Alcoholic Men. J Androl. 9:207-214.

Jaan R, Bo Bergman, Mona-Lisa S, (1997). Sex hormones during alcohol withdrawal:A longitudinal study of 29 male alcoholics during detoxification. Alcohol Alcohol. 32:591-597.

Jackson I M D, Lechan R M, Lee S L, (1990). TRH prohormone: biosynthesis, anatomic distribution and processing. Front Neuroendocrinol. 11:267-312.

Jensen S B, (1984). Sexual function and dysfunction in younger married alcoholics. Acta Psychiat Scand. 69:543-549.

Johnson D E, Chiao Y B, Gavaler J S, Van Thiel D H, (1981). Inhibition of testosterone synthesis by ethanol and acetaldehyde. Biochem Pharmcol. 30:1827-1831.

Kachel V, Ruhenstroth-Bauer G, (1976). Methodik and Ergebissne Optiseher Fromfatorunter-suchungen bei der Zellvolumenmessung nach Coulter. Micros Acta. 75:419-423.

Kader H A, Rostom A Y, (1991). Follicle stimulating hormone levels as predictor of recovery of spermatogenesis following cancer therapy. Clin Oncol. 3:37-40.

Kaler L W, Neaves W B, (1978). Attrition human Leydig cell population with advancing age. Anat Rese. 192:513-521.

Kalla N, Nisula B, Menard R, Loriaux D, (1980). The effects of estradiol on testicular testosterone biosynthesis. Endocrinology. 106:35-39.

Kalra S P, Leadem C A, (1984). Control of luteinizing hormone secretion by endogenous opioid peptides. In: Opioid Modulation of Endocrine Function. Delitala G, Motta M, Serio M (Edrs), New York, Raven Press, pp171-184.

Kane A B, Kumar V, (2004). Environmental and Nutritional Pathology. In: Robbins and Cortran Pathologic Basis of Disease. Vnay Kumar, Abbas A K, Fausto N (Edrs), 7th Ed, Philadelphia, Elsevier, pp415-468.

Karhunen P J, Penttila A, Liesto K, Mannikko A, Valimaki M, Mottonen M, Ylikahri R, (1984). Changes in germinal tissue and Leydig cells correlated with ethanol consumption in males with and without liver disease. Arch Toxicol Suppl. 7:155-158.

Kaufman D W, Helmrich S P, Roseberg L, (1983). Nicotine and cabon monoxide content of cigarette smoke and the risk of myocardial infarction in young men. N Engl J Med. 308:409-415.

Kenten J H, Casadei J, Link J, Lupold S, Willey J, Powell M, Rees A, Massey R, (1991). Rapid electrocehemiluminescence assays of polymerase chain reaction products. Clin Chem. 37:1626-1632.

Kerr J B, (1989). The cytology of human testis. In: Testis. Burger H, de Kretser D (Edrs), New York, Raven, pp197-229.

Kim J H, Kim H J, Noh H S, Roh G S, Kang S S, Cho GJ, Park S K, Lee B J, Choi W S, (2003). Suppression by ethanol of male reproductive activity. Brain Res. 989:91-98.

Kim Y, Park J, Moon Y, (1999). Hematopoietic and reproductive toxicity of 2-bromopropane, a recently introduced substitute for chlorofluorocarbons. Todicol Lett. 108:309-313.

Kimberly M, Leary E, Cole T, Waymack P, (1999). Selection, Validation, Standardization and Performance of a Designated Comparison Method for HDL-Cholesterol for use in the Cholesterol Reference Method Laboratory Network. Clin Chem. 45:1803-1812.

Kirby R S, (2004). Pathophysiology of erectile dysfunction. Rick factors of erectile dysfunction. In: Diagnosis of erectile dysfunction. An atlas of erectile dysfunction. Lue T F (Edr), 1st Ed, New York, The Parthenon Publishing Group, pp20-47.

Klaiber E L, Broverman D M, (1988). Dynamics of estradiol and testosterone and seminal fluid indexes in smokers and non-smokers. Fertil Steril. 50:630-634.

Kulikauskas V, Ablin R J, Blaustein D, (1984). Spermatozoal aberrations in cigarette smokers. IRCS Med Sci. 12:1076-1082.

Kunzle R, Mueller M D, Hanggi W, Brikhauser M H, Drescher H, Bersinger N A, (2003). Semen quality of male smokers and nonsmokers in infertile couples. Fertil Steril. 79:287-291.

Kuo Y J, Shanbour L L, (1978). Effects of ethanol on sodium, 3-o-methyl glucose, and L-alinine transport in the jejunum. American Journal of Digestive Disease. 123:51-56.

Laitinen K, Lamberg-Allardt C, Tnnninen R, (1991). Transient hypoparathyroidism during acute alcohol intoxication. N Engl J Med. 324:721-727.

Langgassner J, (1999). Rauchgewohnheiten der osterrichischen Bevolkerung. Statistische Nachrichten. 5:319-326.

Leland J K, Powell M J, (1990). Electrogenerated chemiluminescence:An oxidative-reduction type ECL reaction sequence using tripropyl amine. J Electrochem Soc. 137:3127-3131.

Lemere F, Smith J W, (1973). Alcohol induced sexual impotence. Am J Psychiat. 130:212-219.

Lennox B, Ahmad K N, (1970). The total length of tubules in human testis. J Anat. 107:191-198.

Lieber C S, (1981). Metabolic Effect of ethanol on the liver and other digestive organs. In: Alcohol and the G I Tract. Clinics in Gastroenterology. Leevy C M (Edr), London, W B Saunders, 10:315-342.

Little J, Vainio H, (1994). Mutagenic lifestyles? A reiew of evidence of associations between germ-cell mutations in humans and smoking, alcohol consumption and use of 'recreational' drugs. Mutat Res. 313:131-151.

Little P J, Adams M L, Cicero T J, (1992). Effects of alcohol on the hypothalamic-pituitary-gonadal axis in the developing male rat. J Pharmacol Exp Ther. 263:1056-1061.

Lue T F, (2002). Physiology of penile erection and pathophysiology of erectile dysfunction and priapism. In: Campbell's Urology. Walsh P C, Retik A B, Vaughan E D, Wein A J (Edrs), 8th Ed, Vol.2, Philadelphia, WB Saunders, pp1591-1671.

Magrini G, Ebiner J R, Burckhardt P, Felber J P, (1976). Study of the relationship between plasma prolactin levels and androgen metabolism in man. J Clin Endocrinol Metab. 43:944-947.

Mak V, Jarvi K, Buckspan M, Freeman M, Hechter S, Zini A, (2000). Smoking is associated with the retention of cytoplasm by human spermatozoa. Urology. 56:463-466.

Makler A, (1978). A new chamber for rapid sperm count and motility estimation. Fertil Steril. 30:313-319.

Marinelli D, Gaspari L, Pedotti P, Taioli E, (2004). Mini-review of studies on the effect of smoking and drinking habits on semen parameters. Int J Hyg Environ Health. 207:185-192.

Marshburn P B, Sloan C S, Hammond M G, (1989). Semen quality and association with coffee drinking, cigarette smoking and ethanol consumption. Fertil Steril. 52:162-165.

Marlatt G A, Miller W R, (1984). Interview Booklet. In: Comprehensive Drinkers Profile. Psychological Assessment Resources, INC, Odessa, Florida, pp66-85.

Martini A C, Molina R I, Estofan D, Senestrari D, de Cuneo F M, Ruiz R D, (2004). Effects of alcohol and cigarette consumption on human seminal quality. Fertil Steril. 82:374-377.

Masters W, Johnson V E, (1970). Human sexuality inadequacy. Little, Brown, pp164-191.

Mateos A, Fermoso J, Agrasal C, Martin I, Paz-Bouza J, Tresguerres J A, Esquifino A I, (1987). Effect of chronic consumption of alcohol on the hypothalamo-pituitary-testicular axis in the rat. Rev Esp Fisiol. 43:33-37.

Matsuzaki Y, Kawaguchi E, Morita Y, (1996). Evaluation of Two Kinds of Reagents for Direct Determination of HDL-Cholesterol. J Anal Bio-Sc. 19:419-427.

McCann S M, (1974). Regulation of secretion of follicle-stimulating hormone and luteinizing hormone. In: Handbook of Physiology. Greep R O, Astwood E B, Knobil E, Sawyer W H, Geiger S R (Edrs), Washington DC, American physiological Society, pp489-517.

McCredie M, Maisonneuve P, Boyle P, (1994). Antenatal risk factors for maligmant brain tumours in New South Wales children. Int J Cancer. 56:6-10.

Mello N K, Mendelson J H, Bree M P, Ellingboe J, Skupny A S T, (1985). Alcohol effects on luteinizing hormone and testosterone in male macaque monkeys. J Pharmacol Exp Ther. 233:588-596.

Melsert R, Hoogerbrugge J W, Rommerts F F C, (1988). The albumin fraction of rat testicular fluid stimulates steroid production by isolated Leydig cells. Mol Cell Endocrinol. 59:221-231.

Mendelson J H, (2003). Effects of intravenous cocaine and cigarette smoking on luteinizing hormone, testosterone, and prolactin in men. J Pharmacol Exp Ther. 307:339-348.

Mendelson J H, Mello N K, Ellingboe J, (1977). Effects of acute alcohol intake on pituitary-gonadal hormones in normal human males. J Pharmacol Exp Ther. 202:676-682.

Mendelson J H, Mello N K, Ellingboe J, (1978). Effects of alcohol on pituitary gonadal hormones sexual function and aggression in human males. In: Psychopharmacology:A Generation of progress. Lipton M A, DiMascio A, Killian K (Edrs), New York, Raven Press, pp1677-1692.

Merino G, Lira S C, Martinez-Chequer J C, (1998). Effects of cigarette smoking on semen characteristics of a population in Mexico. Arch Androl. 41:11-15.

Messing R O, (2001). Alcohol and Alcoholism. In: Harrison's Principles of Internal Medicine. Kasper D L, Fauci A S, Longo D L, Braunwald E, Hauser S L, Jameson J L (Edrs), 15th Ed, Vol. 2, New York , McGraw-Hill International, pp2557-2566.

Mitchell R, Hollis S, Crowley V, McLoughlin J, Peers N, Robertson W R, (1995). Immunometric assays of luteinizing hormone (LH); differences in recognition of plasma LH by anti-intact and beta-subunit-specific antibodies in various physiological situations. Clin Chem. 41:1139-1145.

Mittler J C, Pogaesh L, Ertel N H, (1983). Effects of chronic smoking on testosterone metabolism in dogs. J Steroid Biochem.18:759-63.

Mohan D, Chopra A, Ray R, Sethi H, (2001). Alcohol consumption in India:a cross sectional study. In: Surveys of Drinking Patterns and Problems in Seven Developing Countries. Room R, Demers A, Bourgault C (Edrs), Geneva, World Health Organization, pp103-114.

Morganstern S, Abrahms A, (1994). What Causes Impotence? In: Overcoming Impotence; A doctor's Proven Guide to Regaining Sexual Vitality. Morganstern S, Abrahms A (Edrs), New Jersey, Prentice Hall, pp63-66.

Mosher W D, Pratt W F, (1991). Fecundity and infertility in the United States:Incidence and trends. Fertil Steril. 56:192-193.

Moskova P, Popov I, (1993). Sperm Quality in Smokers and Nonsmokers Among Infertile families. Akusherstvo Ginekologiia. 32:28-30.

Mulligan T, Retchin S M, Chinchilli V, Bettinger C B, (1988). The role of aging and chronic disease in sexual dysfunction. J Am Geriatr Soc. 36:520-524.

Nagy F, Pendergrass P B, Bown D C, Yeager J C, (1986). A comparitive study of cytological and physiological parameters of semen obtained from alcoholics and non-alcoholics. Alcohol Alcohol. 21:17-23.

Norman M A, Holly E A, Preston-Martin S, (1996). Childhood brain tumours and exposure to tobacco smoke. Cancer Epidemiol Biomarkers Prev. 5:85-91.

Obeng Y S, Bard A J, (1991). Electrogenerated chemiluminescence 53:electrochemistry and emission from adsorbed monolayers of a tris(bipyridyl)ruthenium(II)-based surfactant on gold and tin oxide electrodes. Langmuir. 7:195-201.

Ochedalski T, Lachowicz-Ochedalska A, Dec W, Czechowski B, (1994). Evaluating the effect of smoking tobacco on some semen parameters in men of reproductive age. Ginekol Pol. 65:80-86.

Ohl D A, Sonksen J, (1996). What are the changes of infertility and should sperm be banked. Semin Urol Oncol. 14:936-944.

O'Leary M P, Fowler F J, Lenderking W R, (1995). A brief male sexual function inventory for urology. Urology. 5:46-49.

Olsen J, Bolumar F, Boldsen J, (1997). Does moderate alcohol intake reduce fecundability. A European multicenter study on infertility and subfecundity. European Study Group on Infertility and Subfecundity. Alcohol Clin Exp Res. 21:206-212.

O'Malley B W, Strott C A, (1991). Steroid hormones:Metabolism and Mechanism of Action. In: Reproductive Endocrinology. Yen S S C, Jaffe R B (Edrs), Philadelphia, W B Saunders, pp156-157.

Orpana A K, Harkonen M, Eriksson C J P, (1990a). Ethanol-induced inhibition of testosterone biosynthesis in rat Leydig cells:role of mitochondrial substrate shuttles and citrate. Alcohol Alcohol. 25:499-507.

Orpana A K, Orava M M, Vihko R K, Harkonen M, Eriksson C J P, (1990b). Ethanol-induced inhibition of testosterone biosynthesis in rat Leydig cells:Central role of mitochondrial NADH redox state. J Steroid Biochem. 36:603-608.

Pacifici R, Altieri I, Gandini L, Lenzi A, Pichini S, Rosa M, (1993). Nicotine, cotinine, and trans-3-hydroxycotinine levels in seminal plasma of smokers:effects on sperm parameters. Ther Drug Monit. 15:358-363.

Pajarinen J T, Karhunen P J, (1994). Spermatogenic arrest and 'Sertoli cell-only' syndrome- common alcohol-induced disorders of the human testis. Int J Androl. 17:292-299.

Partin A W, Rodriguez R, (2002). The molecular biology, endocrinology, and physiology of the protate and seminal vesicles. In: Campbell's Urology. Walsh P C, Retik A B, Vaughan E D, Wein A J (Edrs), 8th Ed, Vol. 2, Philadelphia, W B Saunders, pp1237-1283.

Patterson T R, Stringham J D, Meikle A W, (1990). Nicotine and cotinine inhibit steroidogenesis in mouse Leydig cells. Life Sci. 46:265-272.

Paz G F, Yavetz H, Hauser R, Yogev L, Lewin L M, Hommonai Z T, (1993). Pathophysiology of the human testis. In: Infertility:Male and Female. Insler V, Lunenfeld B (Edrs), 2nd Ed, London, Churchill Livingstone, pp195-229.

Persijn J P, van der Slik W, (1976). Method of estimation of serum L-Gamma Glutamyl Transferase (GGT) activity. J Clin Chem Clin Biochem. 14:421-427.

Pettersson S, Soderholm B, Persson J E, (1973). Testicular blood flow in man messured with venous occlusion plethysmography and xenon-133. Scand J Urol Nephrol. 7:115-119.

Pickworth W B, Fant R V, (1998). Endocrine effects of nicotine administration, Tobacco and other drug withdrawal in humans. Psychoneuroendocrinology. 23:131-141.

Pohl C R, Guilinger R A, Van Thiel D H, (1987). Inhibitory action of ethanol on luteinizhing hormone secretion by rat anterior pituitary cells in culture. Endocrinology. 120:849-852.

Potts R J, Newbury C J, Smith G, Notarianni L J, Jefferies T M, (1999). Sperm chromatin damage associated with male smoking. Mutat Res. 423:103-111.

Prader A, (1966). Testicular size:Assesment and clinical importance. Triangle. 7:240-243.

Ramnik Sood, (1987). Semen Analysis. In: Medical Laboratory Technology (Methods and Interpretations). Ramnik Sood (Edr), New Delhi, Jaypee Brothers Medical Publishers, pp200-202.

Rantala M L, Koskimies A I, (1987). Semen quality of infertile couples:comparison between smokers and non-smokers. Andrologia. 19:42-46.

Reference Guide V:1.0, (1996). ECL Technology. In: Operator's Manual. Cat No. 1705296001, Germany, Scriptor Dokumentation Service GmbH, Bielefeld, Germany, on behalf of the Boehringer Mannheim, GmbH, pp41-52.

Reynolds R T, Narang B S, (2003). Semen Analysis. In: Medical Laboratory Technology. A Procedure Manual for Routine Diagnostic Tests. Mukherjee K L (Edr), 15th Ed, Vol.2, New Delhi, Tata McGraw-Hill Publishing Company Limited, pp871-879.

Rifai N, Bachorik P S, Albers J J, (2001). Lipids, Lipoprotein and Apolipoprotein. In: Tietz Fundamentals of Clinical chemistry. Burtis C A, Ashwood E R (Edrs), 5th Ed, Philadelphia, W B Saunders, pp488-515.

Ritzen E M, Hsnddon V, French F S, (1989). The Sertoli cell. In: The Testis. Burger H, de Kretser D (Edrs), New York, Raven, pp269-301.

Rivier C, (1999). Alcohol Rapidly Lowers Plasma Testosterone Levels in the Rat: Evidence that a Neural Brain-Gonadal Pathway may be Important for Decreased Testicular Responsiveness to Gonadotropin. Alcohol Clin Exp Res. 23:38-45.

Robins T G, Bornman M S, Ehrlich R I, Cantrell A C, Pienaar E, Vallabh J, (1997). Semen quality and fertility of men employed in a South African lead acid battery plant. Am J Ind Med. 32:369-76.

Room R, Jernigan D, Carlini-Marlatt B, Gureje O, Mäkelä K, Marshall M, Medina-Mora M E, Monteiro M, Parry C, Partanen J, Riley L, Saxena S, (2002). Alcohol in Developing Societies: A Public Health Approach. Finnish Foundation for Alcohol Studies in collaboration with the World Health Organization (Edr), Hakapaino Oy, Helsinki, pp79-114.

Rosen A, Riley A, Wagner G, (1997). The International Index of Erectile Function (HEF): a multidimensional scale for assessment of erectile dysfunction. Urology. 49:822-830.

Rosen R C, (1991). Alcohol and drug effects on sexual response. Human experimental and clinical studies. Ann Rev Sex Res. 2:119-120.

Rosenblum R E, Gavaler J S, Van thiel D H, (1989). Lipid Peroxidation:A Mechanism for alcohol-induced testicular injury. Alcohol Clin Exp Res. 7:569-577.

Rosenfield R L, Moll G W, (1983). Androgenization in women. In: The role of proteins in the distribution of plasma androgens and estradiol. Molinatti G, Martini L, James V H T (Edrs), New York, Raven Press, pp25-45.

Rubes J, Lowe X, Moore D, Perreault S, Slott V, Evenson D, Selevan S G, Wyrobek A J, (1998). Smoking cigarettes is associated with increased sperm disomy in teenage men. Fertil Steril. 70:715-723.

Ruusa J, Bergman B, (1996). Sex Hormones and Alcohol withdrawal:Does a good supply of testosterone prevent serious symptoms during detoxification?. Alcohol. 13:139-145.

Said T M, Geetha R, Agarwal A, (2005). Relationship between semen quality and tobacco chewing in men undergoing infertility evaluation. Fertil Steril. 84:649 -53.

Saleh R A, Agarwal A, Sharma R K, Nelson D R, Thomas Jr A J, (2002). Effect of cigarette smoking on levels of seminal oxidative stress in infertile men:a prospective study. Fertil Steril. 78:491-499.

Salonen I, Eriksson C J P, (1989). Penetration of ethanol into the male reproductive tract. Alcohol Clin Exp Res. 13:746-751.

Salonen I, Huhtaniemi I, (1988). Specific and weight loss-associated effects of one-week exposure to ethanol on pituitary-gonadal function of male rats. Acta Endocrinol. 119:99-105.

Salonen I, Huhtaniemi I, (1990). Effects of chronic ethanol diet on pituitary testicular function of the rat. Biol Reprod. 42:55-62.

Samuel K M, (1989). Notes on Clinical Lab Techniques. In: Laboratory procedure and their interpretation. Samuel K M (Edr), 4th Ed, Madras, M K G Iyyer and Son, pp25-32.

Sanchis R, Esquifino A I, Guerri C, (1985). Choronic ethanol intake modifies estrous cyclicity and alters prolactin and LH levels. Pharmacol Biochem Behrv. 23:221-224.

Sandeman D F, (1966). The effects of X - irradiation on male human fertility. Br J Radiol. 39:901-907.

Sarles H, (1974). Chronic calcifying pancreatitis-chronic alcoholic pancreatitis. Gastroenterology. 66:604-616.

Schade R R, Bonner G, Gay V C, Van Thiel D H, (1983). Evidence for a direct inhibitory effect of ethanol upon gonadotropin secretion at the pituitary level. Alcohol Clin Exp Res. 7:150-152.

Schiavi R C, (1990). Chronic alcoholism and male sexual dysfunction. Journal of Sex and Marital Therapy. 16:23-33.

Schlegel P N, Hardy M, (2002). Male Reproductive Physiology. In: Campbell's Urology. Walsh P C, Retik A B, Vaughan E D, Wein A J (Edrs), 8th Ed, Vol.2, Philadelphia, W B Saunders, pp1437-1474.

Schlorff E C, Husain K, Somani S M, (1999). Dose and Time Dependent Effects of Ethanol on Antioxidant System in Rat Testes. Alcohol 18:203-214.

Schneider G, Kirschner M A, Berkowitz R, (1979). Increased estrogen production in obese men. J Clin Endocrinol Metab. 48:633-638.

Schuckit M A, (2005). Alcohol and Alcoholism. In: Harrison's Principles of Internal Medicine. Kasper D L, Fauci A S, Longo D L, Braunwald E, Hauser S L, Jameson J L (Edrs), 16th Ed, Vol.2, New York, McGraw-Hill International, pp2562-2566.

Sergerie M, Ouhilal S, Bissonnette F, Brodeur J, Bleau G, (2000). Lack of association between smoking and DNA fragmentation of normal men. Hum Reprod. 15:1314-1321.

Setchell B P, Brooks D E, (1988). Anatomy, vasculature, innervation and fluids of male reproductive tract. In: The physiology of reproduction. Knobil E, Neill J D (Edrs), New York, Raven, pp753-836.

Shaarawy M, Mahmoud K Z, (1982). Endocrine profile and semen characteristics in male smokers. Fertil Steril. 38:255-257.

Shen H M, Chia S E, Ni Z Y, New A L, Lee B L, Ong C N, (1997). Detection of oxidative DNA damage in human sperm and the association with cigarette smoking. Reprod Toxicol. 11:675-80.

Shi A, Hales D B, Emanuele N V, Emanuele M A, (1998). Interaction of ethanol and nitric oxide in the hypothalamic-pituitary-gonadal axis in the male rat. Alcohol Clin Exp Res. 22:1754-1762.

Shi Q, Ko E, Barclay L, Hoang T, Rademaker A, (2001). Cigarette Smoking and Aneuploidy in Human sperm. Molecular Reproduction and Development. 59:417-421.

Siedel J, Schmuck R, Staepels J, (1993). Long term stable liquid ready to use mono reagent for the enzymatic assay of serum or plasma triglycerides (GPO-PAP method). AACC Meeting Abstract 34. Clin Chem. 39:1127-1128.

Sigman M, Jarow J P, (2002). Male infertility. In: Campbell's Urology. Walsh P C, Retik A B, Vaughan E D, Wein A J (Edrs), 8th Ed, Vol.2, Philadelphia, W B Saunders, pp1475-1533.

Sinha D N, (2002). Report on the Results of the Global Youth Tobacco Survey in Uttar Pradesh, India - 2002. Patna, India, Dr. Dhirendra N. Sinha, School of Preventive Oncology, A/27, Anandpuri, Boring Canal Road, Patna- 800001, India. 1-11.

Skinner M K, Fetterolf P M, Anthony C T, (1988). Purification of a paracrine factor, P-Mod-S, produced by testicular peritubular cells that modulate Sertoli cell function. J Biol Chem. 263:2884-2890.

Sofikitis N, Miyagawa I, Dimitriadis D, Zayos P, Sikka S, Hellstrom W, (1995). Effects of smoking on testicular function, semen quality and sperm fertilizing capacity. J Urol. 154:1030-1034.

Spira A, (1986). Epidemiology of human reproduction. Hum Reprod. 1:111-115.

Stocco D M, (2000). Intramitochondrial cholesterol transfer. Biochem Biophys acta. 1486:184-197.

Sugiuchi H, Uji Y, Okabe H, Irie T, (1995). Direct Measurement of High-Density Lipoprotein Cholesterol in Serum with Polyethylene Glycol-Modified Enzymes and Sulfated alpha - Cyclodextrin. Clin Chem. 41:717-723.

Sun J G, Jurisicona A, Casper R F, (1997). Detection of deoxyribonucleic acid fragmentation in human sperm:correlation with fertilization in vitro. Biol Reprod. 56:602-607.

Syms A J, Johnson A R, Lipshultz L I, Smith R G, (1984). Studies on human spermatozoa with round head syndrome. Fertil Steril. 42:431-435.

Szasz G, (1974). New substrates for measuring gamma-glutamyl trans peptidase activity. Z Klin Chem u Klin Biochem. 12:228-232.

Telisman S, Cvikovic P, Jurasovic J, Pizzent A, Gavella M, Rocic B, (2000). Semen quality and reproductive endocrine function in relation to biomarkers of lead, cadmium, zinc, and copper in men. Environ Health Perspect. 108:45-53.

Thonneau P, Bujan L, Multigner L, (1998). Occupational heat exposure and male infertility (a Review). Hum Reprod. 13:2122-2125.

Thonneau P, Marchand S, Tallec A, (1991). Incidence and main causes of infertility in a resident population (1,850,000) of three French regions (1988-1989). Hum Reprod. 6:811-816.

Tiemessen C H, Evers J L, Bots R S, (1996). Tight-fitting underwear and sperm quality. Lancet. 347:1844-1845.

Trinder P, (1969). Determination of Glucose in blood using Glucose Oxidase with an alternative oxygen acceptor. Ann Clin Biochem. 6:24-27.

Trummer H, Habermann H, Haas J, Pummer K, (2002). The Impact of Cigarette Smoking on Human Semen Parameters and Hormones. Hum Reprod. 17:1554-1559.

US Congress, (1983). Adverse Social Consequences of alcohol use and Alcoholism. In: Alcohol and Health. 5th Ed, Special report, Maryland, US Department of Health and Human Services, pp83-99.

Valimaki M J, Harkonen M, Eriksson F J P, Ylikahri R H, (1984). Sex hormones and adrenocortical steroids in men acutely intoxicated with ethanol. Alcohol. 1:89-93.

Valimaki M J, Salaspuro M, Harkonen M, Ylikahri R H, (1982). Liver damage and sex hormones in chronic male alcoholics. Clin Endocrinol. 17:469-477.

Valimaki M, Ylikahri R, (1983). The effect of alcohol in male and female sexual function. Alcohol Alcohol. 18:313-320.

Van Thiel D H, Galaver P K, Cobb C F, Santucci L, Graham T O, (1983). Ethanol, a Leydig cell toxIn: evidence obtained in vivo and in vitro. Pharmacol Biochem Behav. 18:317-323.

Van Thiel D H, Galaver P K, Rosenblum E, Eagon Y B, (1987). Effects of Ethanol on Endocrine Cells: testicular Effects. Annals of the New York Academy of Sciences. 492:287-302.

Van Thiel D H, Lester R, (1979). The effect of chronic alcohol abuse on sexual function. Clin Endocrinol Metab. 8:499-510.

Varley H, Gowenlock A H, Bell M, (1980a). Collection of Specimen and some General Techniques. In: Practical Clinical Biochemistry. Varley H, Gowenlock A H, Bell M (Edrs), 5th Ed, Vol.1, London, William Heinemann Medical Books Ltd, pp368-396.

Varley H, Gowenlock A H, Bell M, (1980b). Lipids and Lipoproteins. In: Practical Clinical Biochemistry. Varley H, Gowenlock A H, Bell M (Edrs), 5th Ed, Vol.1, London, William Heinemann Medical Books Ltd, pp625-669.

Veldhuis J, (1991). The Hypothalamic-Pituitary-Testicular Axis. In: Reproductive Endocrinology. Yen S S C, Jaffe R B (Edrs), 3rd Ed, Philadelphia, W B Saunders, pp409-459.

Viczian M, (1988). The effects of cigarette smoke inhalation on spermatogenesis in rats. Experientia. 24:511-513.

Villalta J, Ballesca J L, Nicolas J M, Martinez de Osaba, Antunez E, Pimentel C, (1997). Testicular Function in Asymptomatic Chronic Alcoholics: Relation to Ethanol Intake. Alcohol Clin Exp Res. 21:128-133.

Vine M F, (1996). Smoking and male reproduction: A review. Int J Androl. 19:323-337.

Vine M F, Margolin B H, Morrison H I, Hulka B S, (1994). Cigarette smoking and sperm density:a meta-analysis. Fertil Steril. 61:35-43.

Vine M F, Tse C K, Hu P, Truong K Y, (1996). Cigarette smoking and semen quality. Fertil Steril. 65:835-842.

Vogt H J, Heller W D, Borelli S, (1986). Sperm quality of healthy smokers, ex-smokers, and never smokers. Fertil Steril. 45:106-110.

Wahlefeld A W, Herz G, Bernt E, (1972). Modification of the Malloy-Evelyn method for a simple, reliable determination of total bilirubin in serum. Scand J Clin Lab Invest. 29:11-12.

Wanderly M I, Udrisar D P, (1994). Inhibitory action of in vitro ehanol and acetaldehyde exposuer on LHRH and phorbol ester-stimulated testosterone secretion by rat testicular interstitial cells. APPTLA. 44:135-141.

Weichselbaum T E, (1946). Estimation of total protein. Amer J Clin Path. 16:40-48.

West L A, Horvat R D, Roess D A, (2001). Steroidogenic acute regulatory protein and peripheral-type benzodiazepine receptor associate at the mitochondrial membrane. Endocrinology. 142:502-505.

West R, Mc Neill, Raw M, (2000). Smoking cessation guidelines for health professionals:an update. Thorax. 55:987-999.

Whalley L J, (1978). Sexual adjustment of male alcoholics. Acta Psychiat Scand. 58:281-286.

Willis B R, Anderson Jr R A, Oswald C, Zaneveld L J D, (1983). Ethanol-induced male reproductive tract pathology as a function of ethanol dose and duration of exposure. J Pharmacol Exp Ther. 225:470-478.

Winter J S D, Faiman C, (1972). Pituitary-gonadal relations in male childrens and adolescents. Peiatr Res. 6:126-131.

Wong W Y, Thomas C M G, Merkus H M W M, Zielhuis G A, Doesburg H W, Steegers-Theunissen R P M, (2000). Cigarette smoking and the risk of male factor subfertility:minor association between cotinine in seminal plasma and semen morphology. Fertil Steril. 74:930-935.

World Health Organization, (1999). WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction. WHO (Edrs), 4th Ed, Cambridge University Press, pp1-27.

Wynder E L, (1975). Toward the prevention of laryngeal cancer. Laryngoscope. 85:1190-1196.

Xu X H, Bard A J, (1994). Electrogenerated chemiluminescence 55:emission from adsorbed Ru(bpy) on graphite, platinum and gold. Langmuir. 10:2409-2414.

Yamamoto Y, Isoyama E, Sofikitis N, Miyagawa I, (1998). Effects of smoking on testicular function and fertilizing potential in rats. J Urol. 26:45-48.

Yardimci S, Atan A, Tastan H, Kalender Y, Kalender S, Sunguroglu K, Avunduk M C, (1996). Effects of cigarette smoke exposure on spermatogenesis in rat. J Ankara Medical School. 18:181-186.

Yardimci S, Atan A, Delibasi T, Sunguroglu K, Guven M C, (1997). Long-term effects of cigarette smoke exposure on plasma testosterone, luteinizing hormone and follicle stimulating hormone levels in male rats. Br J Urol. 79:66-69.

Yeh J, Barbieri L R, Freidman J A, (1989). Nicotine and cotinine inhibit rat testis androgen biosynthesis in vivo. J Steroid Biochem. 33:627-630.

Zavos P M, Correa J R, Antypas S, Zarmakoupis-Zavos P N, Zarmakoupis C N, (1998a). Effects of seminal plasma from cigarette smokers on sperm viability and longevity. Fertil Steril. 69:425-429.

Zavos P M, Correa J R, Karagounis C S, Ahparaki A, Phoroglou C, Hicks C L, Zarmakoupis-Zavos, P N, (1998b). An electron microsope study of the axonemal ultrastructure in human spermatozoa from male smokers and nonsmokers. Fertil Steril. 69:430-434.

Zenzes M T, (1995). Cigarette smoking as a cause of delay in conception. Reprod Med Rev. 4:189-205.

Zenzes M T, (2000). Smoking and reproduction:gene damage to human gametes and embryos. Hum Reprod Update. 6:122-131.

Zenzes M T, Bielecki R, Reed T E, (1999). Detection of benzo(a)pyrene diol expoxide-DNA adducts in sperm of men exposed to cigarette smoke. Fertil Steril. 72:330-335.

Zhang J P, Meng Q Y, Wang Q, Zhang L J, Mao Y L, Sun Z X, (2000). Effect of smoking on semen quality of infertile men in Shandong, Chaina. Asian J Androl. 2:143-146.

Zhang J, Savitz D A, Schwingl P J, Cai W W, (1992). A case-control study of paternal smoking and birth defects. Int J Epidemiol. 21:273-278.

7. APPENDICES

APPENDIX – I

ESTIMATION OF BLOOD GLUCOSE

Blood glucose was estimated by glucose oxidase peroxidase method (GOD-POD) using Hitachi-912, autoanalyser, Roche diagnostics, Germany.

Principle

$$\text{Glucose} + O_2 + H_2O \rightarrow \text{gluconolactone} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \rightarrow \text{4 (p-benzoquinone-mono-imino)}$$

$$\text{Phenazone} + 4H_2O.$$

Glucose was oxidized by glucose oxidase to gluconolactone in the presence of atmospheric oxygen. The resultant hydrogen peroxide oxidizes 4-aminophenazone and phenol to 4-(p-benzo-quinone-mono-imino) phenazone in the presence of peroxidase. The color intensity of the red dye was directly proportional to the glucose concentration and was measured photometrically (Greiling and Gressner, 1995; Trinder, 1969).

APPENDIX – II

ESTIMATION OF CREATININE

Creatinine was estimated by Jaffe Method using Hitachi-912, autoanalyser, Roche diagnostics, Germany.

Principle

$$\text{Creatinine} + \text{Picric Acid} \rightarrow \text{Creatinine-Picric Acid Complex.}$$

In alkaline solution, creatinine formed a yellow-orange complex with picrate. The color intensity was directly proportional to the creatinine concentration and was measured photometrically (Foster-Swanson et al, 1994).

APPENDIX – III

ESTIMATION OF SERUM TOTAL BILIRUBIN

Serum bilirubin was estimated by using 2-5 dichlorophenyl diazonium tetrafluroborate (DPD) method using Hitachi-912, autoanalyser, Roche Diagnostics, Germany.

Principle

Indirect bilirubin is liberated by the detergent hydrochloric acid. In the strong acid solution containing 2, 5-dichlorophenyl-diazonium salt, total bilirubin couples to form azobilirubin.

Bilirubin + diazonium ion $\rightarrow$ azobilirubin.

The color intensity of the red azo dye formed was directly proportional to the total bilirubin and was determined photometrically (Wahlefeld et al, 1972).

APPENDIX – IV

ESTIMATION OF CHOLESTEROL

Serum cholesterol was estimated by using cholesterol oxidase-peroxidase method (Greiling and Gressner, 1995; Trinder, 1969) using Hitachi-912, autoanalyser, Roche diagnostics, Germany.

Principle

Cholesterol is determined enzymatically using cholesterol esterase and cholesterol oxidase.

Cholesterol esters + H_2O $\rightarrow$ cholesterol + RCOOH

Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids.

Cholesterol + O_2 $\rightarrow$ cholest-4-en-3-one + H_2O_2

Cholesterol is converted by oxygen with the aid of cholesterol oxidase to cholest – 4- en-3-one and hydrogen peroxide.

$2H_2O_2$ + 4- aminophenazone + phenol $\rightarrow$ 4-(p-benzoquinone-monoimino)-phenazone + $4H_2O$

Hydrogen peroxide formed red dyestuff by reacting with 4-aminophenazone and phenol under the catalytic action of peroxidase. The color intensity was directly proportional to the concentration of cholesterol and was determined photometrically (Greiling and Gressner, 1995; Trinder, 1969).

APPENDIX – V

ESTIMATION OF TRIGLYCERIDE (TG)

Triglyceride was estimated by glycerol phosphate oxidase peroxidase (GPO-PAP) method using Hitachi-912, autoanalyser, Roche diagnostics, Germany.

Principle

$$\text{Triglycerides} + 3H_2O \rightarrow \text{glycerol} + 3RCOOH$$

$$\text{Glycerol} + ATP \rightarrow \text{glycerol-3-phosphate} + ADP$$

$$\text{Glycerol-3-phosphate} + O_2 \rightarrow \text{dihydroxyacetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-aminophenazone} + \text{4-chlorophenol} \rightarrow \text{4-(p-Benzoquinone-monoimino)-phenazone} + 2H_2O_2 + HCl$$

Triglyceride was hydrolyzed rapidly and completely by lipoprotein lipase to glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide produced reacts with 4-aminophenazone and 4-chlorophenol under the catalytic action of peroxidase to form a red dyestuff. This was measured photometrically. The red dye concentration was directly proportional to the TG present in the sample (Siedel et al, 1993; Trinder, 1969).

APPENDIX – VI

ESTIMATION OF SERUM HIGH DENSITY LIPOPROTEIN (HDL) CHOLESTEROL

The Roche direct HDL cholesterol assay meets the 1998 National Institutes of Health (NIH)/ National Cholesterol Education Programme (NCEP) goals for acceptance performance (Kimberly et al, 1999). The results of this method correlate with those obtained by precipitation-based methods and by an ultra centrifugation method. The HDL cholesterol was estimated by Roche direct HDL cholesterol homogeneous enzymatic colorimetric assay using Hitachi-912, autoanalyser, Roche diagnostics, Germany.

Principle

The cholesterol concentration of HDL cholesterol is determined enzymatically by cholesterol esterase and cholesterol oxidase coupled with polyethylene glycol (PEG) to amino groups (approximately 40%).

HDL-cholesterol esters + H_2O → cholesterol + RCOOH

Cholesterol esters are broken down quantitatively into free cholesterol and fatty acid by cholesterol esterase.

HDL-cholesterol + O_2 → Ä^4-cholestenone + H_2O_2

In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Ä^4-cholestenone and hydrogen peroxide.

$2H_2O_2$ + 4-amino-antipyrine + HSDA N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline + H^+ + H_2O → purple-blue pigment + $5H_2O$

In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-amino –antipyrine and HSDA to form a purple-blue dye. The color intensity of the dye was directly proportional to the HDL cholesterol concentration and was measured photometrically (Sugiuchi et al, 1995; Matsuzaki et al, 1996).

APPENDIX – VII

ESTIMATION OF TOTAL PROTEIN

Serum total protein was estimated by Biuret Method using Hitachi-912, autoanalyser, Roche diagnostics, Germany.

Principle

Divalent copper reacts in alkaline solution with protein peptide bonds to form the characteristic purple colored biuret complex. Sodium potassium tartarate prevents the precipitation of copper hydroxide and potassium iodide prevents auto reduction of copper.

Protein + Cu^{2+} → Cu-protein complex

The color intensity was directly proportional to the protein concentration, which was determined photometrically (Weichselbaum, 1946).

APPENDIX - VIII

ESTIMATION OF SERUM ALBUMIN

Serum albumin was determined by bromocresol green (BCG) method using Hitachi-912, autoanalyser, Roche diagnostics, Germany.

Principle

At a pH of 4.1, albumin displays a sufficiently cationic character to be able to bind with bromocresol green (BCG) an anion dyestuff to form blue-green complex.

Albumin + bromocresol green → albumin bromocresol green complex.

The color intensity of the blue green color was directly proportional to the albumin concentration and was determined photometrically (Doumas, 1971).

APPENDIX – IX

ESTIMATION OF SERUM L-GAMMA GLUTAMYL TRANSFERASE (GGT) ACTIVITY (EC 2.3.2.2)

Serum GGT activity was estimated by Szasz, (1974) method using Hitachi-912, autoanalyser, Roche diagnostics, Germany.

Principle

L-γ glutamyl-3-carboxy-4-nitroanilide glyclylglycine → L-γ-glutamyl-glycylglycine + 5-amino-2-nitrobenzoate.

Gamma-glutamyltransferase transfers the γ-glutamyl group of L-γ-glutamyl 3-carboxy-4 nitroanilide to glycylglycine. The amount of 5-amino-2-nitrobenzoate liberated was proportional to the GGT activity and was determined photometrically (Persijn and van der Slik, 1976; Szasz, 1974).

APPENDIX – X

ESTIMATION OF SERUM GLUTAMATE PYRUVATE TRANSAMINASE (SGPT) ACTIVITY (EC 2.6.1.2)

Serum GPT activity was estimated by International Federation of Clinical Chemistry (IFCC) derived reference method using Hitachi-912, autoanalyser, Roche diagnostics, Germany.

Principle

α-ketoglutarate + L-alanine → L-glutamate + pyruvate.

GPT is the enzyme which catalyses this equilibrium reaction. The pyruvate is measured in a subsequent indicator reaction, which is catalysed by lactate dehydrogenase.

$$\text{Pyruvate} + \text{NADH} + H^{+} \rightarrow \text{L- lactate} + NAD^{+}$$

In the second reaction, NADH is oxidized to NAD. The rate of decrease in NADH (measured photometrically) was directly proportional to the rate of formation of pyruvate and thus the GPT activity (Bergmeyer et al, 1985; Greiling and Gressner, 1995).

APPENDIX – XI

ESTIMATION OF ALKALINE PHOSPHATASE (ALP) ACTIVITY (EC 3.1.3.1)

Serum ALP activity was estimated by 'optimized standard method' assay described recommendations of the Deutschen Gessell and Chaff Fu Klinische Chemie (Deutschen Gesellschaft fur Klinische Chemie, German Society of Clinical Chemistry, 1972) using Hitachi-912, autoanalyser, Roche diagnostics, Germany.

Principle

$$\text{P-nitrophenyl phosphate} + H_2O \rightarrow \text{phosphate} + \text{p-nitrophenol}.$$

In the presence of magnesium and zinc ions, p-nitrophenyl phosphate is hydrolysed by phosphates to form phosphate and p-nitrophenol. The P-nitrophenol released is proportional to the ALP activity and is measured photometrically (Deutschen Gesellschaft fur Klinische Chemie, German Society of Clinical Chemistry, Recommended optimized standard method, 1972).

APPENDIX – XII

HAEMATOLOGICAL PARAMETERS

The Coulter Haematology auto analyzer is used which accurately counts and sizes cells by detecting and measuring changes in electrical resistance when a particle such as a cell in a conductive liquid passes through a small aperture (Coulter, 1956).

Each cell suspended in a conductive liquid diluent acts as an insulator. As each cell goes through the aperture, it momentarily increases the resistance of the electrical path between the submerged electrodes on either side of the aperture. This causes a measurable electrical pulse. For counting the vacuum used to pull the diluted suspension of cells through the aperture

must be at a regulated volume. The number of pulses indicates the number of particles and the size of the electrical pulse is proportional to the cell volume (Eckhoff, 1967; Kachel and Ruhenstroth-Bauer, 1976).

In Coulter, RBC and WBC are measured in two different paths. In RBC path 1.6 µl of sample is used and in WBC path 1.4 µl of sample is used for estimation. The unit counts and sizes RBCs and platelets at the aperture of RBC path and WBCs at the WBC aperture and WBC path measure haemoglobin photometrically in the WBC path.

In the RBC path, pulses that represent cells as 36 fl or greater are classified as red cells and are sorted by size into 256 channels and cells in the 2 to 20 fl range are classified as platelets. In the WBC path, pulses that represent cells as 35 fl or greater are classified as white cells.

APPENDIX – XIII

ESTIMATION OF DIFFERENTIAL WBC COUNT

LEISHMAN STAIN PREPARATION

0.15 g of Leishman's stain powder was placed in a glass motor followed by a few ml of methyl alcohol added and was ground to dissolve the stain completely and this was transferred to 100 ml volumetric flask and was made up to 100 ml with methyl alcohol. The stain was stored in clean dry bottle and was closed well. This stain is stable for two to three weeks.

The blood film slides were placed on a flat surface. The entire dry blood film was covered with stain. After one minute double the quantity of buffer solution was carefully added and mixed with the stain by means of a clean pipette. After seven to eight minutes the excess stain was removed by washing with distilled water for two minutes. The film was air dried and was examined microscopically for polymorphs, lymphocytes, eosinophils, basophils and monocytes (Samuel, 1989).

APPENDIX - XIV

HORMONE ANALYSIS

ELECTROCHEMILUMINESCENCE (ECL) TECHNOLOGY

The last decade has seen the development and refinement of many immunoassay measurement principles and systems. The major trend has

been away from liquid phase assays with radioisotopic labels, and towards fast solid-phase assays based on monoclonal antibodies. This development is moving further towards precise and reliable non-isotopic, automated or semi-automated laboratory assays with detection limits measured in picomoles (10^{-12}) and femtomoles (10^{-15}).

ECL- ASSAY PRINCIPLES

ECL-processes are known to occur with numerous molecules including compounds of ruthenium, osmium, rhenium or other elements.

ECL is a process, in which highly reactive species are generated from stable precursors on the surface of an electrode. These highly reactive species react with one another producing light (chemiluminescence).

The development of ECL immunoassay is based on the use of ruthenium(II)-tris (bipyridl) [$Ru(bpy)^{2+}_3$] complex and tripropylamine (TPA). The final chemiluminescent product is formed during the detection step.

The ECL reactions that lead to the emission of light from the ruthenium complex are initiated electrically, rather than chemically. This is achieved by applying a voltage to the immunological complexes (including the ruthenium complex) that are attached to streptavidin-coated microparticles. The advantage of electrically initiated chemiluminescent reaction is that the entire reaction can be precisely controlled.

USE OF THE RUTHENIUM COMPLEX

ECL technology uses a ruthenium chelate as the complex for the development of light. Salts of ruthenium tris(bipyridyl) are stable, water-soluble compounds (Figure 29). The bipyridyl ligands can be readily modified with reactive groups to form activated chemiluminescent compounds.

For the development of ECL immunoassays, [$Ru(bpy)_3^{2+}$] N-hydroxysuccinamide (NHS) ester is used because it can be easily coupled with amino groups of proteins, haptens and nucleic acids. This allows the detection technology to be applied to a wide variety of analytes.

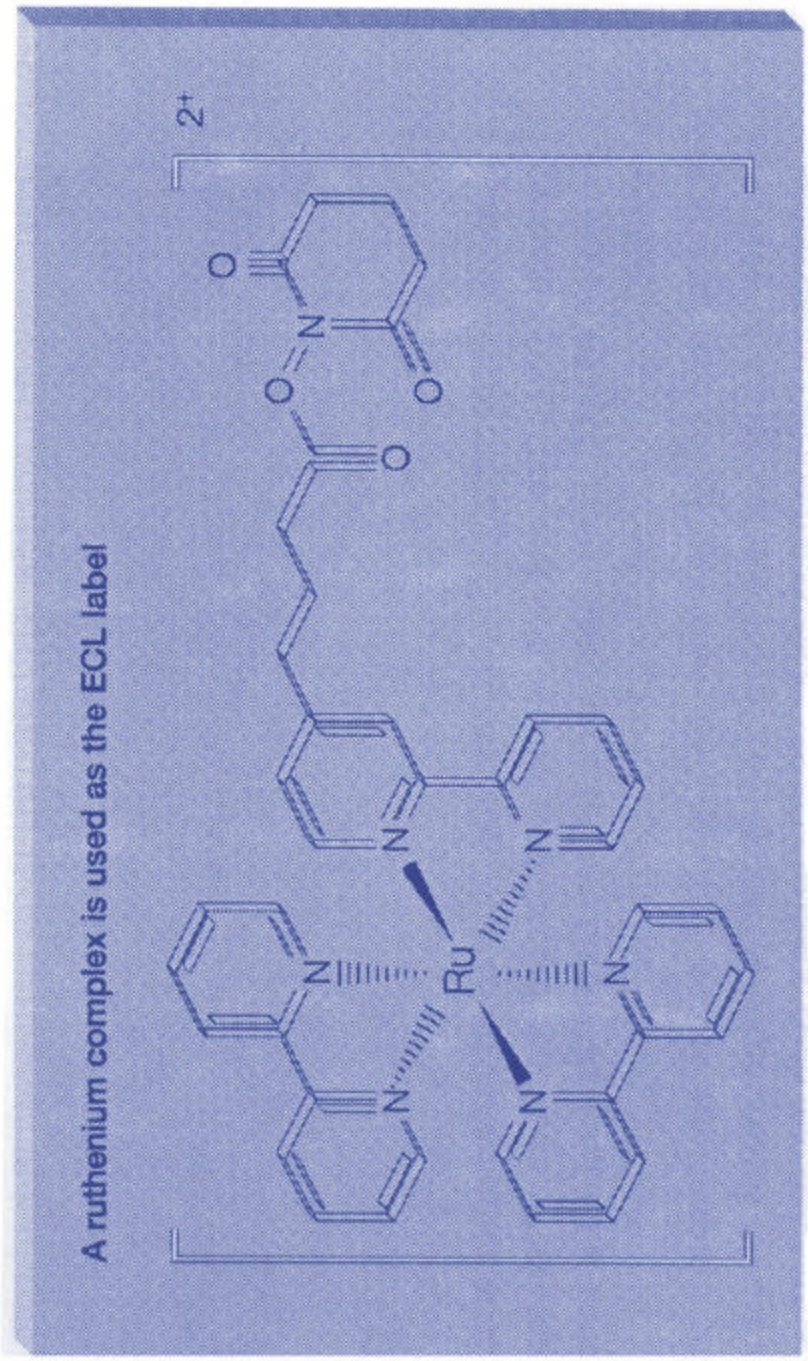


Fig. 29 Ruthenium complex.

THE ECL REACTION AT THE ELECTRODE SURFACE

Two electrochemically active substances, the ruthenium complex and the tripropylamine (TPA), are involved in the luminescent reactions which leads to the emission of light. Both substances remain stable, as long as a voltage is not applied.

The ECL reaction of ruthenium tris(bipyridyl)$^{2+}$ and TPA occurs at the surface of a platinum electrode. The applied voltage creates an electrical field, which causes all the materials in this field to react. Both reaction partners undergo a reaction process. TPA is oxidized at the electrode, releases an electron and forms an intermediate tripropylamine (TPA) radical-cation, which further reacts by releasing a proton (H^+) to form TPA radical (TPA^*).

In turn, the ruthenium complex also releases an electron at the surface of the electrode thus oxidizing to form the [Ru(bpy)3 $^{3+}$] cation. This ruthenium cation is the second reaction component for the following chemiluminescent reaction with the TPA radical. (Figure 30).

TPA^* and [Ru(bpy)3 $^{3+}$] react with one another, whereby [Ru(bpy)3 $^{3+}$] is reduced to [Ru(bpy)3 $^{2+}$] and at the same time forms an excited state via energy transfer. This excited state is unstable and decays with emission of a photon at 620 nm to its original state, ruthenium bipyridyl Ru(bpy)3 $^{2+}$]. The reaction cycle can now start again. The TPA radical reduces to by-products which do not affect the chemiluminescent process. TPA is used up and therefore must be present in excess. The reaction is controlled by diffusion, so that TPA, during the reaction with the ruthenium complex in the ruthenium environment, is depleted and therefore the signal strength (light) is slowly reduced once the maximum is reached. (Figure 31).

Although during measurement, TPA is used up, ruthenium is continually regenerated. This means that the ruthenium complex can perform many light-generating cycles during the whole measurement process, therefore showing an inherent amplification effect. Many signals can be created from one antigen-antibody complex.

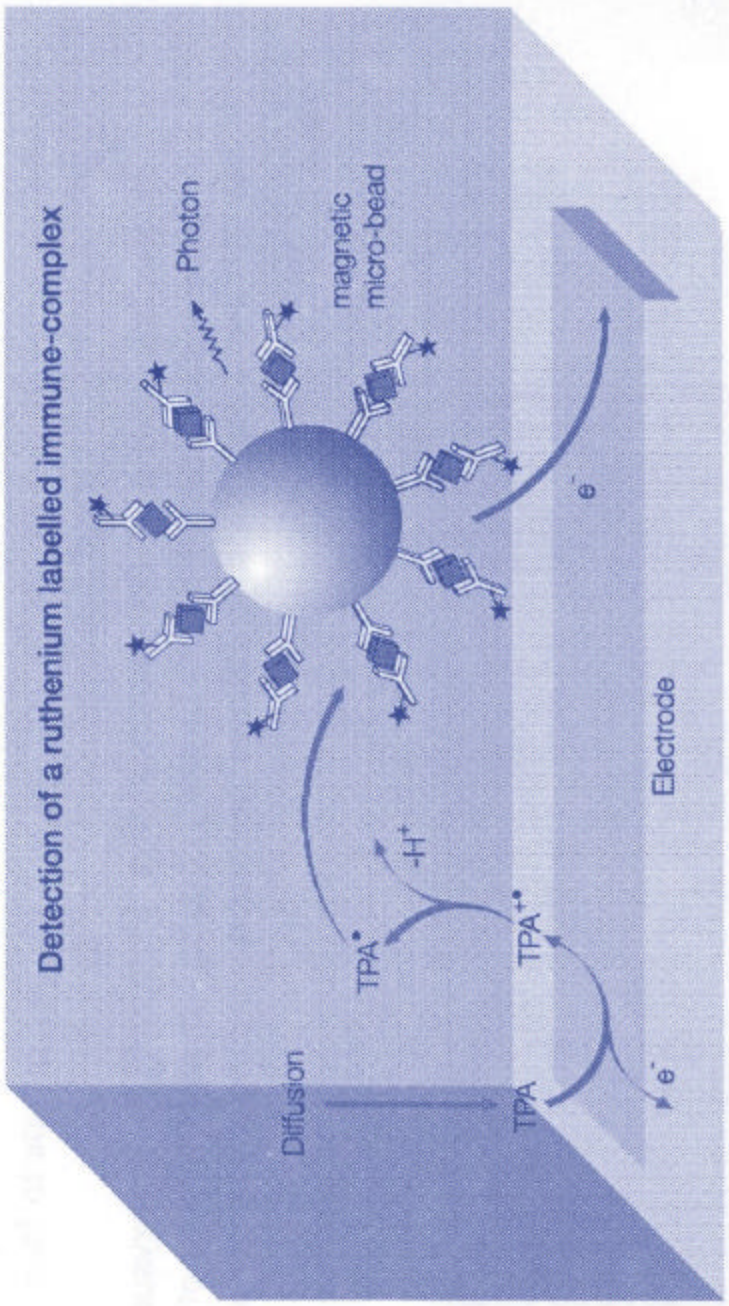


Fig. 30 Ruthenium and Labelled Immune Complex.

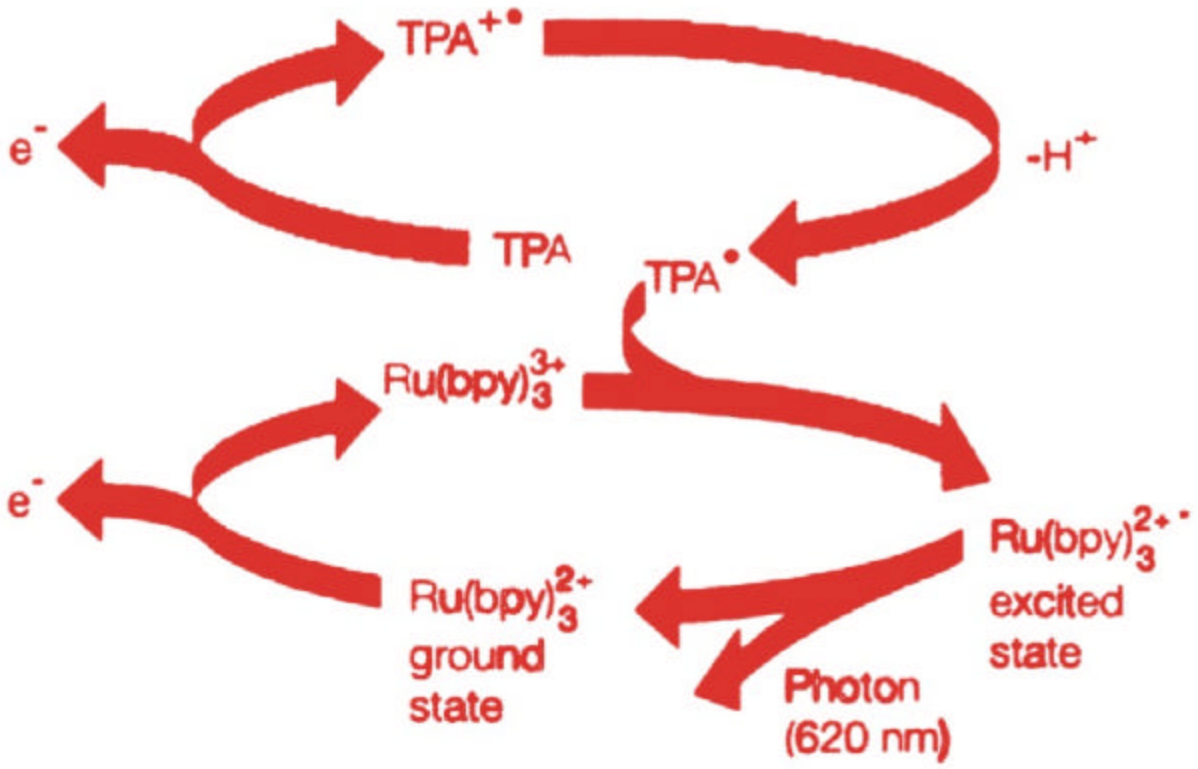


Fig. 31 ECL Technology

ECL MEASURING CELL

The core of the system is the ECL measuring cell, which is designed as a flow-through cell. Essentially, three operating steps are performed in the measuring cell.

Bound/Free Separation

Using a magnet, the streptavidin microparticles, that are coated with antigen-antibody complexes, are uniformly deposited on the working electrode. The magnet is located below the working electrode. A system buffer (ProCell) is used to wash the particles on the working electrode and to flush out the excess reagent and sample materials from the measuring cell.

ECL Reaction

A voltage is then applied to the electrode on which the microparticles, coated with antigen-antibody complexes, are deposited to initiate the ECL reaction. The light emission is measured with a photomultiplier. The system then uses the corresponding signals for the calculation of results.

Release of Microparticles and Cell Cleaning

Once the measurement is completed, the magnetic microparticles are washed away from the electrode surface with a special cleaning solution (CleanCell). The surface of the measuring cell is regenerated by varying the potential on the electrode. The cell is then ready for another measurement (Blackburn et al, 1991; Hoyle, 1994; Kenten et al, 1991; Leland and Powell, 1990; Obeng and Bard, 1991; Xu and Bard, 1994; Reference Guide Elecsys 1010, 1996).

APPENDIX – XV

ESTIMATION OF FOLLICLE STIMULATING HORMONE (FSH)

FSH was estimated by electrochemiluminescence immunoassay (ECLIA) in Elecsys 1010, Roche diagnostics, Germany. As FSH molecular weight is more than 5000 Daltons the sandwich principle (Figure. 32) was selected for assay.

Principle

In the first incubation 40 ìl of sample, biotinylated monoclonal FSH specific antibody and a monoclonal FSH specific antibody labeled with ruthenium complex reacts to form a sandwich.

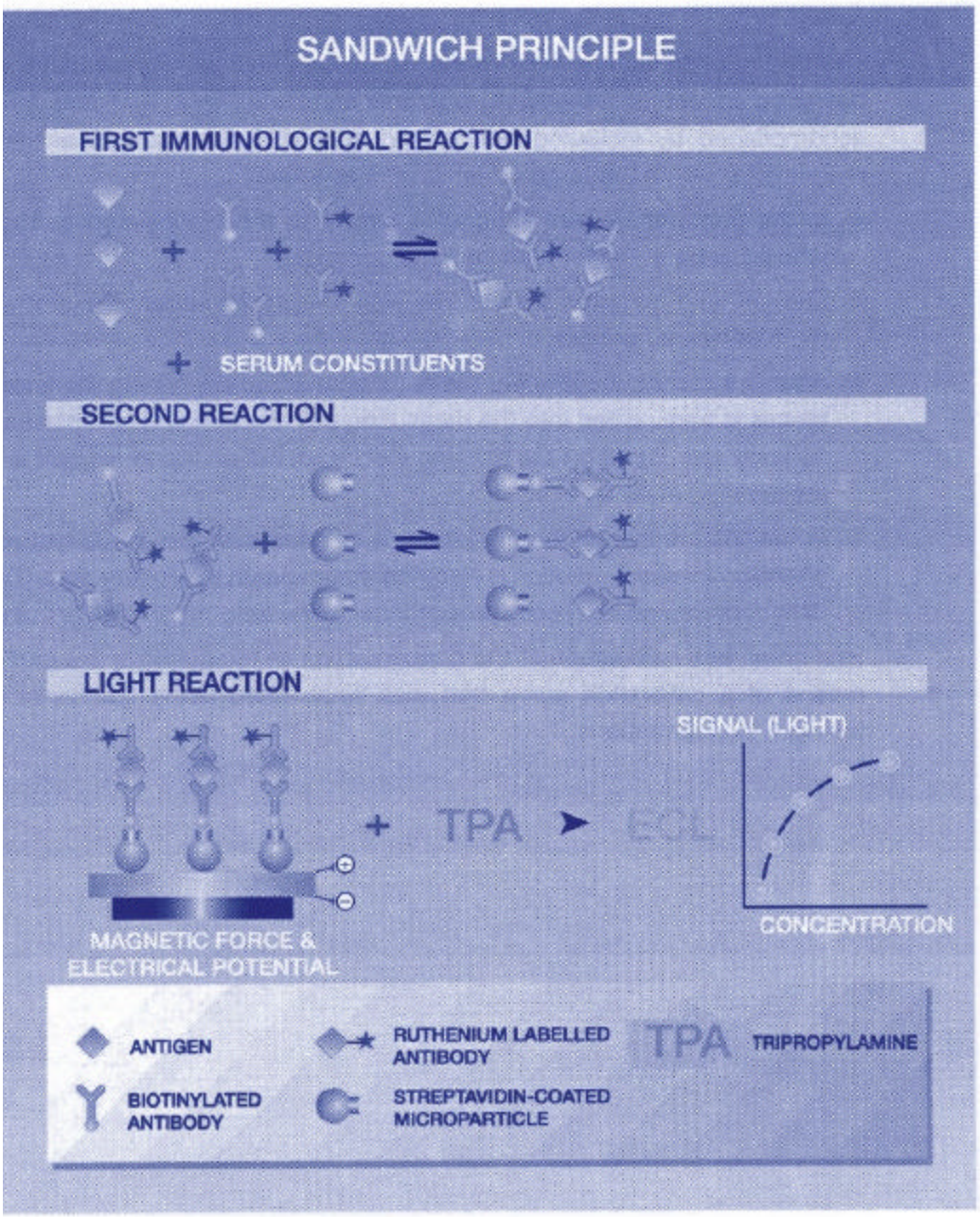


Fig. 32 Sandwich Principle.

In the next incubation streptovidin coated microparticles are added to the complex. These begin to bind to the solid phase via interaction of biotin and streptovidin.

The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are removed with procell. Application of voltage to the electrode induces chemiluminescent reaction which is measured by the photomultiplier.

Calibration

The results are determined via a calibration curve which is specifically generated by 2-point calibration and a master curve provided via the reagent bar code.

FSH has been calibrated using the 2nd RP WHO (Reference Preparation, World Health Organization) reference Standard 78/549.

Quality Control

Before running the study group samples the Precicontrol-U1 normal control and Precicontrol-U2 pathological control were checked.

Elecsys 1010 automatically measured and calculated the FSH concentration and the results were given in mIU/ml (milli International Unit / millilitre) (Blackburn et al, 1991; Hoyle, 1994; Kenten et al, 1991; Leland and Powell, 1990; Obeng and Bard, 1991; Xu and Bard, 1994; Reference Guide Elecsys 1010, 1996).

APPENDIX – XVI

ESTIMATION OF LUTEINIZING HORMONE

LH was estimated by ELISA method. Since, LH has a molecular weight more than 5000 Daltons, sandwich principle has been preferred.

Coba Core II

In Cobas Core II immunoassay auto analyzer a bead solid phase and an enzyme mediated chromogen reaction are employed for the signal production and measurement (EIA).

Technology

The assay test substance (antigen) specifically reacts and binds with the antibody. The specific antibody has specific affinity towards antigen. The

antibody is coated in the glass beads. The conjugate, a specific antibody or antigen which is labeled with horse radish peroxidase enzyme in Cobas Core II serves as the detection system.

Horse Radish Peroxidase (HRP) catalyses the oxidation of the substrate 3,3,5-5-tetramethyl benzidine (TMP) with hydrogen peroxide. The intensity of the resulting color is proportional to the amount of HRP bound to the bead and therefore proportional to the analyte concentration (sandwich tests) (Cobas Core II Method Manual, 2000).

The study groups LH tests were done in Cobas Core II, Roche Germany. Since, LH has 29,500 Daltons molecular weight, the measurement of LH is based on the sandwich solid phase immunoassay principle.

In the first reaction samples, control, calibrators are incubated with a polystyrene bead coated with anti LH antibodies. The antibody has been selected to recognize the different LH subtypes. LH from the specimen is bound to the antibody on the bead. After a washing step, anti L-LH (Labeled) antibodies conjugated to the horse radish peroxidase are added. They react with the antigen bound in the first reaction and form the sandwich complex. After the second reaction, unbound anti L-LH peroxidase is removed by washing and the bound enzyme is reacted with the Cobas Core substrate. The intensity of the resulting color is proportional to the amount of LH in the specimen.

Calibration

The Cobas Core LH EIA II has been calibrated with the WHO 2nd International Standard for Luteinizing hormone Pituitary (code 80/552). Based on the above WHO reference, Roche diagnostics, Germany Specifies a master calibration curve values for each lot of kit was produced. These master calibrator curve values are provided in the kit and are entered into the system. A comparative check is performed against the kit control.

Calculation

The Cobas Core II automatically calculates the results using the individual calibration curve and the values are expressed as mIU/ml (Mitchell et al, 1995).

APPENDIX – XVII

ESTIMATION OF TESTOSTERONE

Testosterone is quantitatively determined by electrochemiluminescence immunoassay (ECLIA) method with Elecsys 1010, Roche diagnostics, Germany. Since Testosterone has a molecular weight less than 5000 Daltons the competitive test principle (Figure 33) is preferred.

Principle

The samples, calibrators and controls were incubated separately with testosterone specific biotinylated antibody and testosterone derivative labeled with a ruthenium complex. The binding sites of the labeled antibody became occupied partly by the sample analyte (depending on its concentration) and partly by the ruthenium labeled hapten to form the respective immunocomplexes.

In the next incubation the streptovidin-coated micro-particles are added and the entire complex becomes bound to the solid phase via interaction of biotin and streptovidin.

The reaction mixture was aspirated into the measuring cell where the microparticles were magnetically captured into the surface of the electrode. Unbound substances were then removed with pro-cell. Application of voltage to the electrode induces chemiluminescent emission which was measured by a photomultiplier.

The results were determined specifically by 2point calibration and a master curve provided via the reagent bar code.

Calibration

Testosterone was calibrated using isotope dilution gas chromatography mass spectrometry (ID-GS/MS) reference standard. The predefined master curve was adapted to the analyser by the use of Elecsys testosterone calset.

Quality Control

Testosterone values were crosschecked with Precicontrol-U1 normal control and Precicontrol-U2 pathological control before running the study group samples.

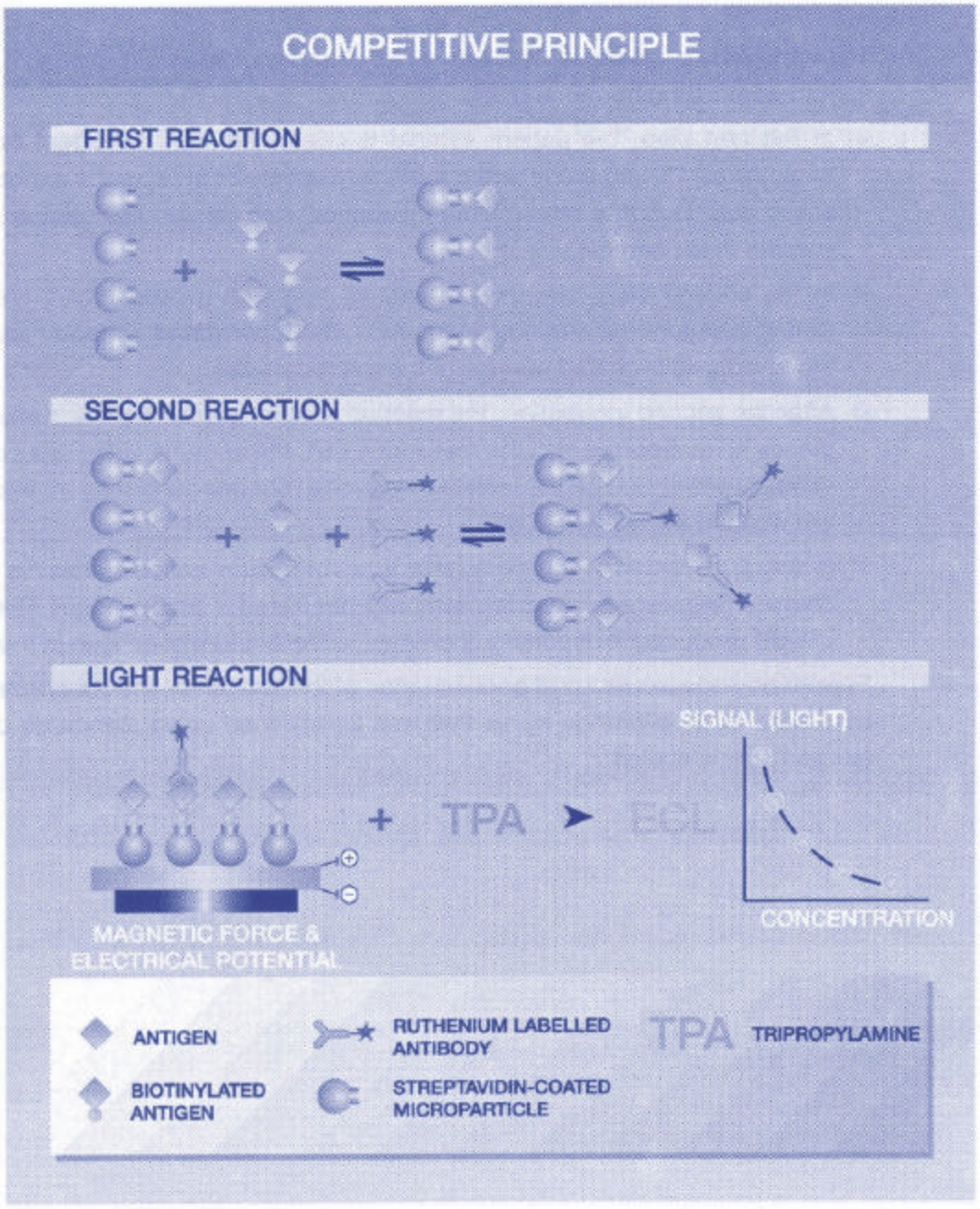


Fig. 33 Competitive Principle.

Calculation

Elecsys 1010 calculates the testosterone concentration of each sample and gives in nanogram/millilitre (ng/ml) unit (Blackburn et al, 1991; Hoyle, 1994; Kenten et al, 1991; Leland and Powell, 1990; Obeng and Bard, 1991; Xu and Bard, 1994; Reference Guide Elecsys 1010, 1996).

APPENDIX - XVIII

ESTIMATION OF PROLACTIN

Prolactin was estimated by electrochemiluminescence immunoassay (ECLIA) in Elecsys 1010, Roche diagnostics, Germany. As the prolactin molecular weight is more than 5000 Daltons the sandwich principle (Figure. 32) is selected for assay.

Principle

The assay sample, calibrators and controls were mixed separately with biotinylated monoclonal prolactin-specific antibody and monoclonal prolactin specific antibody labeled with a ruthenium complex form an antibody antigen antibody sandwich complex. After the addition of streptovidin labeled microparticles, the complex produced was bound to the solid phase via biotin-streptovidin interaction.

The reaction mixture was aspirated into the measuring cell where the microparticles were magnetically captured into the surface of the electrode. Unbound substances were then removed with procell. Application of a voltage to the electrode induces chemiluminescent emission which was measured by the photomultiplier.

Calibration

Results were determined via calibration curve. This curve was specifically generated by 2-point calibration and a master curve provided via the reagent bar code.

Prolactin test has been calibrated against the WHO 3rd IRP (International Reference Preparation) 84/500. The predefined master curve was adapted to the analyser by the use of the Elecsys Prolactin Calset.

Quality Control

Prolactin values were cross checked with Precicontrol-U1 normal control and Precicontrol-U2 pathological control before running the study group samples.

Elecsys 1010 automatically calculated the prolactin concentration of each sample and values given in nanogram/millilitre (ng/ml) (Blackburn et al, 1991; Hoyle, 1994; Kenten et al, 1991; Leland and Powell, 1990; Obeng and Bard, 1991; Xu and Bard, 1994; Reference Guide Elecsys 1010, 1996).

APPENDIX - XIX

ESTIMATION OF ESTRADIOL

Estradiol is a steroid hormone having a molecular weight of 272 Daltons. This hormone was estimated by using electrochemiluminescence immunoassay (ECLIA) in Elecsys 1010, Roche diagnostics, Germany. Competitive test principle (Figure 33) was employed using a polyclonal antibody.

Principle

Estradiol assay sample, calibrators and control serum were incubated separately with estradiol specific biotinylated antibody, an immune complex was formed, the amount of which was dependent upon the analyte concentration in the sample.

After the addition of streptovidin-coated micro-particles and estradiol derivative labeled with a ruthenium complex, the still vacant sites of the biotinylated antibodies became occupied with formation of an antibody hapten complex. The entire complex became bound to the solid phase via interaction of biotin and streptovidin.

The mixture was aspirated into the measuring cell where the microparticles were magnetically captured onto the surface of the electrode. Unbound substances were then removed with procell. Application of a voltage to the electrode induces chemiluminescent emission which was measured by a photomultiplier.

Results were determined via a calibration curve which was specifically generated by 2-point calibration and a master curve provided via the reagent bar code.

Calibration

Estradiol was calibrated using isotope dilution gas chromatography mass spectrometry (ID-GS/MS). The predefined master curve was adapted to the analyser by the use of Elecsys estradiol II calset II.

Quality Control

Estradiol values were crosschecked with Precicontrol-U1 normal control and Precicontrol-U2 pathological control before running the study group samples.

Calculation

Elecsys 1010 automatically calculates the estradiol concentration of each sample and gives in picogram/millilitre (pg/ml) unit (Blackburn et al, 1991; Hoyle, 1994; Kenten et al, 1991; Leland and Powell, 1990; Obeng and Bard, 1991; Xu and Bard, 1994; Reference Guide Elecsys 1010, 1996).

APPENDIX - XX

ESTIMATION OF PROGESTERONE

Progesterone was estimated by electrochemiluminescence immunoassay (ECLIA) with Elecsys 1010, Roche diagnostics, Germany. Since steroid hormone progesterone has a molecular weight of 314.5 Daltons, competitive principle (Figure 33) has been employed.

Principle

In the presence of biotinylated monoclonal progesterone specific antibody and a progesterone derivative labeled with ruthenium complex were incubated with samples / control / calibrators.

Progesterone from the sample competed with the labeled progesterone derivative for the antibody sites.

In the second incubation after the addition of streptovidin coated microparticles, the entire complex became bound to the solid phase via interaction of biotin and streptovidin. The proportion of labeled progesterone derivative bound to the solid phase is inversely proportional to the progesterone content of the sample.

The reaction mixture was aspirated into the measuring cell where the microparticles were magnetically captured onto the surface of the electrode. Unbound substances were then removed by procell. Application of a voltage

to the electrode induces chemiluminescent emission which was measured by a photomultiplier.

Results were determined via a calibration curve which was specifically generated by 2-point calibration and a master curve provided via the reagent bar code.

Calibration

Progesterone test was calibrated by using isotope dilution gas chromatography/mass spectrometry (ID-GC/MS) reference standard. The predefined master curve was adapted to the analyser by the use of Elecsys Progesterone calset.

Quality Control

Progesterone value was crosschecked with Precicontrol-U1 normal quality control and Precicontrol-U2 pathological quality control before running the study group samples.

Calculation

Elecsys 1010 automatically calculates the progesterone concentration and the values were expressed as nanogram/millilitre (ng/ml) unit (Blackburn et al, 1991; Hoyle, 1994; Kenten et al, 1991; Leland and Powell, 1990; Obeng and Bard, 1991; Xu and Bard, 1994; Reference Guide Elecsys 1010, 1996).

APPENDIX – XXI

MAKLER COUNTING CHAMBER METHOD OF SPERMATOZOA CONCENTRATION ESTIMATION

The Makler chamber is a device simple to use for rapid sperm count and motility evaluation directly from undiluted semen sample. The Makler counting chamber comprises of two parts (1) the lower metal base piece and (2) the upper cover glass. The lower part has a metal base and two handles. At the center of the base is a flat disc on which the sample is placed. On the periphery of the discs there are four quartz-coated pins elevated by 10 microns above the surface of the central part. The upper part is the cover glass. At the center of its lower surface there is a one mm^2 grid, subdivided into 100 squares each 0.1 x 0.1 mm. When the cover glass is placed on the four tips, the space bounded by the two surfaces and any row of 10 squares of the grid is exactly 0.001 mm^3 or one millionth of ml.

Preparation of the Chamber

Before the chamber is used, it should be made certain that the opposed glass surfaces are absolutely clean and free of dust. Placing the cover glass on the four tips and observing for color fringes at the four contact points, the cleanliness is tested. They can be seen best against reflected fluorescent light.

SEMEN MICROSCOPIC ANALYSIS

Well-mixed semen sample was placed on the chamber and grasping opposite the two dark points the cover glass was placed. This ensured automatic spreading of the drop into a thickness of 10 microns. The chamber was then placed on the stage of the microscope and observed under 20x objective.

ESTIMATION OF SPERM TOTAL COUNT

Sperms in a strip of 10 squares were counted and the concentration was represented in the unit of million sperms per millilitre. When the sperm count was too dense, they were immobilized by transferring a part of the specimen into another test tube and warming them in a water bath at 50ºC to 60ºC. The

preheated specimen was then mixed and a drop was placed on the chamber and counted the sperms as described above (Makler, 1978).

APPENDIX – XXII

ESTIMATION OF LDL CHOLESTEROL, VLDL CHOLESTEROL AND PHOSPHOLIPIDS

LDL cholesterol, VLDL cholesterol and phospholipids were calculated from the estimations of cholesterol, triglycerides and HDL cholesterol by using the standard formulas (Varley et al,1980b; Rifai et al, 2001).

$$\text{VLDL cholesterol} = \frac{\text{Triglyceride}}{5}$$

LDL cholesterol = Cholesterol – (HDL cholesterol + VLDL cholesterol)

Phospholipids = (Cholesterol x 0.89) + 68.

All the values were expressed as mg/dl.

8. QUESTIONNAIRE BOOK LET

EFFECT OF CHRONIC ALCOHOLISM AND SMOKING IN MALE REPRODUCTIVE FUNCTION

COMPREHENSIVE DRINKERS AND SMOKERS PROFILE

1). Patient Id.No.

2). Address:
Temporary:
Permanent:
Phone Numbers:
Contact Person:

3). Age:

4). Educational Qualification And Occupation:

5). Marital Status:
Date Of Marriage

6). Any Infertility Treatment Taken By The Couples:

7). Children
Name: Age

8). Family Status:
Monthly In Come:

9). Diet Habits:

DRINKING HISTORY

10). About how old were you when you first took drinks:

11). From that whether frequently drinking:

12). What brand of drinks taking:
Past:
Present:

13). What is the frequency in last twelve months:

14) what is the frequency of drinking in the last three months:

Weekly: Once

Twice

Thrice

Four Times

Five Times

Six Times

Daily

15). Pattern of drinking in drinking day (24 Hrs)
Morning Afternoon Evening Night

16). Average volume of drinks consumed per day:

17). Percentage of alcohol content in the brand used:

18). History of drinks induced any diseases:
Diagnosis:
Treatment taken:
Duration of treatment:

SMOKING HISTORY

19). Do you smoke:

20). About how old were you when you first took smoke:

21). From that whether frequently smoking. Specify the frequency:

22). What brand of smoke using
Past:
Present:

23). What was the frequency of the smoking in the past Twelve months:
Daily how many smokes:
Brand wise

24). Smoking only at the time of alcohol in take or always:

25). Average number of smokes per day:

26). Average nicotine content in the brand:

27). Smoke induced any diseases:
Disease:
Treatment:
Duration of treatment:

SEXUAL HISTORY

28). Sexual details:

Before Smoke / Alcohol

After Smoke / Alcohol

Libido details:

Potency and orgasm (duration of erection -time taken for ejaculation):

Frequency of
Intercourse in the last 30 days:

(Marlatt and Miller, 1984).

EVALUATION SHEET

1). Sample Group

2) Sample collected on

3) Sample processed on

4) Routine Parameters Values

(Biochemical and Haematological)

5) Physician Remarks

6) Hormone Assay Value

7) Semen Analysis

Effect of chronic alcoholism on male fertility hormones and semen quality

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The Tamil Nadu Dr. M.G.R. Medical University, Chennai, India

Objective: To evaluate the effects of chronic alcoholism on the male fertility hormones and quality of semen.
Design: Non-probability purposive clinical study.
Setting: Addiction treatment center and an academic research environment.
Patient(s): Sixty-six alcoholics free from smoking and drug abuse who consumed a minimum of 180 mL of alcohol per day (brandy and whisky, both 40%–50% alcohol content) for a minimum of 5 days per week for ≥1 year were included. Thirty nonsmoking nonalcoholics were selected as controls.
Intervention(s): Before starting the addiction treatment for alcoholics, venous blood and semen samples were collected.
Main Outcome Measure(s): Complete blood counts, biochemical parameters, levels of the male fertility hormones FSH, LH, T, PRL, P, and E_2 in blood, and semen parameters.
Result(s): In alcoholics, FSH, LH, and E_2 levels were significantly increased, and T and P levels were significantly decreased. No significant change was noted in PRL levels. Semen volume, sperm count, motility, and number of morphologically normal sperm were significantly decreased.
Conclusion(s): Chronic alcohol consumption has a detrimental effect on male reproductive hormones and on semen quality. (Fertil Steril® 2005;84:919–24. ©2005 by American Society for Reproductive Medicine.)

Key Words: Alcohol, FSH, LH, testosterone, progesterone, estradiol, semen, sperm

Impotence, testicular atrophy, gynecomastia, and loss of sexual interest are often associated with alcoholism in men (1). Sexual disorders have been reported in men who are long-term alcohol users, with the prevalence ranging from 8% to 58% (2). Lemere and Smith (3) reported that 8% of 17,000 patients treated for alcoholism were impotent. The reported prevalence of lack of sexual desire ranged from 31% to 58% in long-term alcohol users (4–6). Fifty-four percent of hospitalized alcoholic men and 24% of healthy controls had erectile impotence (4). In 1984, Jensen (5) reported that 63% of married alcoholic men and 10% of controls had sexual dysfunction, especially lack of sexual desire.

Use of ethanol might cause gonadal disorders, including structural testicular changes and a decrease in testicular and serum levels of T, which might be involved in the hypogonadism and feminization phenotype. Ethanol and its metabolite acetaldehyde cause a reduction in LH binding to Leydig cells, an inhibition of the enzymes responsible for the formation of sex hormones (7, 8).

Van Thiel et al. (9) demonstrated that ethanol acts as a Leydig cell toxin. Moreover, ethanol increases the metabolic clearance rate of T concomitant with an increase in hepatic 5α-reductase activity and increases conversion of androgens into estrogens. Sperm cells might be selectively affected by various substances throughout the process of spermatogenesis to spermiogenesis (10).

Both acute and chronic alcohol intoxication result in dose-dependent suppression of plasma T levels in normal men (11, 12). Alcohol-induced suppression of male T is due to a direct effect on the biosynthetic processes in the testes (13–16). Increased LH levels after alcohol-induced suppression of T in men (11, 12) and male monkeys (17) is consistent with established mechanisms of negative feedback of LH secretory activity.

Alcohol seems to exert a dual effect on the hypothalamic–pituitary–gonadal axis by directly inhibiting testicular steroidogenesis and by blocking the release of LH-releasing hormone/LH from the hypothalamic–pituitary axis (18).

In human semen, ethanol produces a significant decrease in the percentage of motility, straight-line velocity, and curvilinear velocity of sperm. Alcohol causes a significant decrease in the number of spermatozoa with normal morphology and an increase in irreversible tail defects (19).

The sperm of ethanol-consuming animals exhibit alterations in their spermatozoa concentration, abnormal motility and morphology, and a decrease of the fecundation capability (20). It has been reported that ethanol abusers might exhibit sperm alterations, such as changes in the count, morphology, and viability of the spermatozoa (21–23). Alcohol exerts a dose-related toxic effect on testicular function. Spermatogenesis disruption and a primary testicular insuffi-

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ciency and compensatory increase of FSH and LH secretion have been observed in alcoholics (24, 25).

A reduction in sperm concentration and in the percentage of spermatozoa with normal morphology has been detected in chronic alcoholics and in smokers. The above modifications suggest a synergistic or additive effect of both toxic habits on male reproductive function. Men who wish to procreate should be specifically warned of this matter (26).

Drinking alcohol is considered a common social entertainment. In the present study, reproductive function in chronic alcoholics was assessed to know the effects of alcoholism on reproductive function. The intent of the study was also to help physicians treating alcoholics to have a better idea of these patients' reproductive function.

MATERIALS AND METHODS

Subjects

This study was conducted at the Kasthuriba Gandhi Memorial Deaddiction Center in Coimbatore city, Tamil Nadu, India. We screened a total of 1,300 alcoholics who had reported to the Kasthuriba Gandhi Memorial Deaddiction Center and 300 nonalcoholic nonsmoking volunteers (as controls) from Coimbatore city. The study population consisted of 66 nonsmoking alcoholics, aged 36.6 ± 5.7 years (mean ± SD). Alcoholics consuming drugs like diazepam, pethidine, cannabis, and marijuana along with alcohol were excluded from the study. The control population consisted of 30 normal healthy persons aged 35.0 ± 6.1 years.

All subjects were examined by a physician before inclusion in the study. Personal interviews were conducted with all alcoholic and control subjects to obtain relevant clinical data: age, sex, domicile (urban vs. rural dwelling), marital status, diet, history of alcohol consumption, infertility status, past medical illness and treatment, history of smoking, sexual urgency and frequency, and premarital and extramarital sexual history. Sexual function (e.g., erectile function, libido potency, frequency of ejaculation) was also noted in the questionnaire.

Experimental Design

The study included two subject groups, controls and alcoholics. Subjects in the control group were volunteers who were free from any disease and who had never consumed alcoholic drinks and who had never smoked. Subjects in the alcoholic group were nonsmokers who had consumed a minimum of 180 mL of alcohol (brandy and whisky, both 40%–50% alcohol content) per day for a minimum of 5 days per week in the past year.

Seminal Parameters

Semen samples were collected after at least 48 hours but no more than 7 days of sexual abstinence. The semen sample was collected by masturbation and delivered to the laboratory within ½ hour from the time of collection. After liquefaction, semen appearance, volume, consistency, pH, fructose, and sperm motility, concentration, viability, and morphology were analyzed as per the criteria of the World Health Organization (27). Motility was expressed as percentages of rapid progressively motile, slow or sluggishly motile, nonprogressively motile, and immotile sperm. Sperm viability was expressed as percentages of live and dead sperm, and sperm morphology was expressed as percentages of sperm with normal morphology, head-defective morphology, neck-defective morphology, and tail-defective morphology.

Male Fertility Hormones

Ten milliliters of venous blood was collected, and 5 mL of blood was transferred into a clean conical centrifuge tube with no anticoagulant. Serum was separated and stored at −20°C until use. The remaining 5 mL of blood was added to a Vacutainer tube containing ethylenediaminetetraacetic acid, and the complete hemogram in the blood was analyzed with Cell Dyn 1700 (Abbott Laboratories, Abbott Park, IL).

The routine biochemical parameters were analyzed with Hitachi 912 (Roche Diagnostics, Penzberg, Germany). Serum levels of FSH, T, E_2, P, and PRL were measured by the electro-chemiluminescence immunoassay method (Elecsys 1010; Roche Diagnostics), and LH was analyzed by ELISA (Cobas Core II; Roche Diagnostics). All the results were expressed in conventional units.

The results for both groups are expressed as mean ± SD. The results were analyzed statistically with commercial software (SPSS for Windows 7.5.1; SPSS, Chicago, IL). Student's *t*-test was used to determine the degree of significance for the various mean variables obtained. Semen nonparametric values (liquefaction, appearance, volume, consistency, pH, and fructose) were analyzed with the χ^2 test.

RESULTS

For alcoholics, the mean number of days of alcohol consumption per week was 6.1 ± 1.1, for a mean period of 4.5 ± 2.9 consecutive years. The mean volume of alcohol consumption was 441.1 ± 323.9 mL/day.

No significant differences were found between alcoholics and controls for any of the routine biochemical parameters (blood glucose, creatinine, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, very-low-density lipoprotein cholesterol, total bilirubin, total protein, albumin, albumin/globulin ratio, γ glutamyl transferase, alkaline phosphatase, and serum glutamate pyruvate transaminase). Neither were any significant differences found between the two groups for any of the hematologic parameters (hemoglobin, total white blood corpuscle count, differential white blood corpuscle count, platelet count, total red blood corpuscle count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and packed cell volume).

TABLE 1

Fertility hormone levels in controls and alcoholics.

Hormone	Controls (n = 30)	Alcoholics (n = 66)	*t* value
FSH (mIU/mL)	4.60 ± 1.94	7.41 ± 4.16	4.512[a]
LH (mIU/mL)	4.69 ± 2.43	7.22 ± 3.07	4.329[a]
PRL (ng/mL)	19.33 ± 5.03	18.37 ± 12.40	0.536 (NS)
T (ng/mL)	5.88 ± 2.07	4.41 ± 1.03	3.714[a]
E_2 (pg/mL)	34.28 ± 12.39	52.35 ± 28.71	4.306[a]
P (ng/mL)	0.71 ± 0.25	0.52 ± 0.20	3.737[a]

Note: Values are expressed as mean ± SD. NS = nonsignificant.
[a] $P<.001$.

Muthusami. Male fertility profile in alcoholism. Fertil Steril 2005.

Male Fertility Hormones

Regarding the correlation between libido and T, alcoholics showed decreased libido due to decreased levels of T, but controls showed no significant decrease in libido and T. It was also found that 71% of alcoholics and 7% of controls had erectile impotence.

Alcoholics showed a statistically significant increase in FSH ($P<.001$) and LH ($P<.001$) levels compared with controls. Prolactin ($P<.536$) did not show any statistically significant change in alcoholics compared with controls (Table 1).

The mean T ($P<.001$) and P ($P<.001$) levels in the alcoholic group showed statistically significant decreases compared with the control group. The mean E_2 ($P<.001$) level in alcoholics showed a statistically significant increase compared with controls.

Seminal Parameters

In the alcoholic group, sperm count ($P<.001$), percentage of rapid progressively motile sperm ($P<.001$), percentage of live sperm ($P<.001$), and percentage of morphologically normal sperm ($P<.001$) were statistically significantly decreased compared with the control group (Table 2).

The percentages of slow progressively motile sperm ($P<.001$), nonprogressively motile sperm ($P<.001$), immotile sperm ($P<.001$), dead sperm ($P<.001$), head-defective sperm ($P<.001$), neck-defective sperm ($P<.001$), and tail-defective sperm ($P<.022$) were all statistically significantly increased compared with the control group. Chi-square values for semen appearance, pH, viscosity, liquefaction, and fructose between controls and alcoholics did not show any statistical significance.

TABLE 2

Seminal parameter values in controls and alcoholics.

Seminal parameter	Controls (n = 30)	Alcoholics (n = 66)	*t* value
Semen volume (mL)	2.17 ± 0.712	1.56 ± 0.79	3.761[a]
Sperm count ($\times 10^6$/mL)	132.97 ± 89.02	51.99 ± 44.71	4.719[a]
Rapid progressively motile sperm (%)	56.10 ± 8.72	30.38 ± 15.82	10.229[a]
Slow progressively motile sperm (%)	21.48 ± 2.76	29.02 ± 8.37	6.571[a]
Nonprogressively motile sperm (%)	2.50 ± 1.07	4.10 ± 2.21	4.782[a]
Immotile sperm (%)	20.17 ± 8.25	35.91 ± 13.01	7.161[a]
Live sperm (%)	80.80 ± 6.29	60.20 ± 16.61	8.783[a]
Dead sperm (%)	19.20 ± 6.29	39.77 ± 16.62	8.767[a]
Morphologically normal sperm (%)	82.00 ± 9.76	67.17 ± 16.73	5.447[a]
Head-defective sperm (%)	11.30 ± 7.81	18.27 ± 9.43	3.791[a]
Neck-defective sperm (%)	4.27 ± 2.70	9.05 ± 5.82	5.493[a]
Tail-defective sperm (%)	2.43 ± 1.68	5.56 ± 5.02	2.347[b]

Note: Values are expressed as mean ± SD. NS = nonsignificant.
[a] $P<.001$.
[b] $P<.01$.

Muthusami. Male fertility profile in alcoholism. Fertil Steril 2005.

DISCUSSION

It is evident from the report of the physician and from the biochemical and hematologic analysis that all the alcoholics and controls in this study were free from diabetes mellitus, hypertension, renal failure, liver failure, anemia, malnutrition, and chronic infections.

Sexual disorders have been reported frequently in chronic alcoholics. The present study on decreased libido and erectile impotence is supported by the studies of Mulligan et al. (28), Rosen (29), and Gumus et al. (6), which showed that high levels of blood alcohol cause reduced sexual stimulation, inability to enjoy orgasm, and retarded ejaculation. Whalley (4) reported that 54% of hospitalized alcoholic men and 24% of healthy controls had erectile impotence. Jensen (5) reported that 63% of married alcoholic men and 10% of controls had sexual dysfunction due to lack of sexual desire.

The present study showed increased FSH levels in alcoholics. It is the direct toxic effect of alcohol on the testis that leads to decreased seminiferous tubular function. The FSH elevation is due to the absence of testicular feedback regulation at the pituitary level.

The present results correlate with those of Gumus et al. (6), who reported that serum FSH levels are higher in chronic alcoholics. Similar findings have been reported by Van Thiel et al. (30), who found that FSH levels increased in alcohol-fed animals. The decreased total sperm count in semen found in the present study is also confirmed by Lindholm et al. (24), who found increased FSH levels in alcoholics with severe spermatogenesis disruption.

Because alcohol enters into the testis directly, causing decreases in spermatogenesis and T synthesis, it causes the increased level of LH found in chronic alcoholics. The results of this study are supported by those of Heinz et al. (31), Sengupta et al. (32), and Bannister and Lowosky (7), who found that LH levels were increased in chronic alcoholics.

In the present study, the decreased T and increased LH levels in alcoholics suggest that the major effect of alcohol on plasma T in humans is exerted on the testis at a peripheral site rather than on the hypothalamic–pituitary axis at a central site. This finding is supported by those of Mendelson et al. (11), Gordon et al. (33), Lester and Van Thiel (34), and Van Thiel et al. (35), who reported that decreased T levels were accompanied by increased LH levels in alcoholics.

The present study demonstrates that alcohol does not produce any change in PRL levels. This shows that alcohol in the chronic state acts at the peripheral testis level rather than at the central hypothalamic–pituitary level. Contrary to this, Ching and Lin (36) reported that alcohol could increase dopamine stores in the median eminence. An increased dopamine signal from hypothalamic neurons might have suppressed PRL secretion by the pituitary lactotrophs. Decreased plasma T levels, as found in the present study, correlate with study results from Van Thiel (37) and Ida et al. (38).

In alcoholics, decreased T levels are due to decreased synthesis of T in the testis or to increased metabolic clearance of T. Increased metabolic clearance of T by the stimulation of aromatase, a key enzyme in the conversion of T to E_2, leads to an increase in E_2 levels. The statistically significant increase of E_2 levels in chronic alcoholics in the present study is supported by the findings of Van Thiel et al. (39), Gordon et al. (40), Van Thiel et al. (34), and Emanuele et al. (41).

Decreased P levels in chronic alcoholics are due to decreased action or synthesis of 3β hydroxysteroid dehydrogenase, a rate-limiting enzyme for the conversion of pregnenolone to P. It is evident that the sites of alcohol-induced inhibition in the steroidogenic pathway observed in vitro are the reactions from pregnenolone to P by the nicotinamide adenine–dependent 3β-hydroxysteroid dehydrogenase/oxosteroid isomerase and from androstenedione to T by the nicotinamide adenine dinucleotide phosphate–dependent 17-ketosteroid reductase (42–45).

Progesterone is the precursor for the synthesis of T. Alcohol might suppress the action of the enzyme 3β hydroxysteroid dehydrogenase responsible for the synthesis of progesterone and T. The inadequate availability of P might lead to decreased synthesis of T. It is evident from this study that alcohol induces a bidirectional effect on T reduction, by [1] decreasing the synthesis of T in testis by decreasing the progesterone, and/or by [2] enhancing T metabolism.

Because the present study finds no statistically significant changes in alcoholics' semen appearance, pH, viscosity, liquefaction, and fructose, the accessory sex organs ductuli efferentes, seminal vesicle, prostate, bulbourethral (Cowper) gland, and urethral (Littre) gland are found to have normal functions.

The decreased sperm count found in the present study is supported by Brzek (46), who found that alcohol consumption decreases semen volume, density, and motility. The abnormal sperm morphology noted in the present study is supported by Goverde et al. (23), who found that daily alcohol consumption decreases normal sperm morphology. The statistically significant decrease in sperm count in the present study is confirmed by Carlsen et al. (47), who found a significant decrease in mean sperm count, from 113 $\times$ 10^6/mL to 66 $\times$ 10^6/mL.

Gomathi et al. (22) and Goverde et al. (23) reported decreased sperm motility in alcoholics, which is in accordance with the findings of the present study. Goverde et al. (23) observed a significant decrease in the viability of sperm in alcoholics, and this has been confirmed by the present study. The decreased number of morphologically normal sperm and increased number of morphologically defective sperms as found in the present study are supported by the findings of Donnelly et al. (19), Gomathi et al. (22), and Nagy et al. (21), who reported that alcohol consumption decreases the number of sperm with normal morphology

and increases the number of sperm with morphological abnormalities.

CONCLUSIONS

It is evident from the results of the present study that chronic alcoholism suppresses both blood hormonal levels and reduces semen quality. In Leydig cells, alcohol decreases the male sex hormone T. Furthermore, it decreases P and increases E_2 levels in blood. Because of the suppression of blood levels of T in alcoholics, the pituitary feedback regulation is altered. The pituitary LH is increased in blood as a compensatory mechanism. At the seminiferous tubular level, alcohol decreases semen volume, total sperm concentration, motility of sperm, number of morphologically normal sperm, and viability of sperm. The pituitary FSH level in blood is increased because of the loss of seminiferous tubular function in alcoholics.

This study has proved beyond doubt that chronic alcohol consumption has a detrimental effect on male reproductive hormones and on the quality of semen, which, in turn, will make people who are addicted to alcohol impotent and sterile. Hence, men are advised to refrain from chronic alcohol consumption if they want to procreate and lead a normal sexual life.

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REFERENCES

1. Boyden TW, Pamenter RW. Effect of ethanol on the male hypothalamic-pituitary-gonadal axis. Endocr Rev 1983;4:389–95.
2. Schiavi RC. Chronic alcoholism and male sexual dysfunction. J Sex Marital Ther 1990;16:23.
3. Lemere F, Smith JW. Alcohol induced sexual impotence. Am J Psychiatry 1973;130:212–3.
4. Whalley LJ. Sexual adjustment of male alcoholics. Acta Psychiatr Scand 1978;58:281–98.
5. Jensen SB. Sexual function and dysfunction in younger married alcoholics. Acta Psychiatr Scand 1984;69:543–9.
6. Gumus B, Yigitoglu MR, Lekili M, Uyanik BS, Muezzinoglu T, Buyuksu C. Effect of long-term alcohol abuse on male sexual function and serum gonadal hormone levels. Int Urol Nephrol 1998;30:755–9.
7. Bannister P, Losowsky MS. Ethanol and hypogonadism. Alcohol Alcohol 1987;22:213–7.
8. Adler RA. Clinically important effects of alcohol on endocrine function. Clinical review 33. J Clin Endcrinol Metab 1992;74:957–60.
9. Van Thiel DH, Galaver PK, Cobb CF, Santucci L, Graham TO. Ethanol, a Leydig cell toxin: evidence obtained in vivo and in vitro. Pharmacol Biochem Behav 1983;18:317–23.
10. Insler V, Lunenfeld B. Infertility: the dimension of the problem. In: Insler V, Lunenfeld B, eds. Infertility: male and female. 2nd ed. London: Churchill Livingstone, 1993.
11. Mendelson JH, Mello NK, Ellingboe J. Effects of acute alcohol intake on pituitary-gonadal hormones in normal human males. J Pharmacol Exp Ther 1977;202:676–82.
12. Valimaki MJ, Pelkonen R, Harkonen M, Ylikahri R. Hormonal changes in noncirrhotic male alcoholics during ethanol withdrawal. Alcohol Alcohol 1984;19:235–42.
13. Anderson RA, Willis BR, Oswald C, Reddy JM, Beyler SA, Zaneveld LJD. Hormonal imbalance and alterations in testicular morphology induced by chronic ingestion of ethanol. Biochem Pharmcol 1980;29:1409–19.
14. Eriksson CJP, Widenius TV, Leinonen P, Harkonen M, Ylikahri RH. Inhibition of testosterone biosynthesis by ethanol. Biochem J 1983;210:29–36.
15. Willis BR, Anderson RA Jr, Oswald C, Zaneveld LJD. Ethanol-induced male reproductive tract pathology as a function of ethanol dose and duration of exposure. J Pharmacol Exp Ther 1983;225:470–8.
16. Mello NK, Mendelson JH, Bree MP, Ellingboe J, Skupny AST. Alcohol effects on luteinizing hormone and testosterone in male macaque monkeys. J Pharmacol Exp Ther 1985;233:588–96.
17. McCann SM. Regulation of secretion of follicle-stimulating hormone and luteinizing hormone. In: Greep RO, Astwood EB, Knobil E, Sawyer WH, Geiger SR, eds. Handbook of physiology. Washington, DC: American Physiological Society, 1974:489–517.
18. Little PJ, Adams ML, Cicero TJ. Effects of alcohol on the hypothalamic-pituitary-gonadal axis in the developing male rat. J Pharmacol Exp Ther 1992;263:1056–61.
19. Donnelly GP, McClure N, Kennedy MS, Lewis SE. Direct effect of alcohol on the motility and morphology of human spermatozoa. Andrologia 1999;31:43–7.
20. Anderson RA, Willis BR, Oswald C, Zaneweld JL. Ethanol-induced male infertility: impairment of spermatozoa. J Pharmacol Exp Ther 1983;225:479–86.
21. Nagy F, Pendergrass PB, Bown DC, Yeager JC. A comparative study of cytological and physiological parameters of semen obtained from alcoholics and non-alcoholics. Alcohol Alcohol 1986;21:17–23.
22. Gomathi C, Balasubramanian K, Bhanu NV, Srikanth V, Govindarajulu P. Effect of chronic alcoholism on semen studies on lipid profiles. Int J Androl 1993;16:175–81.
23. Goverde HJ, Dekker HS, Janssen HJ, Bastiaans BA, Rolland R, Zielhuis GA. Semen quality and frequency of smoking and alcohol consumption. An explorative study. Int J Fertil Menopausal Stud 1995;40:135–8.
24. Lindholm J, Fabricius-Bjerre N, Bahnsen M, Boiesen P, Bangstrup L, Pedersen ML, et al. Pituitary-testicular function in patients with chronic alcoholism. Eur J Clin Invest 1978;8:269–72.
25. Villalta J, Ballesca JL, Nicolas JM, Martinez de Osaba MJ, Antunez E, Pimentel C. Testicular function in asymptomatic chronic alcoholics: relation to ethanol intake. Alcohol Clin Exp Res 1997;21:128–33.
26. Martini AC, Molina RI, Estofan D, Senestrari D, de Cuneo FM, Ruiz RD. Effects of alcohol and cigarette consumption on human seminal quality. Fertil Steril 2004;82:374–7.
27. World Health Organization. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 4th ed. Cambridge, United Kingdom: Cambridge University Press, 1999.
28. Mulligan T, Retchin SM, Chinchilli V, Bettinger CB. The role of aging and chronic disease in sexual dysfunction. J Am Geriatr Soc 1988;36:520–4.
29. Rosen RC. Alcohol and drug effects on sexual response. Human experimental and clinical studies. Ann Rev Sex Res 1991;2:119–80.
30. Van Thiel DH, Galaver PK, Rosenblum E, Eagon YB. Effects of ethanol on endocrine cells: testicular effects. Ann N Y Acad Sci 1987;492:287–302.
31. Heinz A, Rommelspacher H, Graf KJ, Kurten I, Otto M, Baumgartner A. Hypothalamic-pituitary-gonadal axis, prolactin, and cortisol in alcoholics during withdrawal and after three weeks of abstinence: comparison with health control subjects. Psychiatry Res 1995;56:81–95.
32. Sengupta SN, Ray R, Shetty KT, Desai NG. Pituitary gonadal func-

tioning in male alcoholics in an Indian psychiatric hospital. Alcohol Alcohol 1991;26:47–51.
33. Gordon GG, Southern AL, Lieber CS. The effects of alcoholic liver disease and alcohol ingestion on sex hormone levels. Alcohol Clin Exp Res 1978;2:259–63.
34. Lester R, Van Thiel DH. Gonadal function in alcoholic men. Adv Exp Biol Med 1978;85A:399–414.
35. Van Thiel DH, Lester R, Vaitukaitis J. Evidence for a defect in pituitary secretion of luteinizing hormone in chronic alcoholic men. J Clin Endcrinol Metab 1978;47:499–507.
36. Ching M, Lin CY. Ethanol acutely reduces LH and prolactin secretion: possible involvement by dopamine. Alcohol 1994;11:105–12.
37. Van Thiel DH. Ethyl alcohol and gonadal function. Hosp Pract 1984; 11:152–8.
38. Ida Y, Tsujimaru S, Nakamaura K, Shiro I, Mukasa H Egami H, et al. Effects of acute and repeated alcohol ingestion on hypothalamic-pituitary-gonadal and hypothalamic-pituitary-adrenal functioning in normal males. Drug Alcohol Depend 1992;31:57–64.
39. Van Thiel DH, Gavaler JS, Smith WI Jr, Paul G. Hypothalamic-pituitary-gonadal dysfunction in man using cimetidine. N Engl J Med 1979;300:1012–5.
40. Gordon GG, Altman K, Southern AL, Rubin E, Lieber CS. Effect of alcohol (ethanol) administration on sex-hormone metabolism in normal men. N Engl J Med 1976;295:793–7.
41. Emanuele NV, LaPaglia N, Steiner J, Kirsteins L, Emanuele MA. Reversal of chronic ethanol-induced testosterone suppression in peripubertal male rats by opiate blockade. Alcohol Clin Exp Res 1999;23: 60–6.
42. Widenius TV, Orava MM, Vihko RK, Ylikahri RH, Eriksson CJP. Inhibition of testosterone biosynthesis by ethanol: multiple sites and mechanisms in dispersed Leydig cells. J Steroid Biochem 1987;28: 185–8.
43. Cicero TJ, Bell RD. Effects of ethanol and acetaldehyde on the biosynthesis of testosterone in rodent testes. Biochem Biophys Res Commun 1980;94:814–9.
44. Cicero TJ, Bell RD, Meyer ER, Badger TM. Ethanol and acetaldehyde directly inhibit testicular steroidogenesis. J Pharmacol Exp Ther 1980; 213:228–33.
45. Orpana AK, Orava MM, Vihko RK, Harkonen M, Eriksson CJP. Ethanol-induced inhibition of testosterone biosynthesis in rat Leydig cells: central role of mitochondrial NADH redox state. J Steroid Biochem 1990;36:603–8.
46. Brzek A. Alcohol and male fertility (preliminary report). Andrologia 1987;19:32–6.
47. Carlsen E, Giwereman A, Keiding M, Skakkebaek NE. Evidence for decreasing quality of semen during the past 50 years. Br Med J 1992;305:609–13.