دراسة تشخيصية لبعض أسباب العقم الذكري في منطقة كردستان العراق

أطروحة مقدمة من قبل إدريس محمد أمين

إلى مجلس كلية العلوم بجامعة بابل و هي جزء من متطلبات نيل درجة دكتوراه فلسفه في

علوم الحياة / علم الحيوان

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A Diagnostic Study of Some Causes of Male Infertility in Kurdistan Region of Iraq

A THESIS

SUBMITTED By **EDREES MOHAMMED AMEEN**

TO THE COUNCIL OF THE COLLEGE OF SCIENCE UNIVERSITY OF BABYLON IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN

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Supervised by

Dr. Faris Naji A. Al-Hady

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

لِلَّهِ مُلْكُ السَّمَاوَاتِ وَالأَرْضِ يَخْلُقُ مَا يَشَاءُ يَهَبُ لِمَنْ يَشَاءُ إِنَاثًا وَيَهَبُ لِمَنْ يَشَاءُ الذُّكُورَ(٤٩) أَوْ يُزَوِّجُهُمْ ذُكْرَانًا وَإِنَاثًا وَيَجْعَلُ مَنْ يَشَاءُ عَقِيمًا إِنَّهُ عَلِيمٌ قَدِيرٌ(٥٠)

صدق الله العظيم

سورة الشورى أية ٤٩-٥٠

IN THE NAME OF GOD, THE MERCIFUL
THE COMPASSIONATE

TO GOD BELONGS THE KINGDOM OF THE HEAVENS AND THE EARTH; HE CREATES WHAT HE WILL; HE GIVES TO WHOM HE WILL FEMALES, AND HE GIVES TO WHOM HE WILL MALES (٤٩); OR HE COUPLES THEM, BOTH MALES AND FEMALES ; AND HE MAKES WHOM HE WILL BARREN. SURELY HE IS ALL-KNOWING, ALL-POWERFUL (٥٠).

The Glorious Quran

Surah, Al-Shurah (٤٩-٥٠)

الخلاصة

هدفت الدراسة الحالية تشخيص بعض المشكلات الإدارية و المرضية التي قد تكون من أسباب حالات العقم التي يعاني منها الذكور في منطقة كردستان العراق. شملت الدراسة اختيار مائتين و ثمانون عينة عشوائية جمعت من الذكور الغير الخصيبين والذين راجعوا قسم المختبر في مركز التلقيح الاصطناعي ومعالجة العقم في مدينة اربيل. تم اختيار ثلاثين ذكرا متبرعا سليما استخدمت كمجموعة سيطرة. تم انجاز هذا العمل في فترة ما بين تموز / ٢٠٠٥ – حزيران / ٢٠٠٦ وكان متوسط العمر للاشخاص العقيمين (٣٣.٤٩ ± ٠.٤٣) سنة ، بينما متوسط العمر لمجموعة السيطرة كانت (٣٤.٦٧ ± ٠.٨٤) سنة.

تم جمع عينات السائل المنوي بطريقة الاستمناء الذاتي بعد مدة انقطاع جنسي ٣-٥ ايام ، بعدها تم وضع عينات المني في الحاضنة في درجة حرارة ٣٧ م° لحين التميع ، تم إجراء العديد من الفحوصات على عينات المني المتميعة. المتبقي من عينات السائل المنوي تم وضعها في جهاز النبذ بسرعة ٣٠٠٠ دورة في الدقيقة لمدة ١٠ دقائق للحصول على البلازما المنوية. تم حفظ البلازما المنوية في درجة حرارة – ٨٠ م° لاجراء الفحوصات المتبقية. تم الحصول على النتائج التالية من الدراسة المنجزة :

١- كانت النسبة المئوية لزمن التميع ($p<0.01$) و المظهر و اللزوجة ($p<0.05$) أعلى في الأشخاص العقيمين مقارنة مع الخصيبين.إن حجم السائل المنوي و تركيز النطف والتعداد الكلي للنطف و درجة نشاط النطف والنسبة المئوية للنطف المتحركة و منسب حركة النطف و عيوشية النطف و كذلك الشكل السوي للنطف كان أعلى معنويا ($p<0.01$) في الاشخاص الخصيبين. أما تركيز كريات الدم البيضاء في السائل المنوي و الضغط الاوزموزي و MDA كان اقل معنويا ($p<0.01$) في الخصيبين.

٢- هنالك انخفاض معنوي ($p<0.05$) في درجة نشاط النطف والنسبة المئوية للنطف المتحركة و منسب حركة النطف في عينات السائل المنوي عالية اللزوجة.

٣- إن حجم السائل المنوي كان أعلى معنويا ($p<٠.٠٥$) في عينات السائل المنوي لمرضى العقم ذات الضغط الاوزموزي mOSm / kg ٣٠٠ ≤ . بينما درجة نشاط النطف و منسب حركة النطف كانت اقل ($p<٠.٠٥$) في عينات المني ذات الضغط الاوزموزي ٣٤٠< .

٤- لوحظ إن تركيز MDA في البلازما المنوية كان أعلى معنويا ($p<٠.٠٠١$) لدى مرضى العقم و أيضا كان تركيز MDA لدى مرضى العقم المصابين بالدوالي و بابيضاض المني و المدخنين أعلى ($p<٠.٠٥$) مقارنة مع الغير المصابين بها

٥- إن تقدير كريات الدم البيضاء كانت اقل تركيزا ($p<٠.٠٠١$) باستخدام طريقة Endtz مقارنة مع طريقة القوة الكبرى للعدسة العينية و كان لزيادة كريات الدم البيضاء (ابيضاض المني) في مرضى العقيمين تأثيرا معنويا سلبيا على درجة نشاط النطف والنسبة المئوية للنطف المتحركة و منسب حركة النطف ($p<٠.٠٥$) .

٦- إن وجود القيلة الدوالية لها علاقة معنوية ($p<٠.٠١$) مع انخفاض في تركيز النطف و التعداد الكلي للنطف و درجة نشاط النطف والنسبة المئوية للنطف المتحركة و منسب حركة النطف و عيوشية النطف و الشكل السوي للنطف ($p<٠.٠٥$).

٧- كان للتدخين تأثيرا سلبيا معنويا ($p<٠.٠٥$) على نوعية السائل المنوي في جميع الاشخاص العقيمين.

٨- إن تلازن النطف و انتشار أضداد النطف كلن أعلى في مرضى العقم . إن أعلى نسبة لأضداد النطف وجدت في مرضى العقم المصابين بوهن النطف.

٩- لوحظ من التقدير الهرموني للمرضى زيادة معنوية ($p<٠.٠٥$) في هرمون FSH في مجموعة مرضى اللانطفية مقارنة مع مرضى قلة النطف و الخصيبين.

١٠- تم مشاهدة تحسن معنوي لنوعية السائل المنوي في المرضى المصابين بوهن النطف بعد تنشيطها بوسط تنشيط النطف (Earle's) المحور و الموازن و باستخدام التقنية الطبقية البسيطة و تقنية النبذ و السباحة للأعلى.

Abstract

The present study was aimed to diagnose some management and pathological changes of considerable effects to be the causative of infertility of men in Kurdistan region of Iraq. The study comprised two hundred and eighty randomly selected infertile men who attended laboratory department of the Infertility care and (IVF) Center in Arbil city as well as thirty healthy volunteer fertile men used as control. This work was carried out between July / ٢٠٠٥ and June / ٢٠٠٦. The mean age of infertile men was (٣٣.٤٩ ± ٠.٤٣) years, while the mean age of fertile men was (٣٤.٦٧ ± ٠.٨٤) years.

Semen samples were collected by masturbation after an abstinence of ٣-٥ days, and then incubated at ٣٧ ^{o}C until liquefy. Different semen parameters were assessed in the liquefied samples. The remain semen was centrifuged at ٣٠٠٠ rpm for ١٠ minutes to obtain the plasma. The semen plasma was stored in -٨٠ ^{o}C for further examinations. The following results were obtained from the performed study:

١-The percentage of liquefaction time ($p<٠.٠١$), appearance and viscosity ($p<٠.٠٥$), are higher in infertile men than that of fertile men. The volume of semen, sperm conc., total sperm count, sperm grade activity, sperm motility percent, sperm motility index, sperm viability, and the percent of normal sperm morphology are higher ($p<٠.٠١$) in fertile men, while the conc. of leucocytes in semen, seminal fluid osmolality and MDA are significantly ($p<٠.٠٠١$) lower in fertile men.

٢-There are significant decrease ($p<٠.٠٥$) of sperm grade activity, sperm motility percent, and sperm motility index in hyper-viscous semen samples.

٣-The volume of semen is higher ($p<٠.٠٥$) in patients with seminal plasma osmolality ≤ ٣٠٠ m OSm / kg, while sperm grade activity and sperm motility index ($p<٠.٠١$) are lower in osmolality > ٣٤٠.

٤- The seminal plasma MDA level is significantly higher ($p<٠.٠٠١$) in infertile men and the infertile patients with varicocele, leucocytospermia, and smoking have higher ($p<٠.٠٥$) MDA conc.

٥-Leucocytes determination with Endtz method showed a lower conc. ($p<٠.٠٠١$) than high power field (HPF) method and the increasing of leucocytes in semen of infertile men (leucocytospermia) has significant negative influence on sperm grade activity, sperm motility percent and sperm motility index ($p<٠.٠٥$).

٦- Varicocele is significantly associated with low sperm conc., total sperm count, sperm grade activity, sperm motility percent, sperm motility index, and viability ($p<٠.٠١$), and normal sperm morphology ($p<٠.٠٥$).

٧- Smoking has a significant ($p<٠.٠٥$) negative influence on semen quality of infertile men population.

٨- The agglutination of spermatozoa and the incidence of ASAs among infertile men is higher. The highest percentage of ASAs is seen in asthenozoospermic infertile patients.

٩- Hormonal evaluation indicates that FSH is significantly higher ($p<٠.٠٥$) in azoospermic men compare with fertile and oligozoospermic men.

١٠- Significant improvement of semen quality is observed in patients with asthenozoospermia after activation with modified balanced Earle's sperm

activation medium and using simple layer and centrifugation- swim up techniques.

Dedication:

This thesis is dedicated to my family, especially my mother and father, my wife Zhian, brothers;Abdulbasit, Mustafa, Mazin and Sherwan and sister Shler , my kids; Yousef, Zena, and Aumed, and to all infertile couples in the hope of getting a baby.

Edrees

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I wish to express my thanks to my supervisor, Dr. Faris Naji A. Al-Hady for his guidance in planning and conducting these investigations, encouragement, and continuous help throughout the period of the study.

I would like to thanks the deanery of the College of Science and the Head of Biology Department of Babylon University for their continuous help. I also would like to offer my thanks to the Head of Biology Dept. in the College of Science of Salahaddin University for their help and providence me with advanced physiology laboratory and all devices and chemical compounds for completion of some aspects of this research.

My very thanks also to the staff of the Infertility care and IVF center in Arbil city, especially Mr. Fawze, Nadim, Kurdu, Dashte, and Rostum for their assistance in collection samples from patients. Special thanks are also due to the staff members of Biology Department especially my colleagues; Mr. Sarbaz, Mr. Falah, Mr. Honer, Mr. Dara, Mr. Zana, and Mrs. Ahlam.

Special and great appreciation is due to my mother, father, and wife for their support, encouragement, and patience throught the study.

I certify that this thesis **(A Diagnostic Study of Some Causes of Male Infertility in Kurdistan Region of Iraq)** was prepared under my supervision at the Dept. of Biology, College of Science, University of Babylon and do hereby recommend to be accepted in partial fulfillment of the requirement for the degree of doctor of philosophy Science in **Biology / Zoology.**

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Date: / /

In view of the available recommendations I forward this thesis for debate by the examining committee.

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Head of Biology Dept.

Date: / /

Decision of the Examining Committee

We certify that we read this thesis and as examining committee examined the student in its contents and that in our opinion it is adequate as a thesis for the Degree of **Doctor of Philosophy in Biology / Zoology.**

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and infertile men	

List of Abbreviations

ABP	Androgen-binding protein
AIH	Artificial insemination by husband
ANOVA	Analysis of variance
ART	Assisted reproduction technique
ASA	Antisperm antibody
BTB	Blood-testis barrier
CASA	Computer-assisted semen analysis
CS	Cigarette smoke
DFI	DNA fragmentation index
E_2	Estradiol II
ELISA	Enzyme-linked immunosorbant assay
FSH	Follicle stimulating hormones
GIFT	Gametes intrafalopian transfer
GnRH	Gonadotropic releasing hormone
HPF	High power field
IBT	Immunobead test
ICSI	Intracytoplasmic sperm injection
IUI	Intrauterine insemination
IVF	*In vitro* fertilization
IVF-ET	*In vitro* fertilization embryo- transfer

LH	Luteinizing hormone
LPO	Lipid peroxidation
LSD	Least significant differences
MAR	Mixed agglutination reaction
MDA	Malondialdehyde
OS	Oxidative stress
PBS	Phosphate buffer saline
ROS	Reactive oxygen species
TAT	Tray agglutination titer
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
WHO	World health organization

Introduction

Infertility is defined as failure to conceive after one year of unprotected sexual intercourse (Ross and Niederberger, ١٩٩٥). Infertility is a worldwide public health problem, which affects couples of reproductive age; approximately ١٥ % of couples are infertile. It was estimated that the male factor is solely responsible for inability to conceive in about ٤٠ % to ٥٠ % of infertility cases (Leon *et al*., ١٩٩٩).

Some evidence suggests a decreasing of normal sperm parameters trend of male fertility in the last ٥٠ years such as decreased mean sperm count (Carlsen *et al*., ١٩٩٢). Although the exact factors and mechanisms causing male infertility are still largely elusive, one of the significant development in recent years is the discovery that reactive oxygen species (ROS) and oxidative damage are closely associated with impaired sperm function and male infertility (Sharma and Agarwal , ١٩٩٦ ; Griveau and Le Lannou, ١٩٩٧) .

Antisperm antibodies have been detected in ٥ % to ١٠ % of infertile men and in ٢ % of fertile men. Antibodies against spermatozoa can reduce fertility by decreasing the binding of sperms to the zona pellucida, by interfering with capacitation or acrosome reaction or by immobilizing sperms in cervical mucus (Martin-Du Pan, ١٩٩٧). The pathogenic bacteria in the semen ejaculates can induce a defect in semen parameters also accompanying increase of WBC (leucocytospermia) in the seminal fluid which may predispose to male factor infertility (Nunez-Calonge *et al*., ١٩٩٨).

Varicocele is often cited as the most common cause of male factor infertility. Arguments in support of this statement include reports of increased prevalence of varicocele in populations of infertile men compared with fertile or otherwise unselected men , association of varicocele with abnormal semen parameters, and improvements in semen parameters and / or pregnancy rates after varicocele repair (Redmon *et al* ., ٢٠٠٢) .

Other causes of male infertility including; abnormal environmental factors, cigarette smoking, alcohol consumption, genetic causes, psychological factors, and hormonal causes (Martin-Du Pan, ١٩٩٧). In addition to all these factors, about ٢٠–٢٥ % of male infertility factors remains unexplained and is called unexplained or idiopathic causes (Leon *et al*., ١٩٩٩).

The diagnosis of infertility and its management are depend on accurate analysis of reproductive and hormone profile in both male and female partners (Pal *et al*., ٢٠٠٦). Semen analysis is an extremely important tool for investigating male infertility. A careful semen analysis provides important information concerning the male reproductive hormonal cycle, spermatogenesis and the potency of the reproductive tract (Dale McClure, ١٩٨٦).

In Kurdistan region of Iraq, there are approximately ٨٠٠٠ infertile couples, and near ٣٠٠٠ couples of them were recorded and refer continuously to the Infertility care and (IVF) center in Arbil city for diagnosis and treatment. The semen analysis which was done for patients in the laboratory belong to this center uses very simple, primitive, and limited methods and techniques. So this

study was performed to enter another new techniques and more advanced tests on semen which was not previously used in this center and very important and benefits for patients which refered to this center. The aims of this study were :

١- Determination of reactive oxygen species, which has an important role in male infertility especially in idiopathic men infertility.
٢- Detection and determination of osmolality of semen and vitality of spermatozoa.
٣- Determination of leucocytes in the semen by using new methods (Endtz) test.
٤- Detection of antisperm antibodies in the seminal plasma.
٥- Improvement of semen quality by using different *in vitro* sperm activation techniques which helps to intrauterine insemination (IUI).
٦- The advance diagnoses and management of infertility causes helps to start of true and important treatments of infertile patients according to each case.

٢. Review of literatures:

٢.١. Male reproductive system:

The male reproductive system consists of the testes, epididymis, ductus deferentia (vas deferens), urethra, seminal vesicles, prostate gland, bulbourethral glands, scrotum, and penis Fig (١). Sperm cells are very sensitive to temperature and do not develop normally at usual body temperature. The testes and epididymis, in which the sperm cells develop, are located outside

the body cavity in the scrotum; where the temperature is lower (Seely *et al*., ١٩٩٦).

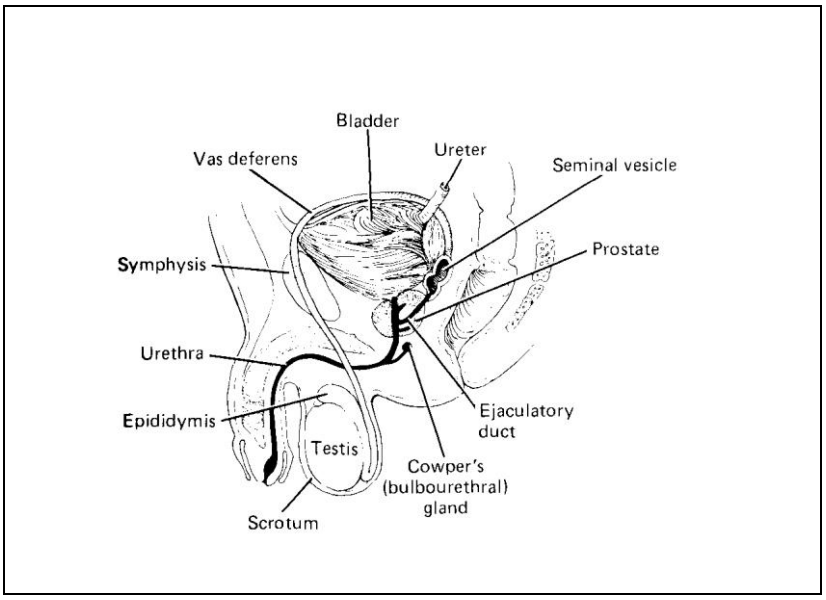


Fig (١): The male reproductive system (Ganong, ١٩٩٥).

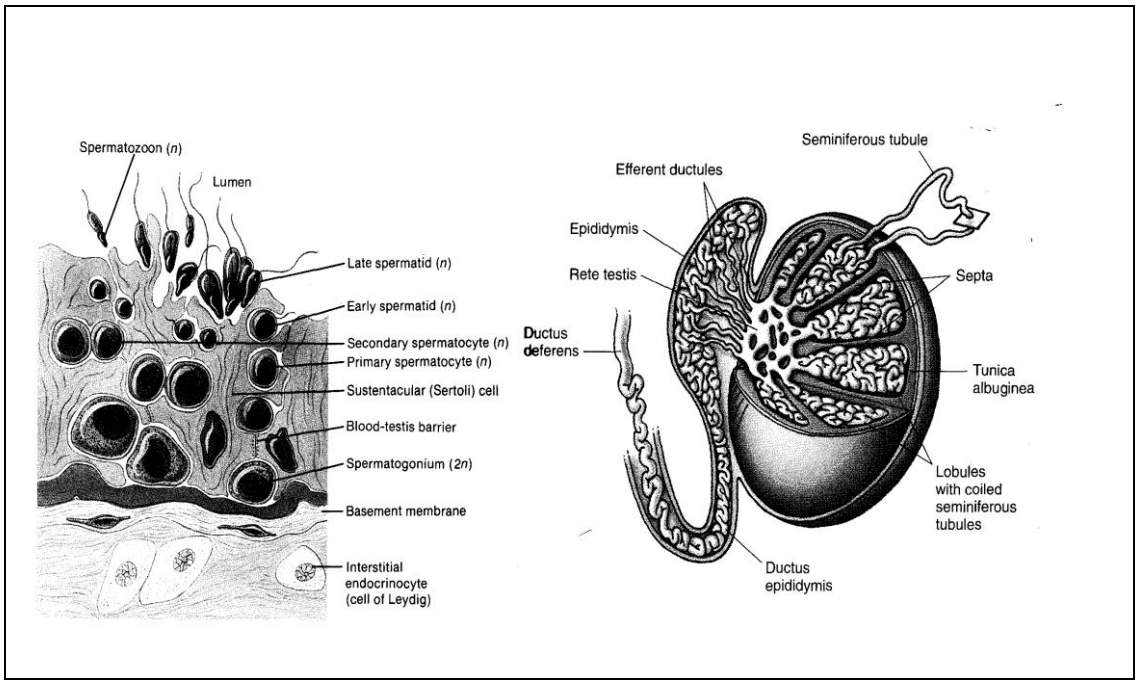
٢.١.١. Scrotum:

The scrotum contains the testes and is divided into two internal compartments by a connectivte tissue septum. Externally the scrotum consists of skin, beneath the skin a layer of loose connective tissue and a layer of smooth muscle, called the dartos (Seely *et al*., ١٩٩٦).

٢.١.٢. Testes:

Each testis is oval, about ٤ cm long and ٢.٥ cm in diameter, and weights ١٠–١٤ gm. Its anterior and lateral surfaces are covered by the tunica vaginalis, a saclike extension of the peritoneum that follows the testes as it descend into the scrotum. The testes itself has a white fibrous capsule called the tunica albuginea. Connective tissue septa divide the testis into ٢٥٠ to ٣٠٠ wedge-shaped lobules (Fig: ٢ b). Each lobule contains ١-٣ seminiferous tubules which has a narrow lumen lined by a thick germinal epithelium. The epithelium

consists of several layers of germ cells producing sperm and a much smaller number of tall Sertoli cells, which protect the germ cells and promote their development. The germ cells depend on the Sertoli cells for nutrients, waste removal, growth factors, and other needs (Fig : ٢ a). A Sertoli cell is shaped a little like a tree trunk whose roots spread out over the basement membrane, forming the boundary of the tubule, and whose thick trunk reaches to the tubule lumen. Tight junctions between adjacent Sertoli cells form a blood-testes barrier (BTB), which prevents proteins and other large molecules in the blood and intercellular fluid from getting to the germ cells. This is important because the germ cells, being genetically different from other cells of the body, would otherwise be attacked by the immune system. Some cases of infertility occur when the (BTB) fails to form adequately in aldoscence and the immune system produces autoantibodies against the germ cells (Saladin, ١٩٩٨).



(a) **(b)**

Fig (٢): Components of the (a), seminiferous tubule. (b), testis (Tortora, ١٩٩٢; Seely et al., ١٩٩٨).

٢.٢. Spermatogenesis:

Spermatogenesis is a complex process involving interaction of multiple cells with several hormones and growth factors over a relatively long period of time which could be studied only *in vivo*. The most peripheral cells are spermatogonia, which divide through mitosis. Some doughter cells were produced from these mitotic divisions remain as spermatogonia and continue to divide by mitosis. Other daughter cells form primary spermatocytes.

Primary spermatocytes contain ٤٦ replicated chromosomes, each consisting of two chromatids. The primary spermatocyte passes through the first meiotic division to produce two secondary spermatocytes. The secondary spermatocyte undergoes a second meiotic division to produce two smaller cells called spermatids, each having ٢٣ chromosomes. After the second meiotic division, the spermatids undergo major structural changes to form sperm cells (Fig: ٣).The cytoplasm of the spermatids is eliminated, and each spermatid develops a head, midpiece, and flagellum to become a sperm cell or spermatozoa (Seely *et al*., ١٩٩٦).

٢.٢.١. Hormonal control of spermatogenesis:

The GnRH was produced from hypothalamus during sex age. The GnRH transport by way of the hypothalamo–hyophyseal portal system to the anterior lobe of the pituitary. Here it stimulates cells called gonadotropes to secrete FSH and LH that stimulate different cells in the testes. LH stimulates the

interstitial cells in (Leydig cells) of the testes to secrete androgens (mainly testosterone). FSH stimulates the Sertoli cells to secrete androgen–binding protein (ABP) (Fig: ٤). ABP is thought to raise androgen levels in the seminiferous tubules and epididymis. (Saladin, ١٩٩٨ ; Alwachi, ٢٠٠٢).

Germ cells have no androgen receptors and don't respond to it. Nevertheless, the androgens have three principal effects: ١. They stimulates spermatogenesis in the presence of ABP. If testosterone secretion ceases, the sperm count and semen volume decline rapidly and caused male infertility; ٢. They suppress the secretion of GnRH by the hypothalamus and reduce the sensitivity of the pituitary to GnRH; ٣. They stimulate development of the secondary sex characteristics and other somatic changes of puberty. It is necessary in any times to reduce FSH secretion without reducing ICSH secretion, particularly when sperm are produced faster than they are used. Inhibin, a hormone secreted from the Sertoli cells, selectively suppresses FSH output from the pituitary. This slows down sperm production without inhibiting testosterone secretion. If sperm count drops below ٢٠×١٠^{٦} / ml, however, Inhibin secretion drops and FSH secretion rises (Saladin, ١٩٩٨ ; Alwachi, ٢٠٠٢).

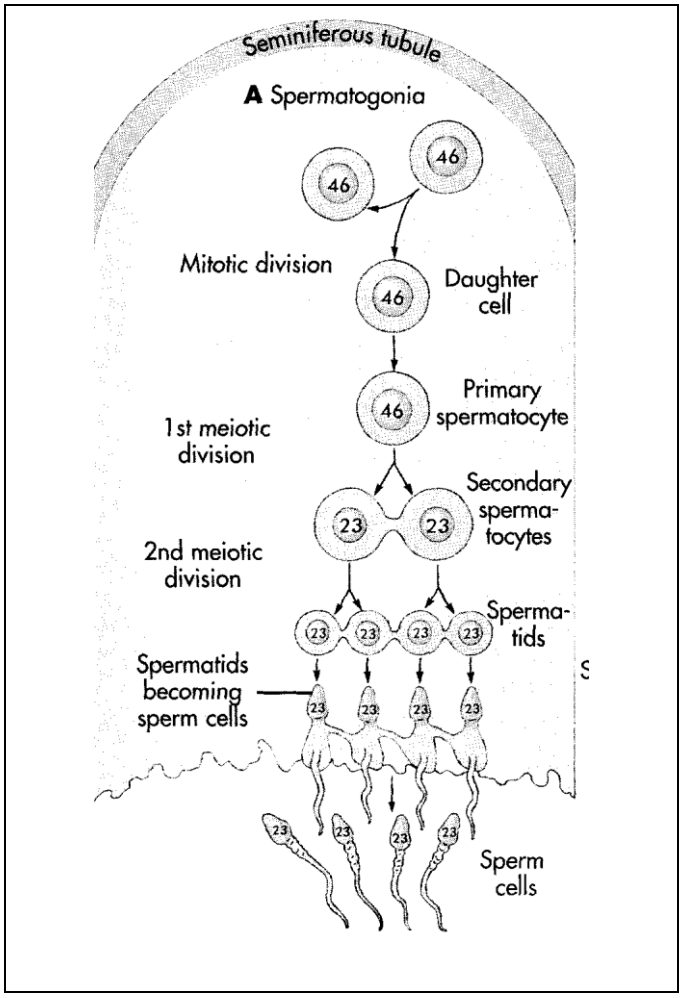


Fig (٣): The formation of the sperm cells (spermatogenesis)

(Seely *et al.*, ١٩٩٦).

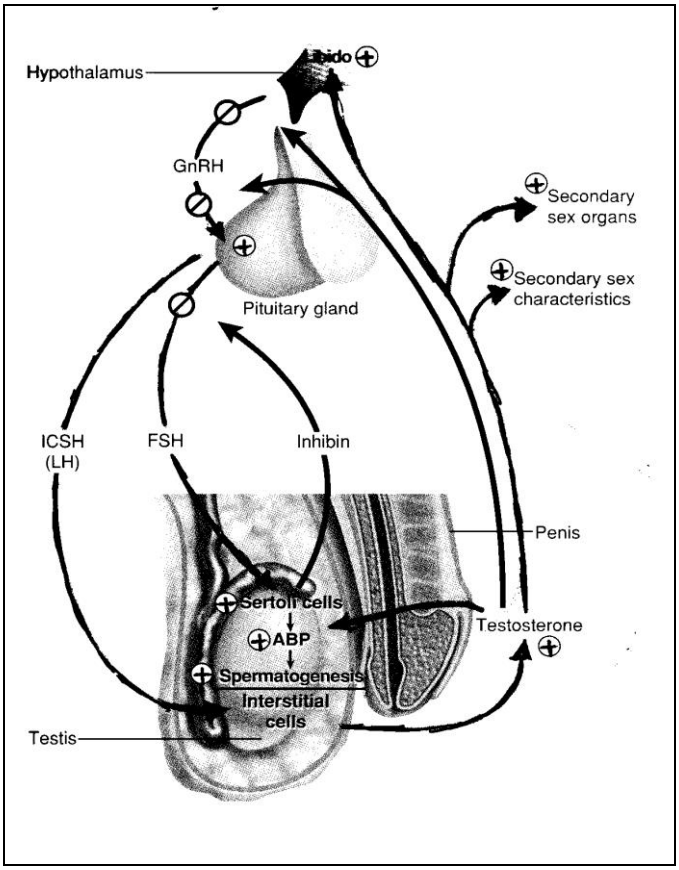


Fig (٤): Hormonal regulation of spermatogenesis (Saladin, ١٩٩٨).

٢.٣. Semen (seminal fluid):

The fluid expelled during orgasm is called semen or seminal fluid. A typical ejaculation volume ٢-٥ ml of semen, composed of about ٦٠ % seminal vesicles fluid, ٣٠ % prostatic fluid, ١٠ % sperms and spermatic duct secretion and a trace of bulbourethral gland secretions (Saladin, ١٩٩٨). The bulbourethral glands and the mucous glands of the urethra produce a mucous secretion, which lubricates the urethra, helps neutralize the contents of the normally acidic urethra, provides a small amount of lubrication during intercourse, and helps reduce the acidity in the vagina (Seely *et al*., ١٩٩٦).

The major constituents of semen are; **spermatozoa; fructose,** this sugar produced by the seminal vesicles, provides a source of energy for sperm motility; **clotting and anti-coagulated factors**. The seminal vesicles secrete

fibrinogen and the prostate produces a clotting enzyme, when these secretions mix during ejaculation, the clotting enzyme converts fibrinogen to fibrin, causing the semen to clot. It becomes very sticky and adheres to the deep recesses of the vagina rather than draining back out. About ١٥-٣٠ minutes later, fibrinolysin in semen produced by the prostatic fluid dissolves the clot. Sperm are then liberated and able to begin their migration up the female reproductive tract. The **prostaglandins**, produced by the prostate and seminal vesicles, stimulate peristaltic contractions of the female reproductive tract that may help draw semen into the uterus or spread it through the uterus. Prostaglandins also reduce the viscosity of the mucus in the cervical canal of the female, making it easier for sperm to travel up this passageway (Saladin, ١٩٩٨). It was also shown that $PGF_{٢}\alpha$ can significantly increase the number of sperms produced by the testis in animals treated with ١.٥ and ٢.٥ mg $PGF_{٢}\alpha$ / kg body weight when compared with control group (Alwachi and Al-Shakarchi, ١٩٨٧ ; Alwachi and Saadi, ٢٠٠٢). **Spermine**, spermine and other alkalines give the semen a pH of ٧.٢ - ٧.٦ .This is important because the vagina has a pH of about ٣.٥-٤, sperm motility is a poor at a pH less than ٦ and thus requires neutralization of vaginal acidity by the semen (Saladin, ١٩٩٨).

In addition of these, semen contain an antibiotic, **seminoplasmin**, these has the ability to destroy a number of bacteria, since both semen and the lower female reproductive tract contain bacteria, the antibiotic activity of seminal plasmin may keep these bacteria under control to help ensure fertilization (Tortora, ١٩٩٢).

٢.٤. Semen analysis:

Spermatozoa were first described by Leeuwenhoek in the ١٧^{th} century, but it was not found that the sperm count to be associated with fertility

potential (Seibel and zilberstain, ١٩٩٥). Since that time a variety of sperm tests and semen parameters have been developed with the hope of clarifying whether or not a man could impregnate his partner.

Macleod (١٩٤٢), Macleod and gold (١٩٥٣), Eliasson (١٩٧١) and Hellinga (١٩٤٩, ١٩٧٦) have led the scientific bases of conventional analysis of spermatozoa and the techniques recommended by them are still considered the reference for more advanced methods (Comhaire and Vermeulen, ١٩٩٥).

Semen analysis comprises a set of descriptive measurements of spermatozoa and seminal fluid parameters that help to estimate semen quality (Campana *et al*., ١٩٩٥). Conventional semen analysis includes measurement of particular aspects of spermatozoa such as sperm conc., motility, morphology and seminal plasma parameters. Quantification and identification of non-spermatozoal cells and detection of antisperm antibodies are also part of basic semen analysis (Comhaire and Vermeulen, ١٩٩٥).

Normal values of semen parameters issued by WHO in ١٩٩٩ are generally used as reference values (table: ١). Table (٢) shows nomenclature for semen variables.

Table (١): Normal values of semen variables (WHO, ١٩٩٩).

Standard tests	Normal values
Volume	$\geq$ ٢.٠ ml
pH	٧.٢ – ٧.٨
Sperm concentration	$\geq$ ٢٠ × ١٠^{٦} / ml

Total sperm count	$\geq ٤٠ \times ١٠^{٦}$ / ejaculate
Motility	٥٠ % or more with forward progression or ٢٥ % or more with rapid progression within ٦٠ minutes of ejaculation.
Morphology	٣٠ % or more with normal forms
Vitality	٥٠ % or more live ,i.e. excluding dye
White blood cells	$\leq ١.٠ \times ١٠^{٦}$ / ml

Table (٢): Nomenclature for semen variables (WHO, ١٩٩٩).

Normozoospermia	Normal ejaculate as defined in table(١)
Oligozoospermia	Sperm conc. fewer than ٢٠×١٠^{٦} / ml
Asthenozoospermia	Fewer than ٥٠ % spermatozoa with forward progression or fewer than ٢٥ % with rapid forward progression.
Teratozoospermia	Fewer than ٣٠ % spermatozoa with normal morphology.
Oligoasthenoteratozoospermia	Signifies disturbance of all three variables (combination of only ٢ prefixes can be used).
Azoospermia	No spermatozoa in the ejaculate
Aspermia	No ejaculate

In general, WHO, (١٩٩٢, and ١٩٩٩), and most studies can depend on the following properties and parameters for the evaluation the principles of seminal analysis:

٢.٤.١. Liquefaction time:

A normal semen samples liquefy within ٦٠ minutes at room temperature, although usually this occurs within ١٥ minutes (WHO, ١٩٩٩).

Liquefaction occurs under the influence of enzymes of prostatic origin. In some cases, liquefaction does not occur within the normal time period and this fact should be recorded, as it may suggests functional disturbance of the prostate. Normal semen samples may contain jelly-like grains which don't liquefy. Samples which don't liquefy need additional treatment such as exposure to bromelin, to make the sample enable to analysis (Comhaire and Vermeulen, ١٩٩٥; WHO, ١٩٩٩).

٢.٤.٢. Appearance:

A normal sample has a homogenous, gray–opalescent appearance. It may appear less opaque if the sperm conc. is very low, red–brown when red blood cells are present (WHO, ١٩٩٩). The presence of mucous streaks may interfere with the counting procedure and suggests inflammation or abnormal liquefaction (Comhaire and Vermeulen, ١٩٩٥).

٢.٤.٣. pH:

The pH is determined by acidic secretions of the prostate and alkaline secretions of the seminal vesicles. It should normally be in the range of ٧.٢–٧.٨ (WHO, ١٩٩٩). If the pH exceeds ٨.٠, infection should be suspected with decreased secretion of acidic products by the prostate, such as citric acid. Abnormal pH may also be recorded in cases of incomplete ejaculation. Extremely acidic pH (< ٦.٥) is found in cases of agenesis (or occlusion) of the seminal vesicles (Rrumbullaku, ٢٠٠٣). If the pH is less than ٧.٠ in a sample with azoospermia, there may be obstruction of the ejaculatory ducts or bilateral congenital absence of the vasa (WHO, ١٩٩٩).

٢.٤.٤. Volume:

The major components of the ejaculate volume are made up of secretions from accessory glands. The bulk of the volume is secreted by the seminal vesicles (Comhaire and Vermeulen, ١٩٩٥). A low ejaculate volume can reflect abnormalities in accessory sex gland fluid synthesis or secretion. It can also be indicative of a physical obstruction somewhere in the reproductive tract (Siegel, ١٩٩٣), or may occur in cases of incomplete or partially retrograde ejaculation. Large volumes are sometimes found in association with varicocele or after relatively long periods of sexual abstinence (Comhaire and Vermeulen, ١٩٩٥). Semen volume affects fertility only when it falls bellow ١.٥ ml (inadequate bufferings of vaginal acidity) or is more than ٥ ml. Low volumes may be associated with retrograde ejaculation, incomplete collection or androgen deficiency (Dale – McClure, ١٩٨٦).

٢.٤.٥. Viscosity (consistency):

The viscosity of the liquefied sample should be recognized as being different from coagulation (WHO, ١٩٩٩). Increase viscosity has the same clinical meaning as abnormal liquefaction, and may be related to prostate dysfunction resulting from chronic inflammation (Comhaire and Vermeulen, ١٩٩٥). High viscosity can interfere with determinations of sperm motility, conc., and antibody counting spermatozoa (WHO, ١٩٩٩), or can impair the availability of fertile sperm at the site of fertilization (Siegel, ١٩٩٣).

Increased viscosity of the ejaculate was reported to occur more frequently among men from infertile couples than in fertile males (Bunge, ١٩٧٠). Meng – Chung *et al*., (١٩٩٢); (Gopalkrishnan *et al*., ٢٠٠٠) concluded that much higher viscosity was observed in the men with asthenozoospermia. Moreover, in post–coital studies, hyper–viscosity was also found to be associated with poor invasion of cervical mucus by spermatozoa (Glass and

Mrouch, ١٩٦٧; Gersh, ١٩٧٠). Study of Elzanaty *et al*., (٢٠٠٤), showed that semen volume was significantly higher in samples with high viscosity and the percentages of total fraction of progressively motile spermatozoa were significantly lower in the sample with high viscosity as compared with samples with normal viscosity.

٢.٤.٦. Sperm concentration (sperm density):

Perhaps the most widely utilized semen parameter is sperm count. Men with < ٢٠ × ١٠^{٦} sperm per ml are typically deemed sub – fertility, and men with counts < ٥ × ١٠^{٦} sperm / ml are often considered infertile (Seibel and Zilberstain, ١٩٩٥).

In ١٩٥٥, MacLeod evaluated sperm counts of ١٠٠٠ men whose wives were pregnant and ١٠٠٠ men whose marriages had been infertile. The greatest contrast between the ٢ groups was seen at a conc. of ٢٠ × ١٠^{٦} / ml, of the fertile men, only ٥ % had sperm counts less than or equal to ٢٠ × ١٠^{٦} compared to ١٦ % of the infertile men; ٨٤ % of the infertile men had counts greater than ٢٠ × ١٠^{٦} sperm / ml, suggesting that men with sperm counts above ٢٠ × ١٠^{٦} may also be infertile. More recently, Lipshultz, evaluated semen from a group of normal men, similar to the findings of MacLeods study, ٦ % of normal men had counts less than or equal to ٢٠ × ١٠^{٦} sperm / ml. According to similar clinical studies, the WHO, established a count of ٢٠ × ١٠^{٦} sperm / ml as the cutoff for normal sperm count (Seaman *et al*., ١٩٩٤).

In report of Carlsen *et al* ., (١٩٩٢), which reviewed ٦١ papers published globally from ١٩٣٨ to ١٩٩١ on sperm quality in fertile men, using a linear regression mode, a meta–analysis of the data was carried out in presumably fertile men from different countries and reported a trend toward decrease in

sperm conc. and quality over the last five decades. According to this report, sperm conc. decreased from ١١٣ × ١٠^٦ to ٦٦ × ١٠^٦ / ml and semen volume decreased from ٣.٤ to ٢.٧ between ١٩٤٠ and ١٩٩٠. Decrease in semen quality has been reported from different regions of USA, Norway, Paris, Scotland, Belgium, Italy, and Finland (Pal *et al*., ٢٠٠٦).

٢.٤.٧. Sperm motility:

Adequate motor activity of the sperm cell is required for normal transport through the female reproductive tract and for penetrating the ovum. Sperm motility is a single most important measure of semen quality and can be a compensatory factor in men with low sperm conc. Sperm motility is usually evaluate in two ways: the number of motile sperm as a percentage of the total, and the quality of sperm movement (how fast and how straight the sperm swim). The degree of forward progression is a classification based on the pattern displayed by the majority of motile spermatozoa and ranges from ٠ (no movement) to ٤ (excellent forward progression). A normal value for sperm motility in the semen is at least ٥٠ % to ٦٠ % motile cells and a quality greater than ٢ (Dale – McClure, ١٩٨٦).

By the same way, Seaman *et al*., (١٩٩٤), descriptive the motility as follows; sperm movements is evaluated both quantitatively and qualitatively. Quantitation is defined as the average percentage of sperm moving in ten random high–power microscopic fields. Qualitative assessment of sperm movement is based on the pattern displayed by the majority of motile spermatozoa, using the following scale of ٠–٤; ٠ = no movement, ١ = movement, but no forward progression, ٢ = movement with slow forward progression, ٣ = movement in an almost–straight line with good speed, ٤ = movement in an almost–straight line with high speed. These ٢ evaluations are

combined with ٥٠ % sperm with forward progression of ٣ or more is considered normal. The same evaluation used by Amelar *et al*., and (١٩٨٠) Levin *et al*., (١٩٩٢).

In recent years, a number of techniques for objective assessment of movement and characteristics of human spermatozoa have been introduced by using computer–assisted semen analysis (CASA) systems (Rrumbullaku, ٢٠٠٣). The microscopic field is scanned systematically and the motility of each spermatozoa encountered is graded a, b, c and d (WHO, ١٩٩٢), according to whether it shows: a = rapid progression motility, b = slow or sluggish motility, c = non–progressive motility, d = immotility.

Abnormalities in motility and quality of movements can arise from infection, the presence of antisperm antibodies, partial ejaculate–duct obstruction, or the subtle testicular alteration that may be cause by gonadotoxins or varicoceles. If none of the sperm are moving, the patients may have necrospermia. This is actually a misnomer, as metabolic studies and special vital stains have revealed that the immobile spermatozoa may not necessarily be dead (Seaman *et al*., ١٩٩٤).

٢.٤.٨. Sperm motility index:

It is important to found a relation between the percentage of motile spermatozoa and grade activity of spermatozoa, because some semen samples has a high percentage of motile spermatozoa with lower grade activity of movement and other samples has a low percentage of motile sperm with high grade movement. According to this, it is probably to determine the sperm motility index to connect between the two important parameters of sperm

motility. The sperm motility index is determined by multiplying the percentage of motility to grade activity of spermatozoa (Makler *et al*., ١٩٧٩).

٢.٤.٩. Vitality (viability):

Sperm vitality is reflected the proportion of spermatozoa that are a (live) as determined by either dye exclusion, or hypo–osmotic swelling. This should be determined if the percentage of immotile spermatozoa exceeds ٥٠ %. Sperm vitality assessments provide a check on the accuracy of the motility evaluation, since the percentage of dead cells should not exceed the percentage of immotile spermatozoa (WHO, ١٩٩٩).

Reduced percentage of motility with a high percentage of viable sperm may reflect structural or metabolic abnormalities of sperm that derived from abnormalities in testicular function or antimotility factors in the seminal plasma (Siegel, ١٩٩٣).

٢.٤.١٠. Morphology:

Sperm cells represent a unique population in which up to ٥٠ % (up to ٧٠٪ according to WHO criteria ١٩٩٢, and up to ٨٦ % according to strict criteria) of the cells can have morphological defects in normal fertile individuals (Campana *et al* ., ١٩٩٥). The normal head should be oval in shape; the length of them should be ٤.٠–٥.٥ µm, and the width ٢.٥–٣.٥ µm. There should be a well – defined acrosomal region comprising ٤٠–٧٠ % of the head area. There must be no neck, midpiece or tail defects and no cytoplasmic droplet more than one – third the size of a normal sperm head. This

classification scheme requires that all borderline forms be considered abnormal (Kruger *et al*., ١٩٨٦; Menkveld *et al*., ١٩٩٠). The following categories of defects should be scored (WHO, ١٩٩٩):

- Head shape / size defects, including large, small, tapering, pyriform, amorphous, vacuolated (> ٢٠ % of the head area occupied by unstained vacular areas), or double heads, or any combination of these.
- Neck and midpiece defects, including absent neck, non inserted**,** or bent neck (the neck and the tail forms an angle of about ٩٠° to the long axis of the head), distended / irregular / bent midpiece, abnormally thin midpiece or any combination of these.
- Tail defects, including short, multiple, hairpin, broken, irregular width, or coiled tails, tails with terminal droplets, or any combination of these.
- Cytoplasmic droplets greater than one–third of the area of a normal sperm head.

Sperm morphology gives information for the function of the reproductive tract and is a predictor of men fertility potential (Rrumbullaku, ٢٠٠٣). Physical sperm aberration may occur during the production of sperm or during storage in the epididymus (Campana *et al*., ١٩٩٥). The increased number of immature spermatozoa may be due to epididymal dysfunction or is a consequence of frequent ejaculations.The increased number of spermatozoa with tapering heads is found in association with varicocele. The recent study indicate that the percentage of tapered spermatozoa, spermatozoa containing cytoplasmic droplets and spermatozoa with bent tail are significantly increased in varicocele patients compared to controls (Rrumbullaku *et al* ., ١٩٩٨).

٢.٤.١١. Agglutination:

Agglutination of spermatozoa means that motile spermatozoa stick to each other, head to head, midpiece to midpiece, tail to tail, or mixed, e.g. midpiece to tail. The adherence of either immotile or motile spermatozoa to mucus threads, to cells other than spermatozoa, or to debris is not considered agglutination and should not be recorded as such

The presence of agglutination is suggestive of, but not sufficient evidence to prove the existence of an immunological factor of fertility (WHO, ١٩٨٧; WHO, ١٩٩٢; Comhaire and Vermeulen, ١٩٩٥). Sperm agglutination could be used also as indication for antisperm antibody testing of infertile men (Comhaire and Vermeulen, ١٩٩٥; Kunathikom *et al*., ١٩٩٥).

٢.٤.١٢. Seminal osmolality:

Mammalian sperm are ejaculated in a heterogonous environment composed of testicular and epididymal fluid together with the secretory products of male accessory glands. Biochemical characteristics of seminal fluid are different in the various portions of the male genital tract especially when considering ionic concentrations and pH (Tuck *et al*., ١٩٧٠; Hinton *et al*., ١٩٨١). Such differences could play an important role in the regulation of important sperm functions as well as motility, capacitation and fertilizing ability acquisition (Eliasson, ١٩٧٠; Eliasson and Lindholmer, ١٩٧٢).

It is well known that whenever along the male genital tract the seminal plasma osmolarity is higher than that of blood (Tuck *et al* ., ١٩٧٠ ; Velazquez *et al* .,١٩٧٧ ; Hinton *et al* ., ١٩٨١ ; Polak and Daunter , ١٩٨٤) and it has been

reported that seminal osmolarity influences sperm motility both in invertebrates and vertebrates including mammals. Human spermatozoa are stored in epididymal fluid close to that measured in the vasa differentia (mean ٣٤٢ m OSm / kg, (Hinton *et al*., ١٩٨١). It is commonly held that the osmotic pressure of human semen is higher than serum (Rossato *et al*., ٢٠٠٢). Spermatozoa are thought to experience a hypotonic challenge upon transfer to fluids in the female tract, the osmolality of which are reported to be in the range ٢٧٦ –٣٠٢ m OSm / kg (Edward , ١٩٧٤ ; Menezo *et al* ., ١٩٨٢ ; Casslen and Nilsson ١٩٨٤ ; Rossato *et al* ., ١٩٩٦) and ٢٨٠–٢٩٠ m OSm / kg (Hinton *et al* ., ١٩٨١). Besides influencing sperm motility, seminal osmolarity variations have been demonstrated to be able to regulated $Ca^{+٢}$ influx, acrosome reaction and fertilizing ability acquisition in human sperm (Rossato *et al*., ١٩٩٦).

The study of Rossato *et al* ., (٢٠٠٢) showed that normospermic fertile men has seminal osmolarity values that are significantly lower with respect to asthenozoospermic patients (٣١٧.٨ vs. ٣٤٥.٢ m OSm / L., P < ٠.٠٠١) and seminal plasma osmolarity negatively correlated with sperm motility percentage and grade activity. Moreover when sperm from fertile subjects were suspended in medium with an osmolarity increasing from ٣٠٠ to ٦٠٠ m OSm sperm motility percentage and grade activity reduced and nearly abolished when medium osmolarity was ٦٠٠ m OSm. On the contrary, when sperm from asthenozoospermic subjects with high semen osmolarity were resuspended in medium with lower osmolarity, sperm motility parameters improved significantly.

Osmolality of normal human seminal plasma ٣٦٦ m OSm / kg was found to be higher than the osmolality of human blood serum. Abnormal seminal

plasma showed a still higher value ٤٢٨ and ٤٥٧ m OSm / kg respectively for astheno and oligoasthenozoospermic subjects (Velazquez *et al*., ١٩٧٧). In addition the study of Gopalkrishnan *et al*., ١٩٩٢, showed that high and low volume semen was associated with incidence of spermatozoa exhibiting subfertile characteristics and there was an inverse correlation between osmolarity and volume of semen sample.

٢.٥. Some causes of male infertility:

٢.٥.١. Oxidative stress and male infertility:

٢.٥.١.١. Reactive oxygen species (ROS):

Recently, the generation of oxidants, also described as reactive oxygen species (ROS), in the male reproductive tract has become a real concern because of their potential toxic effects, at high levels, on sperm quality and function (Sharma and Agarwal, ١٩٩٦). ROS are highly reactive oxidizing agents belonging to the class of free radicals. As is true for all cells living under aerobic conditions, spermatozoa constantly face the oxygen ($O_٢$) paradox. $O_٢$ is required to support life , but its metabolites, such as ROS, can modify cell functions, endanger cell survival, or both (de Lamirande and Gagnon, ١٩٩٥), hence ROS must be inactivated continuously to maintain only the small amount necessary to maintain normal cell function. A battery of different antioxidants normally protects against oxidants (Sies, ١٩٩٣). Oxidative stress develops when oxidants outnumber antioxidants, peroxidation products develop, and these phenomena cause pathologic effects (Spitteler, ١٩٩٣; Sikka, ٢٠٠١).

Recent reports indicate that high levels of ROS are detected in the semen of ٢٥ % to ٤٠ % of infertile men (de Lamirande and Gagnon, ١٩٩٥; Padron *et al*., ١٩٩٧). Most studies have implicated oxidative stress as a mediator of sperm dysfunction. The spermatozoa have a high content of polyunsaturated fatty acids (PUFA) within the plasma membrane and a low concentration of scavenging enzymes within the cytoplasm and they are susceptible to the peroxidation in the presence of elevated seminal ROS (Fraczek *et al* ., ٢٠٠١ ; Dandekar *et al* ., ٢٠٠٢ ; Agarwal and Saleh, ٢٠٠٢ ; Agarwal *et al* ., ٢٠٠٣ ; Zarghami and Khosrowbeygi , ٢٠٠٤). Spermatozoa and seminal plasma contain a number of antioxidant systems which counteract the effects of ROS. Spermatozoa possess a low amount of cellular ROS defense system (Fujii *et al*., ٢٠٠٣; Garrido *et al*., ٢٠٠٤).

The seminal plasma is well endowed with an array of antioxidants that act as free radical scavengers to protect spermatozoa against oxidative stress (Zini *et al*., ٢٠٠٠; Agarwal *et al*., ٢٠٠٤; Sanocka and Kurpisz, ٢٠٠٤). Seminal plasma contains a number of enzymatic antioxidants such as superoxide dismutase (SOD) and catalase (Peeker *et al*., ١٩٩٧; Mruk et al., ٢٠٠٢; Calamera *et al*., ٢٠٠٣). In addition, it contains a variety of non–enzymatic antioxidants such as Vitamin C, Vitamin E, pyruvate, glutathione and carnitine (Meucci *et al* .,٢٠٠٣).

٢.٥.١.٢. Mechanism of oxidant generation in human semen:

A variety of semen components, including morphologically abnormal spermatozoa, precursor germ cells, and leucocytes, are capable of generating ROS. Seminal leucocytes and morphologically abnormal spermatozoa are the main source of ROS in human ejaculates (Kessopoulou *et al*., ١٩٩٢).

٢.٥.١.٢.١. ROS production by spermatozoa:

Clear evidence suggests that spermatozoa can produce ROS (Aitken *et al*., ١٩٩٢; Gil-Guzman *et al*., ٢٠٠١; Hendin *et al*., ١٩٩٩). Levels of ROS production by sperm correlate negatively with quality of sperm in the original semen (Gomez *et al*., ١٩٩٨). The link between poor sperm quality and increased ROS generation lies in the retention of excess residual cytoplasm (cytoplasm droplets) in abnormal spermatozoa. When spermatozoa are impaired, the cytoplasm extrusion mechanism is defective. Spermatozoa released from the germinal epithelium carring surplus residual cytoplasm are thought to be immature and functionally defective (Huszar *et al*., ١٩٩٧). Retention of residual cytoplasm by spermatozoa is positively correlated with ROS generation via mechanism that may be mediated by the cytosolic enzyme glucose–٦–phosphate– dehydrogenase (G_6PD). This enzyme controls the rate of glucose flux through the hexose monophosphate shunt, which in turn, controls the intracellular availability of nicotinamide adenine dinucleotidephosphate (NADPH) (Fig : ٥). The latter is used as a source of electrons by spermatozoa to fuel the generation of ROS by an enzyme system known as NADPH-oxidase (Aitken *et al* .,١٩٩٧).

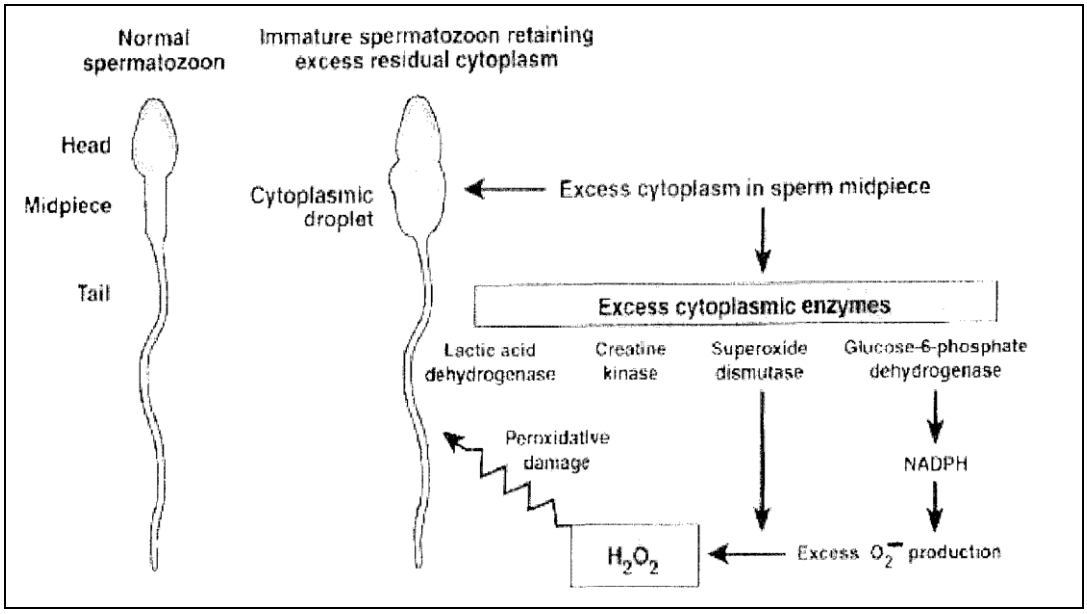


Fig (٥): Mechanism of ROS production by immature spermatozoa (spermatozoa with excess residual cytoplasm). H_2O_2 hydrogen peroxide; NADPH - nicotinamide adenine dinucleotide phosphate; O_2^- superoxide anion. (Aitken *et al.*, ١٩٩٧).

Spermatozoa may generate ROS in two ways: (١) as a result of the NADPH-oxidase system at the level of the sperm plasma membrane, and (٢) as a result of the NADH-dependent oxidreductase at the level of mitochondria (Aitken *et al.*, ١٩٩٢). The mitochondrial system is the major source of ROS in spermatozoa from infertile men (Plante *et al.*, ١٩٩٤). The primary ROS generated in human spermatozoa is the superoxide anion (O_2^-).This one-electron reduction product of O_2 secondarily reacts with itself in a dismutation reaction, which is greatly accelerated by superoxide dismutase, to generate hydrogen peroxide (H_2O_2). In the presence of transition metals such as iron and copper, H_2O_2 and $O_2^{\cdot}$ can interact to generate extremely pernicious hydroxyl radical ($OH^{\cdot}$) as shown in the following equation:

$$O_2^{\cdot} + H_2O_2 \rightarrow OH^{\cdot} + OH + O_2^{\cdot}$$

Alternatively, the hydroxyl radical can be produced from hydrogen peroxide, which requires a reducing agent such as ascorbate or ferrous iron, as

shown in the equation: $H_2O_2 + Fe^{+2} \rightarrow Fe^{+3} + OH^{\cdot} + OH$. The hydroxyl radical is thought to be an extremely powerful initiator of the lipid peroxidation cascade and can precipitate loss of sperm functions.

٢.٥.١.٢.٢. ROS production by leucocytes:

Peroxidase–positive leucocytes are the major source of ROS in semen (Ochsendorf, ١٩٩٩). Peroxidase-positive leucocytes include polymorphonuclear leucocytes, which represent ٥٠ % to ٦٠ % of all seminal leucocytes, and macrophages, which represent ٢٠ % to ٣٠ % of all seminal leucocytes (Fedder *et al*., ١٩٩٣). Peroxidase–positive leucocytes in semen are contributed largely by the prostate and seminal vesicles (Wolff, ١٩٩٥). The capacity of leucocytes to generate ROS depends on their activation, which may occur in response to a variety of stimuli, including inflammation and infection (Saran *et al*., ١٩٩٩). During activation, NADPH production is increased, and the myeloperoxidase system of leucocytes is activated, leading to a respiratory burst with subsequent release of high levels of ROS (Blake *et al*., ١٩٨٧). Such an oxidative burst is thought to be an effective early defense that kills the microbes in cases of infection (Saran *et al*., ١٩٩٩). Sperm damage from ROS produced by leucocytes may occur when seminal plasma is removed during sperm preparation for assisted reproduction (Ochsendorf, ١٩٩٩), or when seminal leukocyte concentrations are abnormally high, such as in leucocytospermia (Shekarriz *et al*., ١٩٩٥).

٢.٥.١.٣. ROS and idiopathic infertility:

Men with idiopathic infertility generally present with significantly higher seminal ROS levels and lower antioxidant properties than healthy control. Therefore, it appears that presence of ROS in infertile normozoospermic men may be the cause behind previously unexplained cases of infertility. Similarly, sperm DNA damage analysis may reveal hidden sperm DNA abnormalities in infertile men with normal standard sperm values who were diagnosed with idiopathic infertility. The increases in sperm DNA damage in these patients may be partly related to high levels of seminal oxidative stress (OS) (Pasqualotto *et al*., ٢٠٠١)**.**

٢.٥.١.٤. ROS and leucocytospermia:

Genital tract infections are usually associated with leucocytospermia and elevated ROS levels, as leucocytes represent the major source of ROS production in ejaculates. Although leucocytes are a constant component of human ejaculates and no semen sample is free of them, if the prevalence of leucocytes exceeds normal values (١ × ١٠^{٦} / ml), spermatozoa can be compromised (Agarwal and Said, ٢٠٠٥). Most important leucocytospermia has been associated with occult sperm DNA damage; this may occur directly in the form of leucocytospermia, a manifestation of inflammation that is associated with cytokines, which can potentially alter spermatogenesis and cause DNA aberrations, or indirectly as a result of pathological ROS levels, which are frequent in leucocytospermic patients. In one study, levels of the oxidative DNA damage marker ٨-hydroxy-٢-deoxyguanosine (٨-OH-Dg) were significantly elevated in infertile male patients (Kodama *et al*., ١٩٩٧).

٢.٥.١.٥. ROS and varicocele:

The exact pathways by a varicocele damages spermatogenesis and sperm quality remain poorly understood. ROS may be an important factor, as elevated levels have been detected in infertile patients with varicocele, along with reduced levels of both seminal and blood plasma antioxidants. Levels of ROS positively correlate with the degree of varicocele and are expected to decrease after varicocelectomy (Barbieri *et al*., ١٩٩٩).

In a recent study from our group, infertile patients with varicocele had a significantly higher sperm DNA fragmentation index (DFI) than healthy controls. In addition, infertile patients with varicocele had significantly higher levels of OS than infertile patients with normal genital examination and the controls. Therefore, it appears that infertile men with varicocele have significantly greater spermatozoal DNA damage, which can be related to high levels of OS in semen (Saleh *et al* .,٢٠٠٣). Another potential cause of sperm DNA damage in patients with varicocele is apoptosis. Levels of apoptosis are higher in ejaculated spermatozoa from such patients than in spermatozoa from healthy men (Agarwal and Said, ٢٠٠٥).

٢.٥.١.٦. ROS and semen quality:

٢.٥.١.٦.١. Teratozoospermia:

Teratozoospermia occurs as a result of defective spermatogenesis and is characterized by an abundance of spermatozoa carrying surplus residual cytoplasm. The retention of residual cytoplasm promotes spermatozoa to generate endogenous ROS via mechanism as mentioned previous. Therefore, patients presenting with teratozoospermia are at greater risk of developing pathogenic levels of ROS apoptosis and sperm DNA damage (Aitken, ١٩٩٩).In general ROS production is highest in immature spermatozoa from

with abnormal semen values. However, immature spermatozoa with cytoplasmic retention are not the only abnormal male germ cells that are associated with high levels of DNA damage and ROS production. Spermatozoa with abnormal head morphology, midpiece defects and tail defects also have the same characteristics. Production of ROS positively correlates with the sperm deformity index, calculated by dividing the total number of deformities observed by the number of sperm evaluated (Aziz *et al*., ٢٠٠٤).

٢.٥.١.٦.٢. Asthenozoospermia:

ROS can directly damage spermatozoa by inducing peroxidation of the lipid-containing sperm plasma membrane, which decreases its integrity, and may also affect sperm motility by damaging the axonemal structure (Saleh and Agarwal, ٢٠٠٢). Therefore, high levels of OS are important in the impairment of sperm motility and the occurrence of asthenozoospermia. (Weng *et al*., ٢٠٠٢).

٢.٥.١.٦.٣. Oligozoospermia:

According to WHO, (١٩٩٩) when sperm concentration is less than ٢٠ × ١٠^{٦} / ml the condition is called oligozoospermia. Kodama et al., (١٩٩٧) and Shen et al., (١٩٩٩), found that the level of sperm ٨-OH-dG in infertile patients was significantly higher than that in healthy subjects. There is a significant inverse correlation between ٨-OH-dG and sperm density and total sperm count. One possible explanation for the inverse ٨-OH-dG levels and total number of sperm counts and sperm density is that oxidative sperm DNA damage is associated with the condition of oligozoospermia (Shen et al., ١٩٩٩). Kodama et al., (١٩٩٧) suggested that those spermatozoa with extensive DNA

damage may tend to degenerate and be absorbed during the spermatogenic process.

٢.٥.١.٦.٤. Azoospermia:

Apoptosis and DNA damage may prevent sperm from maturing, as a result, patients may present with azoospermia as a result of an imbalance in these pathways (Said et al., ٢٠٠٤). Under physiological conditions, apoptosis maintains the number of germ cells within the supportive capacity of Sertoli cells. However disturbances in this pathway can interrupt the spermatogenic cascade. High levels of apoptosis were detected at spermatogenic stages where major developmental blocks occur, and frequencies of DNA damage were higher in less mature germ cells (Tesarik et al., ١٩٩٨).

٢.٥.١.٧. ROS and treatment:

Levels of ROS can be reduced by augmenting the scavenging capacity of the seminal plasma with antioxidants (Agarwal *et al*., ٢٠٠٤). Combined therapy is much more beneficial in managing infertile men, because antioxidants act by different mechanisms on different free radicals. Patients diagnosed with male accessory gland infections may benefit from carnitines (L-carnitine ١ g and acetyle-carnitine ٠.٥ g) twice daily for ٣ months, as it results in a significant reduction in ROS levels in semen samples. Other combinations of vitamins A and E with N-acetyl-cystine may also be used. A few clinical trials report the positive effects of antioxidant administration on sperm DNA integrity. When given for ٢ months, vitamin C (٢٠٠ mg), combined with vitamin E (٢٠٠ mg) and glutathione (٤٠٠ mg), significantly decreased ٨-OH-dG levels, considered as a marker of OS-induced sperm DNA damage. Similarly, N-acetyl-cystine and / or

a mixture of essential fatty acids and vitamins E and A reduced levels of ٨-OH-dG (Agarwal *et al*., ٢٠٠٤).

Sperm preparation methods are used during assisted reproduction techniques (ART_S) which include intrauterine insemination (IUI), in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), media may be supplemented with a variety of antioxidants to guard against OS. Adding different concentrations of vitamin C (٣٠٠ and ٦٠٠ µmol / L) and vitamin E (٤٠ and ٦٠ µmol / L) to sperm preparation medium significantly reduced hydrogen peroxide.The superoxide anion can be also reduced by ٢٩-٧٢ % by adding ١٠ mmol / L pentoxifylline (Agarwal *et al*., ٢٠٠٤). The antioxidants in vitro are more marked in samples that were originally characterized by high levels of ROS. N-acetyl-cystine (٠.١ ١ and ٥ mg / ml) has a dose-dependent effect in reducing ROS levels; the reduction was greater in patients with high levels of ROS than in those with low levels (Oeda *et al*., ١٩٩٧).

Semen samples that contain high levels of DNA damage are often associated with decreased fertilization rates and / or embryo cleavage after IVF and ICSI, and may be linked to early embryo death. Although the most normal appearing and motile spermatozoa are selected during ART, there is always a chance that sperm containing varying degrees of DNA damage may be used. The miscarriage rate is highest after ICSI, which possibly reflects the fact that genomically compromised spermatozoa are sometimes used and lead to irreparable DNA damage in the embryo (Carrell *et al* ., ٢٠٠٣).

٢.٥.١.٨. Malondialdehyde:

Lipid peroxidation (LPO) is the most extensively studied manifestation of oxygen activation in biology. In spermatozoa, production of malondialdehyde

(MDA), an end product of LPO induced by ferrous ion promoters, has been reported (Darley-Usmer *et al*., ١٩٩٥). Formation of MDA can be assayed by the thiobarbituric acid (TBA) reaction which is a simple and useful diagnostic tool for the measurement of LPO for *in vitro* and *in vivo* systems (Taourel *et al*., ١٩٩٢). Available data on the impact of OS on sperm motility are based on the measurement of seminal plasma and sperm MDA (Suleiman *et al* ., ١٩٩٦ ; Fraczek *et al* ., ٢٠٠١ ; Nakamura *et al* ., ٢٠٠٢ ; Keskes-Ammar *et al* ., ٢٠٠٣). The results of these findings are contaversal. Suleiman *et al*., (١٩٩٦) did not observe any correlation between seminal plasma levels of MDA and sperm motility, but observe that the MDA conc. in sperm pellet suspension of asthenospermic and oligoasthenospermic patients was twice that of the normospermic men, and the treatment of asthenospermic patients with oral vitamin E significantly decreased the MDA conc. in spermatozoa and improved sperm motility.

Study of Fraczek *et al*., (٢٠٠١) showed that seminal plasma level of MDA is higher in asthenozoospermic males than this level in normospermic men. Nakamura *et al*., (٢٠٠٢) investigation showed that there is no significant difference in seminal plasma level of MDA between asthenozoospermic and normospermic males. Keskes-Ammar *et al*., (٢٠٠٣), observed that there is an indirect correlation between seminal plasma levels of MDA and sperm motility. Tavilani *et al*., (٢٠٠٥), showed that MDA concentration in the spermatozoa and seminal plasma of asthenospermic was significantly higher than in normospermic males. There are a significant inverse relationships between sperm motility and MDA conc. in normospermic and asthenospermic men, and MDA levels was higher in asthenospermic one (Zarghami and Kosrowbeygi, ٢٠٠٥).

٢.٥.٢. Leucocytospermia:

Leucocytes are present throughout the male reproduction tract and are found in almost every human ejaculates (El-Demiry *et al*., ١٩٨٧). They are thought to play an important role in immunosurveillance (Kiessling *et al* .,١٩٩٥) and phagocytic clearance of abnormal sperm (Tomlinson *et al*., ١٩٩٢).The leukocyte counts in semen is one of the classical measures of semen quality and presence of leucocytes in abnormally high conc. > $١٠^{٦}$ / ml according to (WHO , ١٩٩٩) is defined as leucocytospermia. Increased conc. of leucocytes in semen provides an important clinical indicator of genital tract infection or inflammation (Combaire *et al*., ١٩٨٠). The incidence of leucocytospermia is high among infertile patients (Wolff, ١٩٩٥).

There are different studies and opinions about the role of leucocytospermia in the pathogenesis of male infertility. Some authors have suggested that there is an association between increased seminal WBC conc. and poor semen parameter (Wolff *et al*., ١٩٩٠; Vicino *et al*., ١٩٩٩; Arata de Belabarba *et al*., ٢٠٠٠; Diemer *et al*., ٢٠٠٣) or reduced sperm fertilization capacity (Vogelpoel *et al*., ١٩٩١). Whereas other have found leucocytes to have no negative influence (Tomlinson *et al*., ١٩٩٢; Rodin *et al*., ٢٠٠٣).

Simbini *et al*., (١٩٩٨), showed an inverse relation is apparent between leucocyte conc. and sperm count, the percentage of abnormal sperms was higher and the sperm motility poorer in leucocytospermic samples but leucocytospermia not affects the accessory sex gland functions. Semen parameters including progressive motility rate and sperm conc. were significantly lower in the leucocytospermic group compared with the control group, also ICSI outcome including fertilization and embryo development rates were significantly lower in leucocytospermic group (Yilmaz *et al* ., ٢٠٠٥).

Oxidative stress is a key factor in the aetiology of male infertility and develops when levels of ROS production by leucocytes become high enough to overwhelm the antioxidant strategies present in semen (Sikka *et al*., ١٩٩٥), as mentioned detail in ROS production by leucocytes.

٢.٥.٣. Varicocele:

Varicocele is defined as an abnormal dilatation of the testicular veins within the pampiniform plexus, it occurs on the left side in ٧٨–٩٣ % of cases (Saypol *et al*., ١٩٨٣). The incidence of varicoceles in the general population is approximately ١٥ %, while ١٩ to ٤١ % of men presenting for infertility investigation demonstrate varicoceles (Cockett *et al*., ١٩٨٤). Treatment of varicoceles in infertile men has demonstrated improved semen parameters in ٥٠–٨٠ % of patients (Marks *et al* ., ١٩٨٦), pregnancy rates of ٣–٧١ % (Madger *et al* ., ١٩٩٥), and most recently increased per cycle pregnancy and live birth rates with IUI (Daitch *et al* ., ٢٠٠٠).

There are ٣ accepted theories on the causes of varicoceles. First, that there are anatomical differences between the left and right testicular veins; specifically, that the right testicular vein inserts directly into the inferior vena cava, while the left testicular vein inserts into the left renal vein. The different insertion of the left testicular vein is believed to result in an increase in hydrostatic pressure, which is subsequently transmitted to the pampiniform plexus, causing dilatation and tortuosity of the veins, second, that there is an absence of competent venous valves, resulting in reflux of venous blood. The venograph pattern of ٦٥٩ consecutive men with varicoceles showed that ٧٣ % of these men had absent venous valves (Braedel *et al*., ١٩٩٤). Third, that there is partial obstruction of the testicular vein caused by compression of the left

renal vein between the aorta and the superior mesenteric artery (Naughton *et al*., ٢٠٠١).

The exact mechanism by which the varicocele depress spermatogenesis is unknown but several theories have been advanced to explain the mechanism by which varicocele impairs male infertility. These theories include scrotal hyperthermia, retrograde flow of adrenal or renal metabolities, Leydig cell dysfunction and hypoxia .Varicocele is reported to be associated with elevated ROS production in spermatozoa and diminished seminal plasma antioxidant activities (Mostafa *et al* ., ٢٠٠١).

The studies which explained the relationship between varicocele and semen quantity are contaversal, it is generally accepted that a varicocele influences spermatogenesis and may be linked to a decreased sperm count, abnormal sperm motility, and morphology. Following varicocelectomy, semen quality improves significantly, but the mechanism of action is still unknown (Dubin and Amelar, ١٩٧٧; Hendry, ١٩٩٢). The semen quality including sperm conc. and total sperm count was significantly lower in men with varicocele than that of control subjects, but there are no differences in the volume of semen and sperm motility between the ٢ groups (Lund and Nielsen, ١٩٩٦).

Study of Freiha and Mroueh, (١٩٧٦) showed that there are a significant improvement of sperm count, motility and morphology of patients with varicocele after ligation of spermatic vein. Scrotal cooling at night by means of a prigenital air stream resulted in a scrotal temperature drop by ٠.٨ ć (median), and a significant increase in sperm conc. and total sperm count was achieved by

nocturnal cooling after ٨ weeks and ١٢ weeks. The improvement of sperm motility and sperm morphology was statistically insignificant in oligozoospermic men with a history of testicular maldescent (Jung *et al*., ٢٠٠٥). When comparing pre-operative and post-operative semen parameters of varicocele patients, there was a significant increase in sperm conc., and percentage of motile spermatozoa, as well as a significant reduction in spermatozoa with abnormal morphology, and reduction in the level of MDA, after ٣ months or followed ٣ months of varicocelectomy (Mostafa *et al* .,٢٠٠١).

The influence of varicocele on fertility parameters showed that the mean of testosterone conc. of men aged over ٣٠ years complaining from varicocele was significantly lower than that of younger patients with varicocele, whereas this trend was not observed in men without varicocele (WHO, ١٩٩٢). These findings suggest a detrimental, time dependent effect of varicocele on Leydig cell function. There was significant elevation of FSH in the seminal plasma of the infertile men with varicocele compared with the other infertile men without varicocele and fertile men. Seminal plasma of LH and prolactin values was similar in both infertile groups but significantly higher than in the fertile men. Testosterone and oestradiol levels, in the seminal plasma of infertile men with varicocele were lower than in the fertile and the other infertile males (Micic *et al*., ١٩٨٦).

٢.٥.٤. Smoking:

Cigarette smoking is a serious health problem of our society. According to World Health Organization (WHO) approximately one third of the world's population above ١٥ years of age smoke. It is known that cigarette smoke is a cell mutagen and carcinogen and that it may adversely affect male fertility

(Zenzes, ٢٠٠٠). Cigarette smoke (CS) contains a large number of substances including nicotine, carbon monoxide and recognized carcinogens and mutagens such as radioactive polonium, cadmium, benzo (a) pyrene, dimethylene (a) anthracene and others (Zavos *et al*., ١٩٩٨; Zenzes, ٢٠٠٠). Inhalation of CS, leads to absorption of these agents through vascular and blood circulation throughout the body (Stillman, ١٩٨٦). It is also possible that CS derived substances could end up in the seminal plasma of smokers through various modes of diffusion and active transport (Zavos, ١٩٨٩).

Numerous investigations have been conducted on the relationship between CS and male infertility, but the exact molecular mechanism is not fully understood in most the cases and the data are very disputable.

Infertile men who smoke have higher levels of seminal OS than infertile non-smoker. The link between CS and higher seminal ROS can be attributed in part to the associated increase in seminal leucocyte concentrations by as much as ٤٨ % (Saleh *et al* ., ٢٠٠٢). In addition, cigarette smoke contains a variety of ROS. Smoking may also damage the chromatin structure and produce endogenous DNA strand breaks in human sperm. Levels of DNA damage tend to be higher in smokers (Potts *et al*., ١٩٩٩).

Many investigations have found a significant correlation between smoking and the impairment of semen parameters, such as a decrease in sperm motility or increase in abnormal sperm morphology and lowered sperm density (Alwachi *et al*., ١٩٨٦ ; Vine *et al* ., ١٩٩٤ ; Sofikitis *et al* ., ١٩٩٥ ; Merino *et al* ., ١٩٩٨ ; Zenzes , ٢٠٠٠ ; Zinaman *et al* ., ٢٠٠٠). Recently, it has been proved that CS is associated with a significant decrease in sperm density, total sperm count, and total number of motile spermatozoa (Kunzle *et al*., ٢٠٠٣).

These authors also demonstrated that CS could significant reduce percentage of normal sperm morphology, ejaculate volume and sperm vitality.

The study of Pasqualotto., *et al* (٢٠٠٤) showed no significant differences between non-smoker and smoker fertile men in sperm concentration and motility, but semen volume was the only semen variable which tended to decrease according to the number of cigarette smoked. Also some other studies found no correlation between CS and semen quality, sperm function or sperm nuclear DNA damage (Sergerie *et al*., ٢٠٠٠; Vogt *et al*., ١٩٨٦).

In a meta-analysis of ٢٧ studies on CS and semen quality, mean reduction of ١٣ % in sperm conc., ١٠ % in sperm motility and ٣ % in normal sperm morphology were found in smokers, and CS has also been correlated with poor sperm function in sperm penetration assays (Vine, ١٩٩٦).

٢.٥.٥. Spermatozoa and the immune system:

٢.٥.٥.١. Protection of the spermatozoa:

When spermatocytes and spermatozoa are produced at puberty they express antigens not previously encountered by the immune system. As maturation takes place they become progressively more foreign to their progenitor and migrate towards the lumen of the seminiferous tubule. They are protected from immunological destruction in the testes by tight junctions forming between Sertoli cells, isolating the lumen of the seminiferous tubule from the interstitium. This blood–testes barrier prevents immunoglobulins and immunocompetent cells from entering the lumen of the tubule (Yule *et al*., ١٩٨٨).

The protection provided by the blood – testes barrier is incomplete. It allows the immune system access to the antigens of spermatocytes in the basal compartment of the seminiferous tubule (Yule *et al*., ١٩٨٨). Intra–epithelial lymphocytes are present in the rete testes, epididymis, and vas deferens and macrophages are found in the interstitium of the testis and epididymal lumen. In the testis, macrophages may provide a secondary barrier preventing the escape of none–sequestered antigenic material migrating to the boundary wall of the seminiferous tubule at stages of spermatogenesis where there is a significant amount of degeneration of germ cells (Pundey and Anderson, ١٩٩٣). In the epididymis they have been observed to contain sperm fragments. This constant antigenic (leak) could be responsible for maintaining a state of immune tolerance by generating suppressor T lymphocytes, which account for the most of the intra–epithelial lymphocytes of the rete testes, epididymis and vas deference (Turek and Lipshultz, ١٩٩٤ ; Pollanen and Cooper, ١٩٩٤).

٢.٥.٥.٢. Incidence of antisperm antibodies (ASA):

It is well established that both men and women can produce antibodies which react with human spermatozoa. In infertile men ASA can be detected in seminal plasma and serum and are also located on the surface of spermatozoa. The condition of having ASA has also been found in men with homosexuality, testicular trauma, varicocele, mumps orchitis, spinal cord injury, congenital absence of the vasa and vasectomy (Shibahara *et al* ., ٢٠٠٢).

In ١٩٥٤, Rumke first discovered antibody activity to sperm in the sera from two infertile men using a sperm agglutination technique. They subsequently showed that about ٣ % of infertile men had sperm agglutinating antibody, whereas it was absent from fertile controls (Rumke and Hellinga ١٩٥٩). Afterwards, it has been shown that the incidence of ASA in men

suspected of infertility was ٤–٢١ % (Ayvaliotis et al., ١٩٨٥; Naz and Menge, ١٩٩٤).

The study of Hunter *et al*., (١٩٧٦), showed that sperm–agglutinating antibodies were present in ٣ (٢.٦) % of ١١٧ fertile men and in ١٩ (٣٣.٩ %) of ٥٦ men who had been vasectomized. Twenty–four of the ٥٦ vasectomized men had been studied before vasectomy; sperm – agglutinating antibodies were present in one (٤.٣ %) compared with eight (٣٣.٣ %) after vasectomy. No sperm– immobilizing antibodies were detected before vasectomy but were present in ١٠ (١٧.٩ %) of the ٥٦ men after vasectomy.

٢.٥.٥.٣. Types and origin of the ASA:

ASA found in semen are usually immunoglobulins of the IgG or IgA isotype that are directed to various sites of the spermatozoa, i.e. head, midpiece, tail, or combinations of them (Peters and Coulam, ١٩٩٢). IgGs in semen are mostly regarded as transudates from the systemic circulation via the prostate gland, whereas IgA is usually (٦٠ – ٩٠ %) secretory in type, suggesting intratesticular and / or epididymal synthesis (Adeghe, ١٩٩٢). The binding of these antibodies can be directed toward carbohydrate or peptide moieties of sperm antigens, but the binding can also occur via Fc receptors (Allen and Boune, ١٩٧٨; Isojima, ١٩٨٨).

٢.٥.٥.٤. ASA and fertility:

In general, tail–directed ASA tend to influence and disturb sperm progressive motility, whereas head–directed ASA may alter fertilization by occluding binding sites for zona pellucida binding (Bronson *et al*., ١٩٨٤) or by affecting motility parameters (Zouari *et al*., ١٩٩٣). The acrosome reaction, another crucial step in human sperm function, can also be altered by ASA

(Lansford *et al*., ١٩٩٠). Capacitation involves loss of cholesterol from the sperm surface membrane which induces changes necessary for fertilization. ASA can prevent the loss of cholesterol and inhibit these changes (Benoff *et al*., ١٩٩٣).

From a physiological point of view, immunological infertility due to sperm surface antibodies can result from the effect on sperm transport, the destruction of gametes, acrosome reaction abnormalities, by inhibition of sperm zona–pellucida binding or by prevention of embryo cleavage and early development of the embryo (Bronson *et al*., ١٩٨٤; Shushan and Schenker, ١٩٩٢). Surprisingly, spermatogenesis is apparently not affected by the presence of IgG or IgA ASA since sperm density and / or morphology are not influenced by the presence of these antibodies (Haas *et al*., ١٩٨٣; De Almeida *et al*., ١٩٩١).

٢.٥.٦. Hormonal imbalance:

Endocrine or hormonal disorders represent about ٢-٥ % of male infertility factor (Leon *et al*., ١٩٩٩), and primary endocrine abnormalities are rare in patients with sperm concentration of > ٥ × $١٠^{٦}$ / ml (Butt and Blunt, ١٩٨٨). It is advisable that any infertile male requiring an endocrine evaluation should at least have the following basal hormone measurements: FSH, LH, testosterone, sex–hormone binding globulin (SHBG) and prolactin. Testosterone and gonadotrophins must always be measured simultaneously as the results of either can not be interpreted in isolation (Swerdloff *et al*., ١٩٩١). Low serum FSH, LH, and testosterone values indicate hypothalamo – pituitary disease such as hypogonadotrophic hypogonadism (Trainer and Besser, ١٩٩٥).

A high FSH and LH and low testosterone indicate primary testicular failures, i.e. hypergonadotrophic hypergonadism, involving both Leydig and germinal epithelium. An azoospermic patient with normal serum testosterone, LH and FSH level has either retrograde ejaculation or obstructive azoospermia and may need further evaluation with testicular biopsy and exploration of the vas. In contrast, a patient with oligo / azoospermia and elevated circulating FSH but normal LH and testosterone levels has primary germinal tubular failure with no associated Leydig cell damage, and under these circumstances testicular biopsy is unnecessary. Combined elevation of LH and testosterone in a male indicates that pituitary gonadotrophs and testosterone target–tissue resistance are unresponsive to circulating androgens and suggest partial–androgen–resistant syndrome, i.e.Reifenstein' s syndrome (Islam and Trainer, ١٩٩٨).

Hyperprolactinemia probably causes hypogonadism by disrupting GnRH release from the hypothalamus (Franks *et al*., ١٩٧٨). Elevated circulating prolactin concentrations are associated with impotence and decreased libido rather than spermatogenic failure and decreased of testosterone (Thorner *et al*., ١٩٧٧; Segal *et al*., ١٩٧٩).

٢.٦. *In vitro* human sperm activation:

There are several techniques were discovered and used for sperm activation and improvement of them to further used in artificial insemination. The artificial insemination is used when the seminal fluid is subnormal or the presence of ASA and also used in idiopathic infertility in males and females

(Confino *et al*., ١٩٨٦).

In vitro addition of seminal fluid to the ovum directly without sperm activation lead to failure of fertilization due to incurrence of capacitation process, as well as due to the effects of some abnormal seminal components such as leucocytes and phagocytes (Kanwar *et al* ., ١٩٧٩).

Sperm motility percent and normal sperm morphology are very important to increases the chance of fertilization and pregnancy rates through the Artificial Insemination by Husband (AIH), gametes intrafalopian transfers (GIFT), *in vitro* fertilization and embryo transfers (IVF–ET) (Menkveld *et al*., ١٩٩٠).

The results of Al–Hady, (١٩٩٧), showed a significant improvement of sperm functions which include; sperm motility percent, normal sperm morphology percent, sperm agglutination percent, leucocytes conc., and sperm grade activity after sperm activation compared with the value before activation when used different techniques and two sperm activation medium Ham's F- ١٠ medium and modified balanced Earle's medium .

The malondialdehyde values decreased in seminal fluid which treated with Ham's F- ١٠ medium plus vitamin E and apoptosis of spermatozoa decreased in semen with Ham's F- ١٠ medium only and Ham's F- ١٠ medium plus vitamin E , both of them have protection effect against oxidative stress in human semen (Yenilmez *et al* ., ٢٠٠٦).

٣. Materials and Methods:

٣.١. Patients :

The study included two hundred and eighty selected infertile men who attended to Infertility care and (IVF) center in Arbil, as well as thirty healthy volunteer fertile men who have one or more than one child, as control group.

This work was carried out between July ٢٠٠٥ and June ٢٠٠٦. Other information was taken from the patients before starting the evaluation of semen analysis. This information was recorded in a prepared data form Table (٣). The mean age of infertile men was (٣٣.٤٩٢ ± ٠.٤٣٨) years and the mean duration of infertility was (٧.٤٨٩ ± ٠.٣١٦) years. The mean age of fertile control men was (٣٤.٦٧٣ ± ٠.٨٤٥) years.

Table (٣): Prepared data form for infertile patients.

Number of patients :	Number of file :
Date :	Full address :
Name of patient :	Name of wife :
Age of patient :	Age of wife :
Days of abstinence :	Type of infertility :
Blood group :	Duration of infertility :
Work :	Exposed to chemicals :
Varicocele :	Pubertal mumps :
Inguinal hernia :	Alcohol taking :
Smoking :	
Others :	

٣.٢. Stains:

A – Sperm vitality:

١- Eosin Y ١٠ gm / L in D.W = ١ %.

٢- Nigrosin ١٠٠ gm / L in D.W = ١٠ % (WHO, ١٩٩٩).

B – Working benzidine solution:

١- Ethanol ٩٦ % ٢٠ ml

٢- Benzidine ٠.٦٢٥ mg

٣- D.W ٢٠ ml

C – Hematoxylin and eosin ٠.٥ %

٣.٣. Sperm activation medium:

٣.٣.١. Modified balanced Earle's medium:

Table (٤): Shows the components of the modified Earle's medium**,** ١٠٠ ml of this medium was prepared according to the following steps (Al–Hady, ١٩٩٧):

١- Earle's salt (CMV–١ company, France), Ampicillin, and sodium pyruvate was added to ٩٠ ml of D.W.

٢- Sodium bicarbonate added to the mixture above.

٣- The mixture is well mixed gradually until all they are dissolved.

٤- The osmolality is adjusted between ٢٨٠–٢٩٠ mOSm / kg by using the osmometer type (Knauer, D-١٤١٦٣, Berlin, Germany)

٥- D.W. was added for adjusting the osmolality.

٦- pH adjust at ٧.٦ .

٧- Drops of diluted HCL were used if the solution is alkaline.

Table (٤): Preparation of ١٠٠ ml of modified Earle's medium which used for *in vitro* sperm activation.

Components	Quantity
Earle's salt	٠.٨٨ gm
Ampicillin	O.٠٠٨ gm
Sodium pyruvate	٠.٠٠١ gm
Sodium bicarbonate	٠.٢١ gm
D.W	١٠٠ ml
Osmolality	٢٨٠ – ٢٩٠ mOSm / kg
pH	٧.٦

- Inactivated female serum ٣٠ % was added to the medium during the time of sperm activation (Al – Hady, ١٩٩٧).

٣.٤. Solutions used in malondialdehyde determination:

١ – Thiobarbituric acid (TBA) ٠.٦ %

٢ – Trichloroacetic acid (TCA) ١٧.٥ %

٣ – Trichloroacetic acid (TCA) ٧٠ %

٣.٥. ٠.١ M phosphate buffer saline (PBS) pH ٧.٤:

١- ٠.٢ M $NaHPO_4$ monobasic stock prepared by :

١٣.٥ gm $NaHPO_4$ in ٥٠٠ ml D.W.

٢- ٠.٢ M Na_2HPO_4 dibasic stock prepared by :

٥٣.٥ gm Na_2HPO_4 in ١ L of D.W.

٣- ٠.١ M phosphate buffer prepared by:

٥٧ ml of monobasic and ٢٤٣ ml dibasic phosphate solution were mixed.

٤- ٠.١ M of PBS pH prepared by:

٥٠٠ ml of ٠.١ M phosphate buffer + ٣.٥ gm NaCL

٣.٦. Semen collection:

Semen samples were collected by masturbation after ٣–٥ days of abstinence, in wide mouth disposable plastic container. The semen samples were incubated at ٣٧ ^{o}C for ٣٠ minutes to liquefy (Pal *et a*l., ٢٠٠٦).

The following routine parameters were assessed in the liquefied semen samples according to the methods described in the (WHO, ١٩٩٢; WHO ١٩٩٩). These parameters includes; appearance, volume, pH, color, viscosity, liquefaction time, sperm conc., sperm motility percent, sperm grade activity, sperm vitality percent, sperm morphology percent and agglutination.

The semen was centrifuged at ٣٠٠٠ (rpm) for ١٠ minutes to obtain the plasma. The plasma was stored in –٨٠ ^{o}C (Sanyo ultra Low MDF-١٤٢ Japan) for further examinations, such as antisperm antibodies, osmolality, and malondialdehyde determination. Also a total of ٥ ml of blood was obtained from some patients (azoospermia and severe oligozoospermia), and some control men. Serum was separated by centrifugation at ٣٠٠٠ (rpm) for ١٥ minutes and stored at –٨٠ ^{o}C until hormonal evaluation performation.

٣.٧. Macroscopic examination:

٣.٧.١. Appearance:

Normal semen sample has a gray–opalescent appearance. The normal and abnormal appearance of the semen was recorded.

٣.٧.٢. pH:

The pH of semen sample was estimated by using a pH paper range ٦.١–١٠ was used. Whatever type of pH paper is used, its accuracy should be checked against known standards before the use in routine semen analysis (Comhaire and Vermeulen, ١٩٩٥, WHO, ١٩٩٩). The pH recorded in fresh semen immediately after liquefaction of semen to avoid pH falls as the specimen ages (Hinting, ١٩٨٩).

٣.٧.٣. Volume:

The volume of the ejaculate was measured with a graduated cylinder.

٣.٧.٤. Viscosity:

The viscosity of the liquefied sample was estimated by gentle aspiration into pasture pipette and then allowing the semen to drop by gravity and observing the length of the thread formed. A normal sample leaves the needle as small discrete drops, while in cases of abnormal consistency the drop will form a thread of > ٢ cm (Comhaire and Vermeulen, ١٩٩٥; WHO, ١٩٩٩).

٣.٨. Microscopic examination:

٣.٨.١. Sperm concentration:

The sperm concentration was estimated by multiply the mean of sperm number in ten field with $١٠^{٦}$. Total sperm count = Sperm conc.× volume (Al-Hady, ١٩٩٧).

٣.٨.٢. Sperm motility:

A drop of well mixed undiluted semen was placed on the surface of a warm dry clean slide. A cover slip is placed on the drop of the semen. The slide is allowed to rest on the microscope until the streaming of the sperm has stopped. Then the drop is viewed at ٤٠٠ × (Yaseen, ٢٠٠٤). A minimum of around ٢٠٠ sperm should be counted; both motile and immotile sperms are counted in at least ٥ separate fields (WHO, ١٩٩٩).

The motility of spermatozoa in each sample is graded ٠, ١, ٢, ٣, or ٤ according to whether it shows:

٠ = no movement

١ = movement but not forward progression

٢ = movement with slow forward progression

٣ = movement in an almost straight line with good speed

٤ = movement in a straight line with high speed (Dale McClure, ١٩٨٦;

Levin *et al*., ١٩٩٢; Seaman *et al*., ١٩٩٤).

The percentage of sperm motility was calculated by:

Motility % = number of motile spermatozoa / total number of

spermatozoa (motile and immotile) × ١٠٠ .

The sperm motility index was calculated by multiplying the grade activity with the percentage of motility (Makler *et al*., ١٩٧٩).

٣.٨.٣. Sperm vitality test:

١ - A drop of liquefied semen was mixed with two drops of ١ % eosin Y solution.

٢ – After ٣٠ seconds, three drops of nigrosin solution ١٠ % was added and mixed.

٣ – A thin smear of the semen–eosin–nigrosin mixture was made within the ٣٠ seconds of adding nigrosin .

٤ – Allowed to air - dried and examined under the microscope (١٠٠٠ ×).

One hundred sperm were counted to express the percentage of live spermatozoa. The live spermatozoa are white and the dead are stained red (WHO, ١٩٩٩).

Sperm vitality = number of viable sperm / total number of spermatozoa × ١٠٠.

٣.٨.٤. Sperm morphology:

The sperm morphology was done by using the haematoxylin and eosin stains. These stains available in all laboratories and for the most part is adequate for staining spermatozoa. The method has the advantages of speed and simplicity, it was performed as follows:

١ – Air dry smear of the semen

٢ – Fixed in methanol and washed with water

٣ – Immersed in Harris haematoxylin for ١ minute and then washed with water.

٤– Immersed in alcohol ١ % and in ٧٠ % alcohol for ٢–٣ minutes and washed with water .

٥ – Dipped into ٢ % sodium bicarbonate solution for ١٠–٢٠ seconds, and washed in water.

٦ – Stained with an aqueous ٠.٥ % of eosin solution for ٢٠ seconds and washed with water .

٧ – Dried and examined (Jequier, ١٩٨٦)

The slides were examined at a magnification ١٠٠٠ × using oil immersion. A total of ٢٠٠ sperm were examined. The percentage of normal and abnormal spermatozoa was calculated as follows (Yaseen *et al.*, ٢٠٠٤):

Normal morphology % = number of normal spermatozoa / total number of spermatozoa × ١٠٠

Abnormal morphology % = number of abnormal spermatozoa / total number of spermatozoa × ١٠٠

٣.٨.٥. Leucocytes determination:

The leucocytes were determined in the semen of patients by ٢ methods:

A – High power field (HPF):

This method was used during sperm motility determination. The disadvantage of this method is can not differentiate between leucocytes and other round cells found in the semen such as, spermatocytes, spermatid, epithelial cells , and prostatic cells (Wolff *et al* ., ١٩٩٠).

B – Myeloperoxidase cytochemical test (Endtz method):

This test was performed on suspended cells in liquefied semen specimen and quantities by counting stained cells (Endtz, ١٩٧٤). The leucocytes were determined by the following steps:

١- Adding ٢٠ µL of liquefied semen into a small tube.
٢- Adding ٢٠ µL of sperm prepared medium.
٣- Adding ٤٠ µL of working benzidine solution.
٤- Mixing well at room temperature.
٥- Transfering ٥ µL of prepared solution to Neubaur counting chamber.
٦- Examined under ٢٠ × of objective lens, all leucocytes are stained dark brown in color with round shape.
٧- The cells are counted in all RBCs fields, which equal to ١mm^3. Numbers of WBCs calculated by multiplying total number of cells by ٤ to correct for dilution factor.

× ١٠ (depth in µm of counting chamber)

× ١٠٠٠ (to convert mm^3 to ml)

٣.٩. Osmolality determination:

Seminal plasma osmolality was measured by the freezing point depression method and using an osmometer type (Knauer, D- ١٤١٦٣, Berlin,

Germany). This method requires samples of semen to be centrifuged free of particulate matter (Sweeney and Beuchat, ١٩٩٣). Before the samples osmolality measured, the osmometer must be calibrated between ٠ and ٤٠٠ mOSm / kg using distilled water and standard NaCl solution.

٣.١٠. Assessment of the lipid peroxidation activity:

The assessment of lipid peroxidation process was achieved via determination the end product**,** malondialdehyde (Lunec, ١٩٩٠).

Procedure:

The level of seminal plasma MDA was determined by a modified procedure described by (Guidet and Shah, ١٩٨٩). In brief; to ١٥٠ µl seminal plasma add the following: ١ ml trichloroacetic acid ١٧.٥ %, ١ ml of ٠.٦ % thiobarbituric acid, mixed well by vortex, incubate it in boiling water bath for ١٥ minutes, and then allowed to cool.

Then adding ١ ml of ٧٠ % TCA, then let the mixture to stand at room temperature for ٢٠ minutes, centrifuged at ٢٠٠٠ rpm for ١٥ minutes, and take out the supernatant for scanning spectrophotometriaclly (Muslih *et al* ., ٢٠٠٢).

The conc. of MDA = absorbance at ٥٣٢ nm × D / L × E_{o}

L: light bath (١ cm)

E_{o}: extinction coefficient ١.٥٦ × $١٠^{٥}$ $M^{-١}.Cm^{-١}$

D: dilution factor = ١ ml volume .used in Ref. / ٠.١٥ = ٦.٧

٣.١١. Techniques used for *in vitro* human sperm activation:

In this study two methods were used for improvement of semen quality.

A – Layering technique:

١- One ml of semen taken from asthenozoospermic patient and put in test tube, and covered with ١ ml of prepared modified Earle's medium which provided with ٣٠ % of inactivated female serum.

٢- The tube was incubated at ٣٧ ^{o}C for at least ٣٠ minutes.

٣- After incubation period, one drop of superior medium was taken to a clean slide and cover with cover slip and examined at ٤٠٠× (Al – Sultani , ١٩٩٧) .

B–Centrifugation – swim up techniques (Single wash technique):

١- One ml of semen taken from asthenozoospermic patient and mixed with ٢ ml of prepared Earle's medium which provided with ٣٠ % inactivated female serum .

٢- Centrifuge at ٢٠٠٠ rpm for ١٠ minutes.

٣- Remove the supernatant and the pellet was covered with ١ ml of modified Earle's medium .

٤- The sample was incubated at ٣٧ ^{o}C for at least ٣٠ minutes.

٥- One drop of superior medium was taken to a clean slide and examined at ٤٠٠ ×. (Al – Hady, ١٩٩٧).

Semen was examined before and after activation including evaluation of sperm conc., motility, morphology, agglutination and leucocytes.

٣.١١.١. Inactivation of human serum:

The modified Earle's medium which used for *in vitro* sperm activation in this study, was provided by inactivated female serum which prepared as follows:

١- Blood samples are taken of a female at ١٣ days of menstrual cycle.

٢- Centrifuge at ٣٠٠٠ rpm for ١٠ minutes to obtain the serum.

٣- The serum is put in water bath at ٥٦ ć for ٣٠ minutes to remove decomplementation.

٤- The serum is stored at – ٨٠ ^{o}C until used (Shibahara *et al*., ١٩٩٣).

The inactivated serum was added with conc. ٣٠ % to the modified Earle's medium during in *vitro* sperm activation (Al–Hady, ١٩٩٧).

٣.١٢. Techniques used for detection of antisperm antibodies:

In this study two methods were used to detection of ASAs which including:

A- Sperm agglutination test:

The presence of agglutination is suggestive of, but not sufficient evidence to prove the existence of an immunological factor of fertility. Agglutination was assessed at the time of determining sperm motility (Comhaire and Vermeulen, ١٩٩٥; WHO, ١٩٩٩).

Agglutination % = number of sperm agglutinated / total number of spermatozoa × ١٠٠.

The disadvantages of this method was that, sometimes the motile and non - motile spermatozoa was agglutinated due to the presence of bacteria, fungi, and other substances in semen such as mucus threads, fibers, non–spermatic cells and debris, or due to the effects of sex steroid hormones or non – immunoglobulin proteins and in all these cases the agglutination was considered pseudo–agglutination to differentiate them with true agglutination (Beer and Neaves, ١٩٧٨; Jones, ١٩٨٠).

B- Tray agglutination test (TAT) or Micro- agglutination test (MAT):

This test was firstly performed by (Friberge, ١٩٧٤), and it used widely and considered the reference test for detection of ASA in serum and seminal plasma (Linnet and Suominen, ١٩٨٢)

The principle of this test is that, when the ASAs are presence in serum or seminal plasma of infertile men, this lead to agglutination of normal spermatozoa which added to them. The type of agglutination (head to head, tail to tail or mixed) is depending on the type of antibodies found in serum or plasma. This method has different advantages such as; TAT is more sensitive than gelatin agglutination test (GAT), large number of semen samples can be examined in one time, and this test not requires large amount of normal semen sample (Friberge, ١٩٧٤).

Procedure:

١- Put normal semen sample (> ٥٠ × ١٠^{٦} spermatozoa / ml and motility ≥ ٥٠ %), in a centrifuge tube and add an equal amount of phosphate buffer saline (PBS) solution on the wall of the tube to form a separate layer on the semen sample. Incubate at ٣٧ ć for ١ hr or more until it used. The active spermatozoa move toward the superior of the solution.

٢- Perform a serial dilutions for each sample of patients seminal plasma which used to detection of ASA in them using Micro-titer plates which contains different spots and as the follows :

A- Put ١٧٥ µl of PBS solution to the ١st spot and ١٠٠ µl of them to the spots ٢-٥.

B- Put ٢٥ µl of patient seminal plasma to the ١st spot and mixed well, in this case the dilution done was ١: ٨.

C- ١٠٠ µl of this dilution is transfer to the ٢nd spot and mixed well to making the dilution ١: ١٦.

D- ١٠٠ µl of the ٢nd dilution is transfer to the ٣rd spot to making the dilution ١: ٣٢.

E- Repeat the same steps for obtaining the other dilutions ١: ٦٤ and ١: ١٢٨.

٣- Transfer ٦ µl of each dilution by using Micro-Hamilton syringe and put in the spot of the Terasaki plates after covering them with a thin layer of fluid paraffin.

٤- Transfer ١ µl of the superior layer of normal semen-PBS mixture, and to each spot of Terasaki plates which contains the dilutions of the patient's seminal plasma ١: ٨, ١: ١٦, ١: ٣٢, ١: ٦٤, and ١: ١٢٨.

٥- Incubate at ٣٧ ^{o}C for at least ٤ hrs.

٦- Examine and record the degree and type of agglutination using ٤٠٠ × of microscopic magnification power. The highest seminal plasma dilution which the agglutination observed is called agglutination titer.

٣.١٣. Hormone determination:

The hormones FSH, LH, Testosterone, Prolactin, and Estradiol II ($E_٢$) of some azoospermic and severe oligozoospermic, in addition of some fertile control men were measured in the Central Laboratory of Rizgary Teaching Hospital in Arbil city.

Blood samples was taken by vein puncture and immediately transferred into the plastic tubes, then centrifuged and kept at – ٨٥ ^{o}C (Sanyo ultra low MDF -١٤٢ Japan) until assay. The above hormones were measured using Enzyme Linked Immunosorbant assay (ELISA) (Thomas, ١٩٩٨).

٣.١٤. Statistical analysis:

Analysis of data was performed by using SPSS (Version ١٠) in the home computer. Results are expressed as mean ± S.E. Statistical differences were determined by Least Significance Differences (LSD) test for multiple comparisons between different groups after performing Fisher (F-test) and ANOVA making.

Dependent paired student t-test was used to analyze the differences between HPF and Endtz method for detection of leucocytes in the semen and also used for analyze of spermatozoal improvement by using layering and swim – up techniques and comparison the two methods. Independent unpaired t-test was used to compare the parameters between the infertile men's and also

between fertile and infertile groups. Chi-square test was used to compare of semen liquefaction time, appearance and viscosity, and agglutination of spermatozoa between fertile and infertile men. P value < ٠.٠٥ was considered statistically significance, (Freund,١٩٨١).

٤. Results:

٤.١. General criteria of infertile men:

Table (٥) shows the general criteria of infertile patients which recorded in the presence work. The most patients who attended to the Infertility care and IVF center were from the Arbil city (١٨٠ samples which represent about ٦٤.٢٨٥ % of total samples). Primary infertility includes ٢٤٥ patients and secondary infertility includes only ٣٥ men. The number of smokers was ١٢١ which represent ٤٣.٢١٤ %. About ١٠ infertile men was drinking alcohol. The blood group of ١٣٠ men was O^+ but the less numbers which record were O^-. The number of infertile men which has hard work are ١٩٠ and others with easy and moderate work. Some patients have varicocele and represent ٢٧.١٤٢ % of all infertile men.

Figure (٦) includes the numbers and percentages of infertility male factors classified according to the WHO, ١٩٩٩.Idiopathic infertile men represent the most number between all groups ٣٥ % and asthenoteratozoospermic patients represent only ٤.٦٤٢ % which are the smallest group among all infertile men.

٤.٢. Semen quality and sperm parameters of fertile and infertile men:

٤.٢.١. Liquefaction time, appearance, viscosity and pH:

As shown in table (٦) and figure (٧), the liquefaction time of semen was significantly differences (p<٠.٠١) between fertile men and infertile men. ٩٣.٣٣٣ % of fertile men have liquefaction time less than ٣٠ minutes (normal) while ٧٥ % of infertile men have liquefaction time less than ٣٠ minutes. ٩٠ % of fertile men and ٧٧.٨٥٧ % of infertile men has normal semen appearance; the differences between the two groups are significant at (p<٠.٠٥) (table ٧ and figure ٧). When viscosity of semen was measured significant differences appears between both groups (p<٠.٠٥), the percentage of normal viscosity among fertile men are higher than that of infertile men (table ٨ and figure ٧). Table (٩) shows the results of semen pH which recorded between fertile men and infertile men. No abnormal pH recorded among fertile men while ٥ % of infertile men have abnormal semen pH.

٤.٢.٢. Semen parameters, volume, leucocytes, osmolality, and malondialdehyde:

There are high significance differences of sperm parameters between fertile volunteer control men and infertile men , table (١٠).The volume of semen, sperm conc., total sperm count, grade activity, motility, sperm motility index, viability, and normal sperm morphology are higher in fertile men compare with infertile men (p<٠.٠٠١) . The number of leucocytes in semen with both methods HPF and Endtz, osmolality, and malondialdehyde are significantly higher in infertile men (p<٠.٠٠١).

٤.٢.٣. Semen quality of fertile men and different infertile groups:

٤.٢.٣.١. Fertile, oligoasthenozoospermic and oligoasthenote-ratozoospermic men:

As shown in table (١١), there are significant differences (p<٠.٠٠١) of semen quality between fertile men and the two infertile groups, oligoasthenozoospermia and oligoasthenoteratozoospermia. Semen volume, sperm conc., total sperm count, grade activity, motility, sperm motility index, viability, and normal sperm morphology, are higher in fertile men than the other groups.Leucocytes with both HPF and Endtz methods and osmolality of seminal fluid are lower in fertile men than the other two infertile groups.

The results of comparison between oligoasthenozoospermic and oligoasthenoteratozoospermic infertile men observed a significant differences (p<٠.٠٥) of semen volume, in which is lower in oligoasthenozoospermic one. No differences appear in sperm conc., total sperm count, sperm motility index, osmolality, and semen leucocytes between the ٢ groups. Grade activity, viability (p<٠.٠١) and normal sperm morphology (p<٠.٠٠١) are higher in oligoasthenozoospermic men.

Table (٥): General criteria of infertile men which recorded in the presence work .

Criteria	Type of criteria	Number	Percentage
١-Samples in	a-Arbil	١٨٠	٦٤.٢٨٥
	b-Sulaymaniah	٦٠	٢١.٤٢٨
	c-Duhok	٤٠	١٤.٢٨٣
٢-Type of infertility	a- primary	٢٤٥	٨٧.٥٠٠
	b-secondary	٣٥	١٢.٥٠٠
٣-Smoking	a- smokers	١٢١	٤٣.٢١٤
	b- none smokers	١٥٩	٥٦.٧٨٥
٤-Alcohol	a-drinking	١٠	٣.٥٧١
	b- none drinking	٢٧٠	٩٦.٤٢٨
٥- Blood group	A^+	٦٨	٢٤.٢٨٥
	B^+	٤٧	١٦.٧٨٥
	AB^+	١٨	٦.٤٢٨
	O^+	١٣٠	٤٦.٤٢٨
	A^-	٣	١.٠٧١
	B^-	٤	١.٤٢٨
	AB^-	٨	٢.٨٥٧
	O^-	٢	٠.٧١٤
٦-Occupation	a- hard work (stress)	١٩٠	٦٧.٨٥٧
	b- easy & moderate work	٩٠	٣٢.١٤٢
٧-Varicocele	a- with	٧٦	٢٧.١٤٢
	b- without	٢٠٤	٧٢.٨٥٧

Number of patients = ٢٨٠

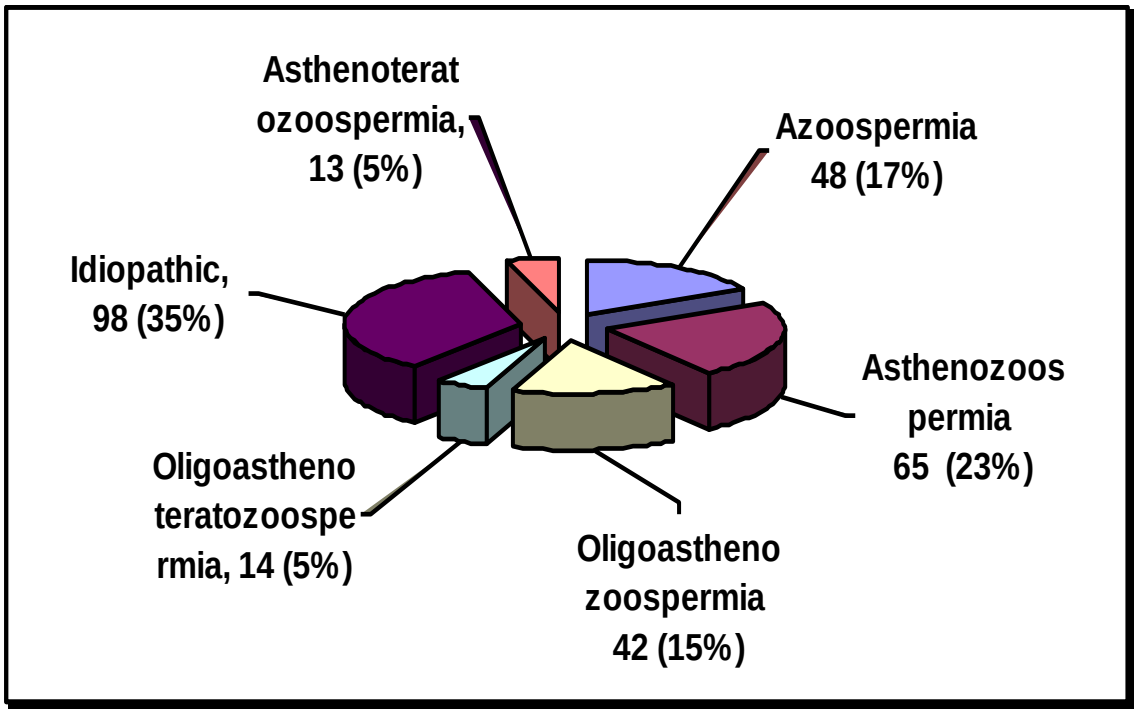


Figure (٦): The numbers and the percentages of different nomenclature of semen variables.

Table (٦): Comparison of liquefaction time of semen between fertile and infertile men.

	Liquefaction time of semen	
	Less than ٣٠ minutes (normal)	More than ٣٠ minutes (abnormal)
Fertile men n = ٣٠	٢٨ (٩٣.٣٣٣ %)	٢ (٦.٦٦٦ %)
Infertile men n = ٢٨٠	٢١٠ (٧٥ %)	٧٠ (٢٥ %)

Calculated x^2 = ١٢.٦١٠ p < ٠.٠١

Tabulated x^2 = ٦.٦٣

Table (٧): Comparison of semen appearance between fertile and infertile men.

	Semen appearance	
	Normal	**Abnormal**
Fertile men n = ٣٠	٢٧ (٩٠ %)	٣ (١٠ %)
Infertile men n = ٢٨٠	٢١٨ (٧٧.٨٥٧ %)	٦٢ (٢٢.١٤٢ %)

Calculated x^2 = ٥.٤٦٤ **p < ٠.٠٥**

Tabulated x^2 = ٣.٨٤

Table (٨): Comparison of semen viscosity between fertile and infertile men.

	Viscosity of semen	
	Normal	**Abnormal (low or high)**
Fertile men n = ٣٠	٢٨ (٩٣.٣٣٣ %)	٢ (٦.٦٦٦ %)
Infertile men n = ٢٨٠	٢٣٤ (٨٣.٥٧١ %)	٤٦ (١٦.٤٢٨ %)

Calculated x^2 = ٤.٦٦٢ **p < ٠.٠٥**

Tabulated x^2 = ٣.٨٤

Table (٩): The percentage of semen pH between fertile and infertile men.

	Semen pH			
	Normal (٧.٢ – ٧.٨)		Less than ٧.٢ or more than ٧.٨	
	number	%	number	%
Fertile men n = ٣٠	٣٠	١٠٠	٠	٠
Infertile men n = ٢٨٠	٢٧٥	٩٨.٢١٤	٥	١.٧٨٥

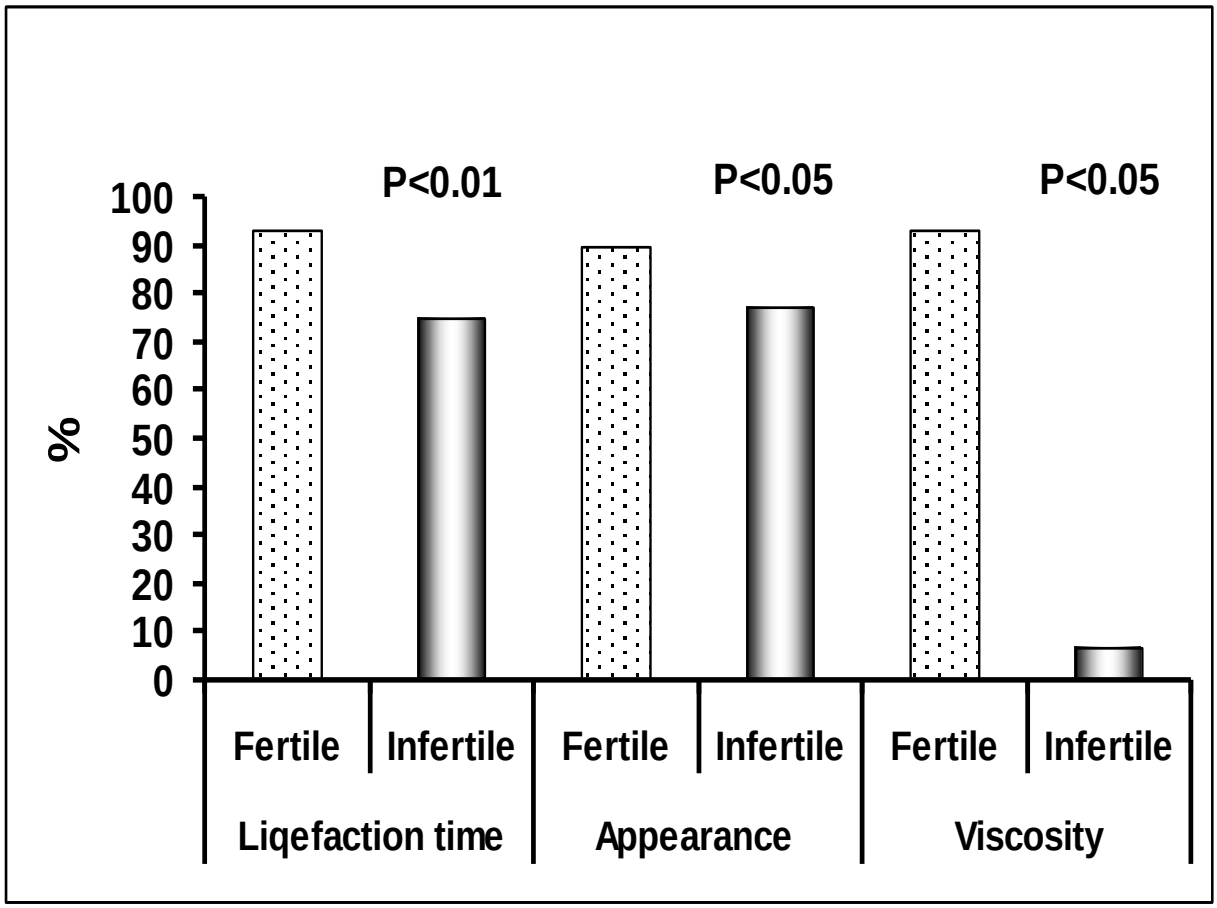


Figure (٧): The percentage of normal liquefaction time, appearance and viscosity of fertile and infertile men.

Table (١٠): Semen quality of fertile and infertile men (Means ± S.E).

Semen quality	Fertile men (control)	Infertile men	p- value

١- Volume (ml)	٤.١٣٣ ± ٠.١٤٧	٢.٨٥٠ ± ٠.٠٨٥	٠.٠٠٠
٢-Sperm conc. (× ١٠^{٦} / ml)	٩٧.٠٠ ± ١.٦٠١	٥٣.٦٦٥ ± ٢.١٣٥	٠.٠٠٠
٣- Total sperm count (× ١٠^{٦} / Ejaculate)	٤٠٠.٣٣ ± ١٥.١٧	١٥٠.٥٢ ± ٧.٧٤	٠.٠٠٠
٤- Grade activity	٣.٨٠٠ ± ٠.٠٧٤	٢.٠٤٣ ± ٠.٠٧٦	٠.٠٠٠
٥- Motility (%)	٥٨.٠٠ ± ٠ .٧٤٢	٣٣.٤٤٨ ± ١.٢١٥	٠.٠٠٠
٦-Sperm motility index	٢٢٢.٠٠ ± ٦.٦٨٥	٦١.٩٣٥ ± ٣.٨٩٧	٠.٠٠٠
٧- Viability (%)	٧٣.١٦٦ ± ٠.٦٩٨	٤٩.٧٨٠ ± ١.٣٢٢	٠.٠٠٠
٨- Normal morphology (%)	٧١.٥٠٠ ± ٠.٨٣٥	٥٨.٢٠٦ ± ١.١٩٤	٠.٠٠٠
٩- Leucocytes (× ١٠^{٦} / ml) ; **HPF :** **Endtz method :**	 ١.٨٠٠ ± ٠.٤٨٠ ٠.٤٣٣ ± ٠.١٤٩	 ٨.٠٦٩ ± ٠.٥٦٤ ٢.٢٧٠ ± ٠.٢٢٥	 ٠.٠٠٠ ٠.٠٠٠
١٠-Osmolality (m OSm/ kg)	٣٠٣.٧٠ ± ٢.٤٤٢	٣٣٩.٤٦ ± ٣.٩٦٨	٠.٠٠٠
١١- Malondialdehyde (µ mol / L)	١.٢٨٢ ± ٠.٠٥٣	٣.٢٠٥ ± ٠.١٦٩	٠.٠٠٠
	N=٣٠	N=٢٣٢	

٤.٢.٣.٢. Fertile, asthenozoospermic and asthenoteratozoospermic men:

The differences of semen parameters between fertile, asthenozoospermic and asthenoteratozoospermic men are demonstrated in table (١٢). Sperm conc., total sperm count, grade activity, motility, sperm motility index, viability, and normal sperm morphology are significantly higher ($p<٠.٠٠١$) in fertile men than the other ٢ groups, while semen volume of fertile men is higher than asthenozoospermic ($p<٠.٠٠١$) and asthenoteratozoospermic ($p<٠.٠٥$) men. Leucocytes with HPF method are lower in fertile men than asthenozoospermic ($p<٠.٠٠١$) and asthenoteratozoospermic ($p<٠.٠٥$) men. Leucocytes with Endtz method in asthenozoospermic is significantly ($p<٠.٠٥$) higher than fertile men. Osmolality of fertile men is lower than that of asthenozoospermic ($p<٠.٠١$) and asthenoteratozoospermic ($p<٠.٠٥$) men.

Multiple comparisons with LSD test, showed no significant differences of semen volume, sperm conc., total sperm count, sperm motility index, osmolality, and leucocytes between asthenozoospermic and asthenoteratozoospermic men. Sperm grade activity, motility, viability ($p<٠.٠١$) and normal sperm morphology ($p<٠.٠٠١$) are higher in asthenozoospermic than asthenoteratozoospermic men.

٤.٢.٣.٣. Fertile and idopathic infertile men:

The results of comparison of semen parameters between fertile men and idiopathic infertile men are observed in table (١٣). Semen volume, sperm conc., total sperm count, sperm grade activity, sperm motility percent, sperm motility index, and viability are significantly higher ($p<٠.٠٠١$) in fertile men than idiopathic infertile men. Leucocytes with both HPF and Endtz methods, also osmolality of seminal plasma fluid are lower ($p<٠.٠٠١$) in fertile men.

٤.٢.٣.٤. Fertile and azoospermic infertile men:

As shown in table ١٤, semen volume is significantly higher ($p<٠.٠٠١$), while leucocytes with both methods HPF ($p<٠.٠١$) and Endtz ($p<٠.٠٥$) and osmolality ($p<٠.٠٥$) are lower in fertile men than azoospermic infertile men.

Table (١٣): Semen quality of fertile and idiopathic men (Means ± S.E).

Semen quality	Fertile men (control)	idiopathic	p- value
١- Volume (ml) :	٤.١٣٣ ± ٠.١٤٧	٢.٨٦٣ ± ٠.١٢٩	٠.٠٠٠
٢-Sperm conc. (× ١٠٦ / ml)	٩٧.٠٠ ± ١.٦٠١	٨٠.٢٢٤ ± ١.٧٦٤	٠.٠٠٠
٣- Total sperm count (× ١٠٦ / Ejaculate)	٤٠٠.٣٣ ± ١٥.١٧	٢٢٨.٠٩ ± ١٠.٦٦	٠.٠٠٠
٤- Grade activity	٣.٨٠٠ ± ٠.٠٧٤	٣.٢٣٤ ± ٠.٠٤٣	٠.٠٠٠
٥- Motility (%)	٥٨.٠٠ ± ٠ .٧٤٢	٥٢.٣٤٦ ± ٠.٤٣٠	٠.٠٠٠
٦-Sperm motility index	٢٢٢.٠٠ ± ٦.٦٨٥	١٠٥.٤٠٨ ± ٦.٤٩٨	٠.٠٠٠
٧- Viability (%)	٧٣.١٦٦ ± ٠.٦٩٨	٦٨.٨١٦ ± ٠.٦٥٥	٠.٠٠٠
٨- Normal morphology (%)	٧١.٥٠٠ ± ٠.٨٣٥	٧٠.٨١٦ ± ٠.٥٥٠	٠.٥٣٥
٩- Leucocytes (× ١٠٦ / ml) ; HPF : Endtz method :	١.٨٠٠ ± ٠.٤٨٠ ٠.٤٣٣ ± ٠.١٤٩	٦.٨٨٢ ± ٠.٧٥٦ ١.٩٧٠ ± ٠.٣٦٠	٠.٠٠٠ ٠.٠٠٠
١٠-Osmolality (m OSm/ kg)	٣٠٣.٧٠ ± ٢.٤٤٢	٣٢٦.٨٢٦ ± ٥.٤٧٥	٠.٠٠٠
	N= ٣٠	N= ٩٨	

Table (١٤): Semen quality of fertile and Azoospermic men (Means ± S.E).

Semen quality	Fertile men (control)	Azoospemic men	p- value
١- Volume (ml)	٤.١٣٣ ± ٠.١٤٧	٢.٤٣٥ ± ٠.٢٢٢	٠.٠٠٠
٢-Sperm conc. (× ١٠^{٦} / ml)	_____	_____	_____
٣- Total sperm count (× ١٠^{٦} / Ejaculate)	_____	_____	_____
٤- Grade activity	_____	_____	_____
٥- Motility (%)	_____	_____	_____
٦-Sperm motility index	_____	_____	_____
٧- Viability (%)	_____	_____	_____
٨- Normal morphology (%)	_____	_____	_____
٩- Leucocytes (× ١٠^{٦} / ml) ; HPF : Endtz method :	١.٨٠٠ ± ٠.٤٨٠ ٠.٤٣٣ ± ٠.١٤٩	٦.٧٠٨ ± ١.٦٧١ ١.٨٣٣ ± ٠.٥٩٧	٠.٠٠٧ ٠.٠٢٥
١٠-Osmolality (m OSm/ kg)	٣٠٣.٧٠ ± ٢.٤٤٢	٣٢٥.٥٦٢ ± ٨.٠٤١	٠.٠١٢
	N= ٣٠	N= ٤٨	

٤.٣. Viscosity and semen quality:

As shown in table (١٥), most semen parameters not affected with viscosity of semen. The parameter which affected only related with sperm motility percent. There are significant differences ($p<٠.٠٥$) in sperm grade activity, sperm motility percent and sperm motility index between normal and abnormal semen viscosity of infertile men. These parameters are lower in abnormal semen viscosity than the normal one.

٤.٤. Osmolality and semen quality:

The osmolality of seminal plasma is divided into ٣ groups' ≤ ٣٠٠, ٣٠١-٣٤٠, and > ٣٤٠ m OSm / kg. LSD test was used for multiple comparisons of semen parameters between all groups. The results showed a significant higher ($p<٠.٠٥$) in semen volume of osmolality ≤ ٣٠٠ m OSm / kg than the other two groups (table ١٦). Grade activity and sperm motility index ($p<٠.٠١$) and sperm motility percent ($p<٠.٠٥$) was significantly decrease in seminal plasma osmolality of > ٣٤٠.

No significant differences appears in semen volume between osmolality ٣٠١-٣٤٠ and > ٣٤٠, but grade activity is significantly higher ($p<٠.٠١$) in osmolality of ٣٠١-٣٤٠ compare with osmolality > ٣٤٠. Other results include a significant ($p<٠.٠٥$) higher percentage of motility in osmolality ٣٠١-٣٤٠ than > ٣٤٠. No significance differences appear in, sperm density, viability and morphology of spermatozoa between all groups.

Table (١٥):Viscosity and semen quality of infertile men(Means ± S.E).

Semen quality	Viscosity of semen		p- value
	Normal	Abnormal	
١- Volume (ml)	٢.٧٦٢ ± ٠.١٥١	٢.٨٧٦ ± ٠.١٠٣	٠.٥٦٥
٢-Sperm conc. (× ١٠^{٦} / ml)	٥٣.٩٢٦ ± ٢.٢٦٩	٥٢.٥٨٨ ± ٦.٢٥١	٠.٨٢٥
٣- Total sperm count (× ١٠^{٦} / ejaculate)	١٥٣.٤٦ ± ٨.١٥٦	١٤٣.٥٣ ± ٢٠.٨٢١	٠.٦٤٤
٤- Grade activity	٢.١١١ ± ٠.٠٨٢	١.٦١٧ ± ٠.١٨٨	٠.٠٢٢
٥- Motility (%)	٣٤.٥٧٥ ± ١.٣٠١	٢٦.٨٨٢ ± ٣.١٧٥	٠.٠٢٥
٦-Sperm motility index	٩٤.٢٣٢ ± ٥.٥٤١	٦٣.٢٦٤ ± ١٠.٩٢٣	٠.٠١٥
٧- Viability (%)	٥٠.٠٠٠ ± ١.٤٣٥	٤٧.٠٥٨ ± ٣.٤٧٥	٠.٤٣٤
٨- Normal morphology (%)	٥٦.٩٣٩ ± ١.٣٠٠	٦٠.٠٠٠ ± ٣.١٢٤	٠.٣٦٨
٩-Osmolality (m OSm/ Kg)	٣٤٠.٣٩٣ ± ٤.٤١٨	٣٣٤.٠٨٨ ± ٨.٥٣١	٠.٥٧٥
	N = ١٧٤	N = ٥٨	

Table (١٦): Osmolality and semen quality of infertile men (Means ± S.E).

Semen quality	Osmolality (m OSm / kg)			L.S.D
	≤ ٣٠٠	٣٠١ - ٣٤٠	> ٣٤٠	
١- Volume (ml)	٣.٢١٥ ± ٠.١٨٨	٢.٧٣٢ ± ٠.١٣٣ *	٢.٧٠١ ± ٠.١٢٧ *	٠.٤٥٢
٢-Sperm conc. (× ١٠^{٦} / ml)	٦٠.٣٥٣ ± ٣.٩٩٤	٦٠.٠١٤ ± ٣.٨٤١	٥٨.٢٢٠ ± ٣.١٧٩	١٧.٠١٣
٣- Total sperm count (× ١٠^{٦} / ejaculate)	١٩٤.٤٧± ١٥.٧٢٧	١٦٦.٣٧ ± ١٤.٥٤٠	١٥٦.٦٠ ± ٩.٥١٧	٥٩.٥٩٦
٤- Grade activity	٢.٢٦٩ ± ٠.١٥٨	٢.٢٥٠ ± ٠.١٣٣	١.٧٢٢ ± ٠.١٠٦ **	٠.٤٩٨
٥- Motility (%)	٣٦.٧٩٣ ± ٢.٤٥٧	٣٦.٥٠٠ ± ٢.٢٢٧	٢٩.٣١٦ ± ١.٧٦٦ *	٧.٠١٢
٦-Sperm motility index	١٠٧.٥٥ ±١٠.٥٢٩	١٠٢.٤٥ ± ٩.٤٥٢	٧٠.٩٦٠ ± ٦.٧٢١ **	٣٠.٢٢٥
٧- Viability (%)	٥١.٨٨٩ ± ٢.٦٤٤	٥٣.٤٥٥ ± ٢.٣٨٤	٤٦.٥٣٤ ± ١.٩٣٨	١٠.٦٧٥
٨- Normal morphology (%)	٦٠.٧٦١ ± ٢.١٣٩	٦١.٤٤١ ± ٢.٠٨٦	٦٠.٢٢١ ± ١.٨٨٧	٩.٥٨٦
	N=٦٣	N=٦٨	N=١٠١	

*** P < ٠.٠٥ ** P < ٠.٠١ *** P < ٠.٠٠١ LSD test was used for multiple comparisons**

٤.٥. Some causes of male infertility:

٤.٥.١. ROS and infertility:

٤.٥.١.١. ROS and different infertility groups:

Malondialdehyde, the end product of lipid peroxidation was measured in seminal plasma of fertile men and different groups of infertile men. As shown in table (١٧), significant differences appears between them .The seminal plasma MDA is significantly decrease in fertile men than oligoasthenozoospermic ($p<٠.٠١$),oligoasthenoteratozoospermic($p<٠.٠٠١$), asthenozoospermic ($p<٠.٠٠١$), asthenoteratozoospermic ($p<٠.٠٠١$). Also azoospermic ($p<٠.٠٥$) and idiopathic ($p<٠.٠٥$) infertile men showed a significant increase in MDA level than fertile men.

٤.٥.١.٢. Relationship of ROS with varicocele, leucocytes, and smoking:

The relationships between seminal plasma MDA with varicocele, leucocytes and smoking are demonstrated in figure (٨). The MDA level is ٢.٨٥٩ μ mol / L in none-varicocele and ٤.٧٠ μ mol / L in varicocele infertile men, the data is significant at ($p<٠.٠٥$).

Semen of infertile men which has leucocytes > ١×١٠^{٦} / ml showed a significant increase ($p<٠.٠٥$) of MDA level (٣.٨٧٨ μ mol / L) compare with normal leucocytes level (٢.٩٣٢ μ mol / L) . The seminal plasma MDA level in smokers is high ($p<٠.٠٥$) than none- smokers infertile men (٣.٦٩٢ and ٢.٨٥٦ μ mol / L) respectively.

٤.٥.٢. Leucocytospermia:

٤.٥.٢.١. Comparison between HPF and Endtz methods for determination of semen leucocytes:

Two methods are used for determination of semen leucocytes, high power field (HPF) and Endtz methods. The results which are recorded in table (١٨) showed a significant difference between the ٢ methods in fertile men and all infertile groups. Semen leucocytes with Endtz method are significantly lower (p<٠.٠٠١) in fertile men, asthenozoospermia, oligoasthenozoospermia,

Table (١٧): Seminal plasma MDA levels between fertile men and different infertile groups (Means ± S.E) .

Fertile and infertile groups	Seminal plasma MDA (µ mol / L)	p-value
Fertile men	١.٢٨٢ ± ٠.٠٥٣	
Oligoasthenozoospermia	٣.٢٧٨ ± ٠.٦٠١	٠.٠٠٩
Oligoasthenoteratozoospermia	٣.٥٣٤ ± ٠.٤٨٧	٠.٠٠٠
Asthenozoospermia	٣.١٢٤ ± ٠.٣٠١	٠.٠٠٠
Asthenoteratozoospermia	٤.٤٧١ ± ١.٠٢٩	٠.٠٠٠
Azoospermia	٢.٦٣٨ ± ٠.٢٥٩	٠.٠٢٥
Idiopathic	٢.٩٤٠ ± ٠.٢٢٥	٠.٠٤٣

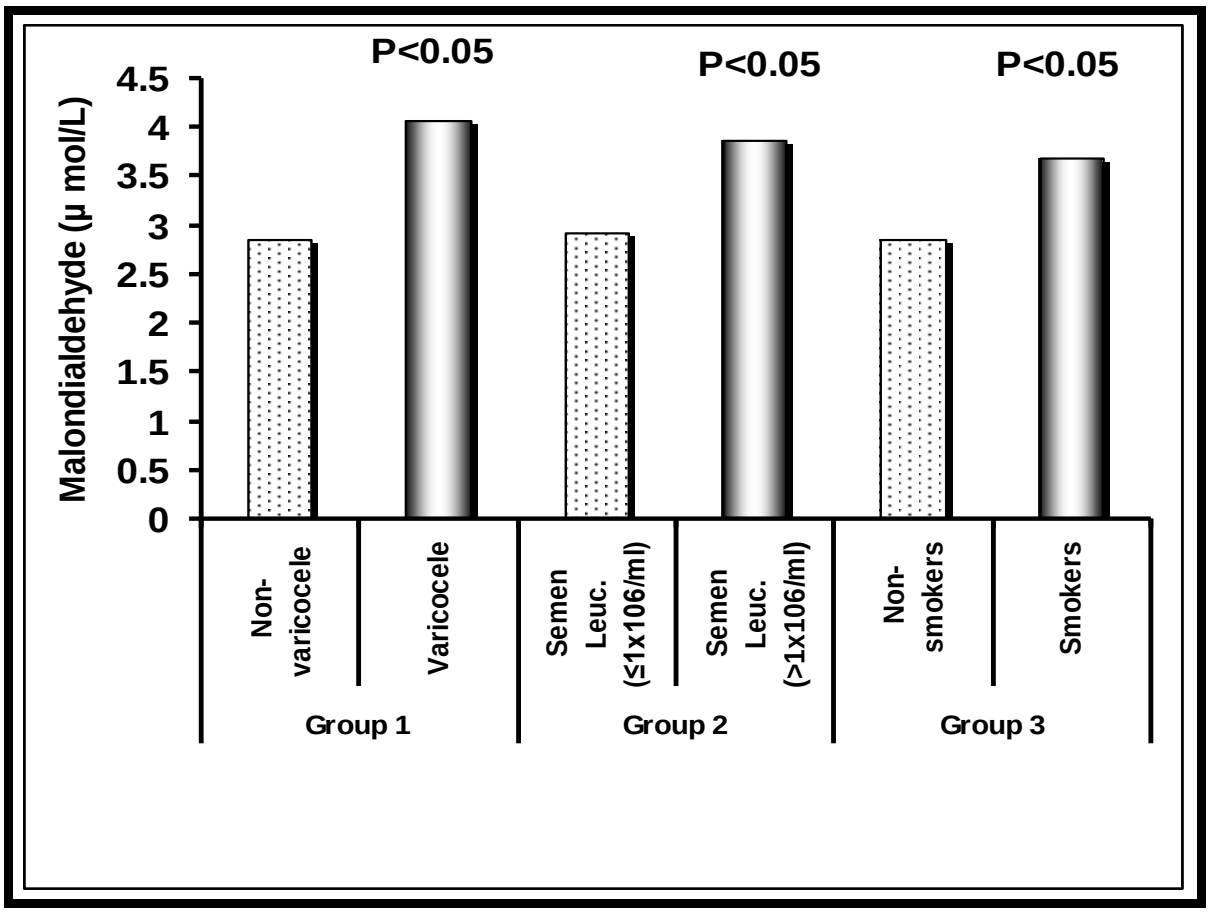


Figure (٨): Comparison of seminal plasma malondialdehyde between different groups of infertile men.

oligoasthenoteratozoospermia, azoospermia and idiopathic . Also in asthenoteratozoospermic men the leucocytes with Endtz method is lower ($p<٠.٠٥$) than HPF method.

٤.٥.٢.٢. Leucocytes and semen quality:

The effect of leucocytes on semen quality is observed in table (١٩). Most semen parameters are not affected with leucocytes. Only the motility of spermatozoa is affected with leucocytes. There are significant decrease ($p<٠.٠١$) of sperm grade activity and sperm motility percent, and sperm motility index ($p<٠.٠٠١$) in semen with leucocytospermia (leucocytes > ١×١٠^{٦}/ ml) compare with normal semen leucocytes.

٤.٥.٣. Varicocele and semen quality:

As shown in table (٢٠), no significant differences appear of semen volume between varicocele and none-varicocele infertile men. Sperm conc., total sperm count, grade activity, motility, and viability are decrease ($p<٠.٠١$) in varicocele men compare with none-varicocele men. Also sperm motility index ($p<٠.٠٠١$) and normal sperm morphology ($p< ٠.٠٥$) showed a significant decrease in varicocele infertile men.

٤.٥.٤. Smoking and its effects on semen quality:

Low significant effects of smoking on semen quality are recorded, table (٢١). Semen volume, sperm conc., total sperm count, grade activity, motility, viability, and normal sperm morphology are significantly lower ($p<٠.٠٥$) in smoker's infertile men than none-smokers men. Also sperm motility index is higher ($p<٠.٠١$) in none-smoking men.

Table (١٨): Comparison between high power field (HPF) and Endtz methods for determination of leucocytes (Means ± S.E).

Fertile and infertile groups	Leucocytes (× ١٠^٦ / ml)		p- value
	HPF	Endtz method	
١-Fertile men	١.٨٠٠ ± ٠.٤٨٠	٠.٤٣٣ ± ٠.١٤٩	٠.٠٠٠
٢-Asthenozoospermia	١٠.٤٩٢ ± ١.٣٤٨	٢.٩٦٩ ± ٠.٥٠٩	٠.٠٠٠
٣-Oligoasthenozoospermia	٧.٥٩٥ ± ١.٢٦٢	٢.١٠٧ ± ٠.٥٠٤	٠.٠٠٠
٤-Asthenoteratozoospermia	٩.١٥٣ ± ٢.٣٩٠	٢.٥٣٨ ± ٠.٩٨٤	٠.٠٠١
٥-Oligoasthenoteratozoospermia	١٠.٢١٤ ± ٢.٦١٢	٢.٨٧١ ± ١.٠٨٥	٠.٠٠٠
٦-Azoospermia	٦.٧٠٨ ± ١.٦٧١	١.٨٣٣ ± ٠.٥٩٧	٠.٠٠٠
٧-Idiopathic	٦.٨٨٢ ± ٠.٧٥٦	١.٩٧٠ ± ٠.٣٦٠	٠.٠٠٠

Table (١٩): Effects of leucocytospermia on semen quality of infertile men (Means ± S.E).

Semen quality	Leucocytes (× ١٠^٦ / ml)		p- value
	≤ ١	> ١	
١- Volume (ml)	٢.٨٥١ ± ٠.١٣٩	٢.٨٠٩ ± ٠.١٠٧	٠.٨١١
٢-Sperm conc. (× ١٠^٦ / ml)	٥٤.٦٤٢ ± ٢.٧٩١	٥١.٨٠٠ ± ٣.٢٦٣	٠.٥٢٢
٣- Total sperm count (× ١٠^٦ / Ejaculate)	١٥١.٥٣١ ± ٩.٣٥٠	١٥٢.٩٥ ± ١٢.٩٦٨	٠.٩٢٨
٤- Grade activity	٢.٢١٠ ± ٠.٠٩٨	١.٧٤١ ± ٠.١١٣	٠.٠٠٣
٥- Motility (%)	٣٦.٠٠٦ ± ١.٥٤٤	٢٨.٨٤٧ ± ١.٨٦٦	٠.٠٠٤
٦-Sperm motility index	١٠١.٩١٨ ± ٦.٥٤٥	٦٦.٤٠٠ ± ٧.٢٨٤	٠.٠٠٠
٧- Viability (%)	٥٠.٨٤٣ ± ١.٧١٥	٤٨.٠٥٨ ± ٢.٠٦٣	٠.٣١٢
٨- Normal morphology (%)	٥٨.٥٤٤ ± ١.٥٢٠	٥٦.٤٤٧ ± ١.٩٩٤	٠.٤٠٤
	N= ١٤٧	N= ٨٥	

Table (٢٠): Semen quality of varicocele and none varicocele infertile men.

(Means ± S.E).

Semen quality	None varicocele men	Varicocele men	p- value
١- Volume (ml)	٢.٨٦٣ ± ٠.١٠٥	٢.٨٠٠ ± ٠.١٤٨	٠.٧٨٩
٢-Sperm conc. (× ١٠^{٦} / ml)	٥٧.٨٧٨ ± ٢.٥٠٩	٤٢.٦٠٩ ± ٣.٧٥٦	٠.٠٠١
٣- Total sperm count (× ١٠^{٦} / Ejaculate)	١٦٣.٥٣٠ ± ٨.٩١٦	١١٨.٤٠٦ ± ١١.٨٩١	٠.٠٠٦
٤- Grade activity	٢.٠٦٥ ± ٠.٠٨٨	١.٥٩٣ ± ٠.١٢٧	٠.٠٠٤
٥- Motility (%)	٣٥.٩٤٦ ± ١.٤٢٨	٢٦.٩٠٦ ± ٢.١١٦	٠.٠٠١
٦-Sperm motility index	١٠٠.٦٩٦ ± ٦.٠٥٣	٦٠.٨٤٣ ± ٧.٩٩٧	٠.٠٠٠
٧- Viability (%)	٥٢.٠٠٦ ± ١.٥٩٥	٤٣.٥٩٣ ± ٢.٢٨٣	٠.٠٠٥
٨- Normal morphology (%)	٥٩.٦٤٢ ± ١.٣٧٢	٥٣.٣٥٩ ± ٢.٣١٢	٠.٠١٨
	N= ١٦٨	N= ٦٤	

Table (٢١): Semen quality of smoking and none-smoking infertile men

(Means ± S.E).

Semen quality	None smoking infertile men	Smoking infertile men	p- value
١- Volume (ml)	٢.٩٨٤ ± ٠.١١٢	٢.٦١٦ ± ٠.١٣٠	٠.٠٣٤
٢-Sperm conc. (× ١٠^{٦} / ml)	٥٧.٦٧٧ ± ٢.٤٣٧	٤٨.٠٨٢ ± ٣.٧٦١	٠.٠٢٤
٣- Total sperm count (× ١٠^{٦} / Ejaculate)	١٦٤.٥٥ ± ٩.١٥	١٣١.١٦ ± ١٢.٧٢	٠.٠٣٤
٤- Grade activity	٢.٢٠٧ ± ٠.٠٩١	١.٧٩٣ ± ٠.١٣١	٠.٠١٠
٥- Motility (%)	٣٦.٥١١ ± ١.٤٠٢	٢٩.٥١٥ ± ٢.١٣٥	٠.٠٢٢
٦-Sperm motility index	١٠٠.٤٢٢ ± ٦.٦١٧	٧٧.١٥٤ ± ٧.٦٠٦	٠.٠٠٧
٧- Viability (%)	٥٢.٠٦٦ ± ١.٦٥٠	٤٥.٧٧٣ ± ٢.٢٠٧	٠.٠٢١
٨- Normal morphology (%)	٦٠.٤٣٧ ± ١.٤٤٤	٥٥.٣٠٩ ± ٢.٠٠٢	٠.٠٣٩
	N= ١٣٥	N= ٩٧	

٤.٥.٥. Immunological factors and infertility:

٤.٥.٥.١. Agglutination:

The agglutination of spermatozoa is observed in table (٢٢), and figure (٩). Significant differences ($p<٠.٠٥$) of agglutination appears between fertile and infertile men. Two semen samples which represent ٦.٦٦٦ % of fertile men, and ٣٢ samples which represent ١٣.٧٩٣ % of infertile men showed an agglutination of spermatozoa.

٤.٥.٥.٢. Antisperm antibodies:

٤.٥.٥.٢.١. Antisperm antibodies detection in fertile and infertile men:

No ASA_S are observed in fertile men, but ١٤ samples from ٢٣٢ of infertile men which represent about ٦.٠٣٤ % showed the positive detection of ASA, (table ٢٣).

٤.٥.٥.٢.٢. Incidence of ASA_S in different group of infertile men:

The detection and incidence of ASA_S among infertile groups are differs, (table ٢٤). Asthenozoospermia is a larger group which ASA_S has been observed. ٩ samples which represent ١٣.٨٤ % showed positive detection of ASA_S. Other groups which ASA_S has been detected are asthenoteratozoospermia (٧.٦٩ %) and idiopathic (٤.٠٨ %).

٤.٥.٥.٢.٣. Distribution of ASA_S between different seminal plasma dilutions:

The seminal plasma dilutions which are used in ASA_S detection are ١:٨, ١:١٦, ١:٣٢, ١:٦٤, and ١:١٢٨. As shown in table (٢٥) , ٥ samples from ٩ of asthenozoospermia reach to the dilution ١:١٢٨ which is called agglutination titer, while ٢ samples from ٤ of idiopathic and ١ sample from ١ of asthenoteratozoospermia reach to agglutination titer .

Table (٢٢): The percentage of spermatozoal agglutination of fertile and infertile men.

	Agglutination of spermatozoa	
	Negative	**Positive**
Fertile men n=٣٠	٢٨ (٩٣.٣٣٣ %)	٢ (٦.٦٦٦ %)
Infertile men n=٢٣٢	٢٠٠ (٨٦.٢٠٦ %)	٣٢ (١٣.٧٩٣ %)

Calculated x^2 = ٣.٩٦٤ **p < ٠.٠٥**

Tabulated x^2 = ٣.٨٤

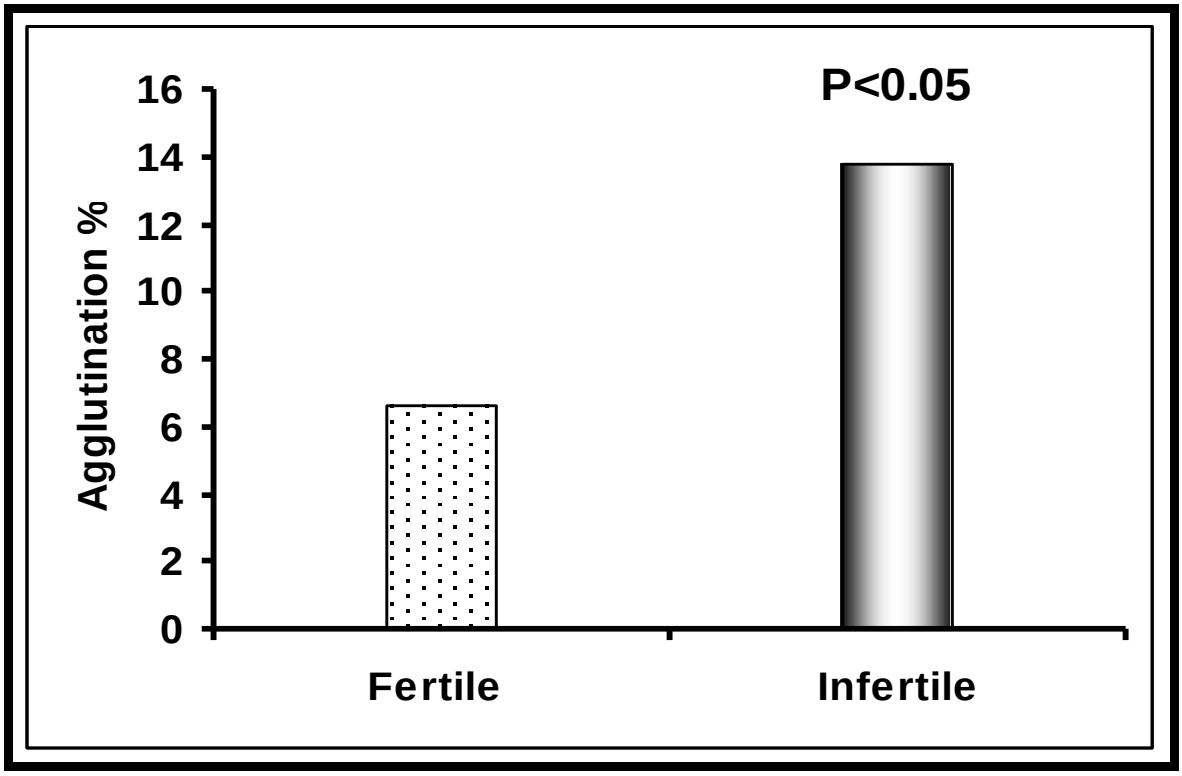


Figure (٩): The percentage of spermatozoal agglutination in fertile and infertile men.

Table (٢٣): The percentage of antisperm antibodies detection in semen of fertile and infertile men .

	Antisperm antibodies	
	Negative	**Positive**
Fertile men n=٣٠	٣٠ (١٠٠ %)	٠ (٠ %)
Infertile men n=٢٣٢	٢١٨ (٩٣.٩٦٥ %)	١٤ (٦.٠٣٤ %)

Table (٢٤): Incidence of ASA_S among different group of infertile men.

Infertile groups	**Antisperm antibodies**	
	Negative	**Positive**
Asthenozoospermia	٥٦ (٨٦.١٥ %)	٩ (١٣.٨٤ %)
Asthenoteratozoospermia	١٢ (٩٢.٣٠ %)	١ (٧.٠٨ %)
Idiopathic	٩٤ (٩٥.٩١ %)	٤ (٤.٠٨ %)
Oligoasthenozoospermia	٤٢ (١٠٠ %)	٠ (٠ %)
Oligoasthenoteratozoospermia	١٤ (١٠٠ %)	٠ (٠ %)

Table (٢٥): Distribution of ASA_S among different seminal plasma dilutions.

Infertile groups	**Seminal plasma dilutions**				
	١:٨	**١:١٦**	**١:٣٢**	**١:٦٤**	**١:١٢٨**
Asthenozoospermia n=٩	٩	٩	٨	٦	٥

Idiopathic n=٤	٤	٤	٢	٢	٢
Asthenoteratozoospermia n =١	١	١	١	١	١

٤.٥.٦. Hormonal evaluation of infertility:

The hormones which are measured and related to the infertility are LH, FSH, Testosterone, Prolactin, and Estradiol II ($E_٢$) .These hormones are measured in some severe oligozoospermic and azoospermic infertile men and some fertile men to compare with infertile men. The results are illustrated in table (٢٦). No significant differences appear of LH, Testosterone, Prolactin, and Estradiol II ($E^{٢}$) between fertile men, oligozoospermic and azoospermic infertile men. Except FSH is significantly higher ($p<٠.٠٥$) in azoospermia compare with fertile men and severe oligozoospermia. Table (٢٧) shows the percentage of abnormal hormone value of oligozoospermia and azoospermia .The highest percentage of abnormal value is recorded in FSH with azoospermia (٤٠٪) and oligozoospermia (٣٣.٣٣ %), while some hormones such as Prolactin, and Estradiol II ($E_٢$), the abnormal value of them are very low ٠ % and ٦.٦٦ % of prolactin in oligozoospermia and azoospermia respectively.

٤.٦. *In vitro* human sperm activation:

٤.٦.١. Human sperm activation using simple layer technique:

Table (٢٨), shows the semen parameters of ٣٠ asthenozoospermic men before and after activation with modified balanced Earle's sperm activation medium by using simple layer technique. Sperm conc., agglutination of spermatozoa and leucocytes were significant decrease ($p<٠.٠٠١$) after semen activation compare with them before activation. Grade activity, sperm motility percent, and sperm motility index increase significantly ($p<٠.٠٥$) after activation. Also normal sperm morphology showed a high improvement ($p<٠.٠٠١$) after activation.

٤.٦.٢. Human sperm activation using centrifugation – swim up single layer technique:

Other technique which used for human sperm activation is centrifugation swim up single layer method which illustrated in table (٢٩). Sperm conc., agglutination of spermatozoa, and leucocytes showed a significant decrease ($p<٠.٠٠١$) after sperm activation compare with them before activation. Grade activity, sperm motility percent, and sperm motility index increase significantly ($p<٠.٠٥$) after activation. There are a high percentage ($p<٠.٠٠١$) of normal sperm morphology after sperm activation.

٤.٦.٣. Comparison between simple layer and centrifugation swim up techniques:

When simple layer technique compare with centrifugation-swim up technique for their effects on semen quality, significant differences appears between them, (table ٣٠). Sperm conc., and leucocytes are lower ($p<٠.٠٠١$) in centrifugation-swim up than that of simple layer, also agglutination of spermatozoa is lower ($p<٠.٠١$) in centrifugation-swim up method. Grade activity, motility, sperm motility index, and normal sperm morphology are higher ($p<٠.٠٠١$) in centrifugation-swim up compare with simple layer technique.

Table (٢٦): Hormonal evaluation of fertile men, severe oligozoospermic and azoospermic infertile men (Means ± S.E).

Hormones	Fertile men (control)	Severe oligozoospermia	Azoospermia	L.S.D.
١-LH (µIU / ml)	٥.٦٨٨ ± ٠.٥٣٩	٦.٧٢٨ ± ١.٢٠١	٩.٠٣٢ ± ١.٨٣١	٣.٢٣٤
٢-FSH(µIU/ ml)	٦.٧٠٨ ± ١.٢١٩	١٢.٤٢٥ ± ١.٩٦٩	٢٢.٧٤٤ ± ٣.٩٠٨*	١٣.٢١
٣-Testosterone (ng / ml)	٦.٥٥٠ ± ٠.٦٢٤	٧.٠٤١ ± ١.٣٥٢	٤.٢١٢ ± ٠.٦٩٦	٣.٤٢٢
٤-Prolactin (ng / ml)	١١.٩٨٢ ± ١.٠٣٧	١١.٣٩٦ ± ١.٣٧٠	١٥.٢٧٨ ± ٤.٥٢٦	٥.٠٢١
٥-Estradiol II (pg / ml)	٣٣.٢٢٠ ± ٩.٦٤٨	٥٠.٦٦٤ ± ١٠.٣٢٥	٥٢.٩٤٠ ±١٠.٢٣٣	٢٥.٦٢
	N= ١٠	N=١٥	N=٢٠	

* P < ٠.٠٥ LSD was used for multiple comparisons

Table (٢٧): The number and percentage of abnormal hormone value in severe oligozoosperima and azoospermia.

Hormones	Severe oligozoospermia	Azoospermia
LH	١ (٦.٦٦ %)	٢ (١٠ %)
FSH	٥ (٣٣.٣٣ %)	٨ (٤٠ %)
Testosterone	١ (٦.٦٦ %)	٣ (١٥ %)
Prolactin	٠ (٠ %)	١ (٦.٦٦ %)
Estradiol II ($E_٢$)	١ (٦.٦٦ %)	٠ (٠ %)
	N=٨	N=١٤

Table (٢٨): Improvement of seminal parameters using Earle's sperm activation medium and simple layer technique (Means ± S.E).

Seminal parameters	Before activation	After activation	P - value
١.Sperm conc.(× ١٠٦/ ml)	٥٨.٨٦٦ ± ٣.٨٦٣	٣٤.٠٦٦ ± ٢.٨٢٤	٠.٠٠٠
٢. Grade activity	١.٤٣٣ ± ٠.٠٩٢	٢.٦٣٣ ± ٠.٠٨٩	٠.٠٢٣
٣. Motility (%)	٢٣.٨٦٦ ± ١.٧١٥	٤٤.٧٦٦ ± ١.٣٧٨	٠.٠٢٠
٤. Sperm motility index	٣٨.٧٦٦ ± ٤.٨٧٧	١٢١.٢٠ ± ٧.١٠٤	٠.٠١٥
٥. Normal sperm morphology (%)	٥٦.٠٠٠ ± ٢.٩٨٨	٦٧.٨٠٠ ± ٢.٠٦٦	٠.٠٠٠
٦. Agglutination (%)	١٠.٣٣٣ ± ٣.٣٣٨	٣.١٣٣ ± ١.١١٠	٠.٠٠٠
٧. leukocytes (× ١٠٦ / ml)	٩.٣٦٦ ± ١.٦٨٢	٢.٩٣٣ ± ٠.٦٥٩	٠.٠٠٠
N=٣٠			

Table (٢٩): Improvement of seminal parameters using Earle's sperm activation medium and centrifugation – swim up single layer technique (Means ± S.E).

Seminal parameters	Before activation	After activation	P - value
١.Sperm conc.(× ١٠٦/ ml)	٥٨.٨٦٦ ± ٣.٨٦٣	١٩.٢٣٣ ± ٢.١٥٧	٠.٠٠٠
٢. Grade activity	١.٤٣٣ ± ٠.٠٩٢	٣.٢٣٣ ± ٠.١١٤	٠.٠١٧
٣. Motility (%)	٢٣.٨٦٦ ± ١.٧١٥	٥٢.١٠٠ ± ١.٢٤٤	٠.٠١٩
٤. Sperm motility index	٣٨.٧٦٦ ± ٤.٨٧٧	١٦٩.٤٦٦ ± ١٠.٢٢٩	٠.٠١١
٥. Normal sperm morphology (%)	٥٦.٠٠٠ ± ٢.٩٨٨	٧٨.٨٣٣ ± ١.٥٣٢	٠.٠٠٠
٦. Agglutination (%)	١٠.٣٣٣ ± ٣.٣٣٨	٠.٧٦٦ ± ٠.٣٧٦	٠.٠٠٠
٧. leukocytes (× ١٠٦ / ml)	٩.٣٦٦ ± ١.٦٨٢	٠.٥٦٦ ± ٠.٢٢٣	٠.٠٠٠
N=٣٠			

Table (٣٠): Comparison between Simple layer and centrifugation–swim up single layer techniques for human sperm activation (Means ± S.E).

Seminal parameters	Simple layer technique	Centrifugation swim up technique	P - value
١.Sperm conc.(× ١٠٦/ ml)	٣٤.٠٦٦ ± ٢.٨٢٤	١٩.٢٣٣ ± ٢.١٥٧	٠.٠٠٠
٢. Grade activity	٢.٦٣٣ ± ٠.٠٨٩	٣.٢٣٣ ± ٠.١١٤	٠.٠٠٠
٣. Motility (%)	٤٤.٧٦٦ ± ١.٣٧٨	٥٢.١٠٠ ± ١.٢٤٤	٠.٠٠٠
٤. Sperm motility index	١٢١.٢٠ ± ٧.١٠٤	١٦٩.٤٦٦ ± ١٠.٢٢٩	٠.٠٠٠
٦. Normal sperm morphology (%)	٦٧.٨٠٠ ± ٢.٠٦٦	٧٨.٨٣٣ ± ١.٥٣٢	٠.٠٠٠
٧. Agglutination (%)	٣.١٣٣ ± ١.١١٠	٠.٧٦٦ ± ٠.٣٧٦	٠.٠٠٦
٨. leukocytes (× ١٠٦ / ml)	٢.٩٣٣ ± ٠.٦٥٩	٠.٥٦٦ ± ٠.٢٢٣	٠.٠٠٠
N=٣٠			

٥. Discussion:

٥.١. Semen quality of fertile and infertile men:

٥.١.١ Macroscopic evaluation:

٥.١.١.١. Liquefaction time:

The results of the present study showed that liquefaction time of infertile men was longer than fertile one. The seminal vesicles secrete fibrinogen and the prostate produces a clotting enzyme, the clotting enzyme converts fibrinogen to fibrin, causing the semen to clot like blood. About ١٥-٣٠ minutes later, fibrinolysin in the prostatic fluid dissolves the clot (Saladin, ١٩٩٨). In cases, which liquefaction does not occur within the normal period, should be regarded, as it may suggests functional disturbance of the prostate (Comhaire and Vermeulen, ١٩٩٥).

٥.١.١.٢. Appearance:

The normal semen appearance which recorded among fertile men is higher than infertile men. The abnormal semen which observed is heterogenous, red-brown, yellow, and white-clear (colorless). In agreement with the present results Katz *et al*., (١٩٨٦); Jequier and Crich, (١٩٨٦), are also noted that yellow, pink or red colorations of the semen is an indicator for semen abnormality. A normal sample has a homogenous, gray-opalescent appearance. It may appear less opaque if the sperm conc. is low, red-brown when red blood cells are present or yellow in patients with jaundice or taking some vitamins (WHO, ١٩٩٩).

٥.١.١.٣. Viscosity:

When semen viscosity was measured in the present study, significant differences appear between fertile and infertile men. ٦.٦٦ % of the fertile and ١٦.٤٢ % of infertile men showed abnormal semen viscosity. Increased viscosity of the ejaculate was reported to occur more frequently among men from infertile couples than fertile males (Bunge, ١٩٧٠). Increased viscosity has the same clinical meaning as abnormal liquefaction, and may be related to prostate dysfunction resulting from chronic inflammation (Comhaire and Vermeulen, ١٩٩٥).

٥.١.١.٤. Semen pH:

No abnormal pH recorded among fertile men, while ٥ % of infertile men have abnormal semen pH (less than ٧.٢ or more than ٨.٠). When the pH exceeds ٨.٠, infection should be suspected with decreased secretion of acidic products by the prostate, such as citric acid. Extremely acidic pH (< ٦.٥) is found in cases of agenesis (or occlusion of the seminal vesicles) (Rrumbullaku, ٢٠٠٣).When the pH is less than ٧.٠ in a sample with azoospermia, then may be obstruction of the ejaculate ducts or bilateral congenital absence of the vasa (WHO, ١٩٩٩).

٥.١.١.٥. Volume:

The volume of the ejaculate from fertile men is higher than infertile men and different groups of infertile men, oligoasthenozoospermia, oligoasthenoteratozoospermia, asthenozoospermia, asthenoteratozoospermia, idiopathic, and azoospermia. However the results were within normal ranges for both according to the (WHO) standardization for seminal fluid analysis which estimated the normal range of the volume ٢-٥ ml. A low ejaculate volume can reflect abnormalities in accessory sex gland fluid synthesis or

secretion. It can also be indicative of a physical obstruction somewhere in the reproductive tract (Siegel, ١٩٩٣), or may occurs in cases of incomplete or partially retrograde ejaculation. Semen volume affects fertility only when it falls bellow ١.٥ ml (incomplete bufferings of vaginal acidity), or is more than ٥ ml (Dale-McClure, ١٩٨٦).

٥.١.٢. Microscopic evaluation:

٥.١.٢.١. Sperm concentration:

Infertile men showed a lower sperm conc., and total sperm count than fertile men. In addition, the results of the current study observed that oligoasthenozoospermia, oligoasthenoteratozoospermia, asthenozoospermia, asthenoteratozoospermia, and idiopathic has lower sperm conc. and total sperm count than that of fertile men. These results was in agreement with the findings of ; Shen *et al*., (١٩٩٩); Chia *et al*., (٢٠٠٠) and Okonofua *et al*., (٢٠٠٥) who recorded that sperm conc. and total sperm count from healthy fertile men are higher than infertile men. Also the study of Khosrowbeygi *et al*., (٢٠٠٤), showed that sperm conc. is higher in fertile control men than asthenozoospermic, asthenoteratozoospermic**,** & oligoasthenoteratozoospermic men.

The sperm count may be affected by many factors such as hormonal and seminal microbial infections. Some causes of azoospermia related to hormonal imbalance, while obstructive azoospermia associated with bacterial infections (Kondoh *et al*., ١٩٩٩). In addition to these causes, we record that varicocele, and smoking is higher among infertile men than that of fertile men, that is may be due to another causes for reduction of sperm density among infertile men.

٥.١.٢.٢. Sperm motility:

The results of the present study showed that grade activity, the percentage of sperm motility and sperm motility index are lower in infertile men and different groups of infertile men including oligoasthenozoospermia, oligoasthenoteratozoospermia, asthenozoospermia, asthenoteratozoospermia, and idiopathic than that of control fertile men.

Adequate motor activity of the sperm cell is required for normal transport through the female reproductive tract and for penetrating the ovum. Sperm motility is one of the most important parameters utilized in the evaluation of a semen samples fertilizing ability *in vitro* (Mahandevan and Trounson, ١٩٨٤) and *in vivo* (Barratt *et al*., ١٩٩٣). Also sperm motility is the single most important measure that can be consider a compensatory factor in men with low sperm conc.(Dale-McClure, ١٩٨٦).Abnormalities in motility and quality of movements can arise from infection, the presence of antisperm antibodies, partial-duct obstruction, or the subtle testicular alteration that may be caused by gonadotoxins and varicoceles (Seaman *et al*., ١٩٩٤).

The sperm motility result of this study was agreement with the observations of, Chia *et al*., (٢٠٠٠); Okonofua *et al*., (٢٠٠٥), who observed that sperm motility in infertile men, are lower than that of fertile men. The study of Khosrowbeygi *et al*., (٢٠٠٤) recorded that asthenozoospermic, asthenoteratozoospermic, and oligoasthenoteratozoospermic men have sperm motility lower than that of idiopathic fertile men. Also Zarghami and Khosrowbeygi, (٢٠٠٤) showed a significant differences between idiopathic ferile and asthenozoospermic infertile men, and sperm motility was inversely correlated with seminal plasma MDA levels. These results are in accordance with our study. Infertile men of the present study showed an increase in MDA

compare with the normal control fertile men; this may be another cause of decreasing sperm motility.

٥.١.٢.٣. Viability:

If none of the sperm are moving, the patients may have necrospermia. This is actually a misnomer, as metabolic studies and special vital stains have revealed that the immobile spermatozoa may not necessarily be dead (Seaman *et al*., ١٩٩٤). Sperm viability should be determined if the percentage of immotile spermatozoa exceeds ٥٠ % (WHO, ١٩٩٩). In the present work the viability of the sperm from fertile men are higher than that of infertile men and also different infertile groups. These results are in accordance with those of Chia *et al*., (٢٠٠٠) who record that infertile men has ٥٨.٩ % and fertile men has ٧٣.٦ % viable sperm; and with Okonofua *et al*., (٢٠٠٥) who observed that fertile men has higher viable spermatozoa than that of infertile men.

Pal *et al*., (٢٠٠٦) observed that fertile men have ٧٥.٦٧ % of viable sperm, while Shen *et al*., (١٩٩٩) showed no significant differences between fertile and infertile Singapore men. Reduced percentage of motility with a high percentage of viable sperm may reflect structural or metabolic abnormalities of spermatozoa that derived from abnormalities in testicular function or antimotility factors in the seminal plasma (Siegel, ١٩٩٣).

٥.١.٢.٤. Sperm morphology:

As shown in the findings of the results, significant differences appears of spermatozoal morphology between fertile and infertile men.The normal

sperm morphology is higher in fertile control men compared with infertile men and other infertile groups including oligoasthenozoospermia, oligoasthenoteratozoospermia, asthenozoospermia, and asthenoteratozoospermia. The results of the current study is in agreement with the observations of Shen *et al*., (١٩٩٩) and Chia *et al*., (٢٠٠٠), recorded that the percentage of normal sperm morphology is higher in fertile men compare to infertile men. Also the study of Khosrowbeygi *et al*., (٢٠٠٤), showed that normozoospermic fertile men has a higher normal sperm morphology than oligoasthenoteratozoospermic, asthenozoospermic, and asthenoteratozoospermic infertile men. In addition of these, the study of Pale *et al*., (٢٠٠٦) on fertile Indian men observed that the normal sperm morphology of them is ٧٥.٦٧ %.

Different defects of sperm morphology was recorded from infertile men in the present study, these defects including; large head, small head, tapering head, amorphous head, sperm without head, double head, bent neck, short tail, long tail, double tail, without tail, coiled tail, tails with terminal droplets, and large cytoplasmic droplets.

It was found that prostate and seminal fluid infection lead to morphology abnormalities, dysfunction of sperms and changes in semen parameters (Giblin *et al*., ١٩٨٨). The increased number of immature spermatozoa may be due to epididymal dysfunction and a consequence of frequent ejaculations.The increased number of spermatozoa with tapering heads is found in association with varicocele. The recent study indicate that the percentage of tapered sperm, sperm containing cytoplasmic droplets and sperm with bent tail are significantly increased in varicocele patients compare with controls (Rrumbullaku *et al*., ١٩٩٨).

٥.٢. Viscosity and semen quality:

From the persent study and as shown in table١٥, among most semen parameters only the motility of spermatozoa was affected with viscosity. The infertile men with abnormal viscosity showed lower grade activity. The percentage of motility, and sperm motility index is lower than with infertile men with normal viscosity. Although seminal viscopathy was shown to be associated with male infertility, the pathogenesis of seminal hyper-visco-elasticity and the mechanism by which it influences male fertility are still not well understood (Elzanaty, *et al*., ٢٠٠٤).

High viscosity can interfere with determination of sperm motility, sperm conc., and antibody counting spermatozoa (WHO, ١٩٩٩) or can impaired the availability of fertile sperm at the site of fertilization (Siegel, ١٩٩٣). Results are in accordance with the findings of, Ming-Chung *et al*., (١٩٩٢), and Gopalkrishnan *et al*., (٢٠٠٠), who concluded that much higher viscosities were observed in the men with asthenozoospermia. Moreover, in post-coital test studies, hyper-viscosities were also found to be associated with poor invasion of cervical mucus by spermatozoa (Gersh, ١٩٧٠). Gonzales *et al*., (١٩٩٣), postulated that hyper-viscosity of seminal fluid associated with hypo-functions of seminal vesicles. However subsequent investigations have not confirmed these assumptions (Carpino and Siciliano, ١٩٩٨; Siciliano *et al*., ٢٠٠١).

Prostate-specific antigen (PSA) is considered to be the main seminal plasma proteolytic enzyme and was found to degrade semenogelin I (sgI) and semenogelin II (sgI) which have been to be the dominating proteins

components of semen coagulum into smaller fragments. The primary source of these proteins being the seminal vesicles (Lundwall *et al*., ٢٠٠٢). Another factor which might be implicated in the process of semen viscosity is zinc, mainly originating from the prostate. This metal may be crucial for modulation of the three dimensional structure sgI and sgII rendering them more susceptible to proteolytic breakdown by seminal proteases and in particular by the PSA (Lilja, ١٩٨٥). Fructose has been shown to be the main source for energy of spermatozoa. Its concentration in seminal plasma has been recommended as a marker of the seminal vesicles secretion activity (WHO, ١٩٩٩) and its amount was found to correlate positively with the other seminal vesicles proteins (Montagnon *et al*., ١٩٨٢). Fructose is an indicator of seminal vesicles secretion, which contains semenogelins. Unfortunately, no reliable assays for measurements of the sgI and sgII levels are available for which reason we cannot evaluate the secretion of these proteins (Elzanaty et al., ٢٠٠٤).

Elzanaty *et al*., (٢٠٠٤), found that the conc. of PSA and zinc were significantly lower in hyper-viscosity semen samples, than in those with normal viscosity, while total amount of fructose was significantly higher in samples with high viscosity. High semen volume and low the total fragments of sperm motility were recorded in samples with high viscosity. Finding of decreased conc. of PSA in the hyper-viscosity samples supports the idea of the association between the prostatic enzymes and semen viscosity. Another cause might be an increased seminal volume and high total amount of sgI and sgII as caused by hyper-secretion from the seminal vesicles.

Mendeluk *et al*., (١٩٩٧), observed that in case of asthenozoospermia, sperm in ejaculated with high viscosity show better response to swim-up

technique than those with normal. Treatment of hyper-viscous samples with proteolytic enzymes which could results in improved sperm motility and render the samples, more suitable for IUI.

٥.٣. Osmolality and semen quality:

Epididymal fluid is characterized by progressive decreases in Na^+ and increases in K^+ concentrations along the duct. Osmolality is made up by high amounts of small organic molecules including amino acids such as glutamate and taurine, carnitine, glycerophosphocholine (GPC) and myoinositol. The increase in osmolality from testicular to epididymal fluid should induce uptake of osmolytes by epididymal sperm to counteract cell shrinkage. These compounds then are utilized by sperm upon ejaculation into a relatively hypotonic environment in the female tract, by mechanism of regulatory volume decreases resulting in efflux of osmolytes and cellular water (Cooper and Yeung, ٢٠٠٣).

Yeung *et al*., (٢٠٠٣), observed that incubation of sperm with L-carnitine, myoinositol and taurine did not change sperm volume or kinematics (the parameters related to motility), but the presence of glutamate and K^+ decreased the efficiency of forward progression indicative of volume increase, suggesting them as potential osmolytes for human sperm.

The results of the carried study showed that semen osmolality in both fertile and infertile men is higher than serum, and the osmolality of fertile men is lower than that of infertile men and all infertile groups except idiopathic and azoospermic. The results is in accordance with the findings of Tuck *et al*., (١٩٧٠); Hinton *et al*., (١٩٨١); Polak and Daunter, (١٩٨٤) and Rossato *et al*.,

(٢٠٠٢), which has been reported that the seminal plasma osmolality is higher than that of blood and osmolality influences sperm motility. Besides influencing sperm motility, seminal osmolality variations have been demonstrated to be able to regulated $Ca^{+٢}$ influx, acrosome reaction and fertilizing ability acquisition in human sperm. Human sperm possess an osmosensitive $Ca^{+٢}$ influx pathway that are activated by exposure to a hypo-osmolar medium. The influx of extracellular $Ca^{+٢}$ within sperm following exposure to hypo-osmolar medium is able to activate the acrosome reaction leading to fertilizing ability acquisition (Rossato *et al*., ١٩٩٦).

The study of Rossato *et al*., (٢٠٠٢) on a large numbers of idiopathic fertile and asthenozoospermic infertile semen specimens showed that idiopathic fertile men has seminal osmolality values significantly lower with respect to asthenozoospermic patients. Furthermore a significant negative correlation was recorded between seminal osmolarity and percentage of total sperm motility and kinetic characteristics of spermatozoa evaluated by a computerized system for sperm motility analysis. Also no correlation of Na^{+}, K^{+}, Cl^{-} and sperm motility, while a significant positive linear correlation was shown between seminal ionized Ca^{+} concentrations and curvilinear velocity. When sperm from fertile subjects were suspended in medium with an osmolarity increasing from ٣٠٠ to ٦٠٠ m OSm sperm motility percentage and grade activity reduced and nearly abolished when medium osmolarity was ٦٠٠ m OSm. On the contrary, when sperm from asthenozoospermic subjects with high semen osmolarity were resuspended in medium with lower osmolarity, sperm motility parameters improved significantly (Rossato *et al*., ٢٠٠٢). Abnormal seminal plasma showed a still higher value ٤٢٨ and ٤٥٧ m OSm / kg respectively for asthenozoospermic and oligoasthenozoospermic subjects

(Velazquez *et al*., ١٩٧٧)., compare with ٣٤٣ and ٣٦٠ m OSm / kg for asthenozoospermic and oligoasthenozoospermic subjects in our study.

٥.٤. ROS and infertility:

Oxidative stress is a condition associated with an increased rate of cellular damage induced by oxygen and oxygen-derived oxidants commonly known as reactive oxygen species (ROS) (Sikka, *et al*., ١٩٩٥). ROS have been implicated in over a hundred of disease states which range from arthritis and connective tissue disorders to carcinogenesis, aging, toxin exposure, physical injury, infection and acquired immunodeficiency syndrome (Joyce, ١٩٨٧). O_2 is required to support life, but its metabolities, such as ROS, can modify cell functions, endanger cell survival, or both (de Lamirande and Gagnon, ١٩٩٥), hence ROS must be inactivated continuously to maintain only the small amount necessary to maintain normal cell function. Different antioxidant normally protects against oxidants (Sies, ١٩٩٣). Oxidative stress develops when oxidants outnumber antioxidants, peroxidation products develop and these phenomena cause pathologic effects (Sikka, ٢٠٠١).

As mentioned in the review of literature, recent reports indicate that high levels of ROS are detected in the semen of ٢٥ to ٤٠ % of infertile men (de Lamirande and Gagnon, ١٩٩٥; Padron *et al*., ١٩٩٧). Spermatozoa and seminal plasma contain a number of antioxidant system which counteract the effects of ROS, spermatozoa possess a low amount of cellular ROS defense system (Fujii *et al*., ٢٠٠٣; Garrido *et al*., ٢٠٠٤). The mechanism of ROS production by spermatozoa and leucocytes is well mentioned in the review of literature. Lipid peroxidation (LPO) is the most extensively studied manifestation of oxygen

activation in biology. In spermatozoa, production of MDA, an end product of LPO induced by ferrous ion promoters, has been reported (Darley-Usmer *et al*., ١٩٩٥). Available data on the impact of oxidative stress on sperm motility are based on the measurement of seminal plasma and sperm MDA (Nakamura *et al*., ٢٠٠٢; Keskes-Ammar *et al*., ٢٠٠٣).

The results of the current study which are shown in tables ١٠ and ١٧ indicate that the seminal plasma MDA conc. of fertile men is significantly lower than that of infertile men and all groups of infertile men starting from oligoasthenozoospermia and ending to idiopathic. Also the results showed that asthenozoospermic infertile men which including oligoasthenozoospermia, oligoasthenoteratozoospermia, asthenozoospermia, and asthenoteratozoospermia has a higher MDA levels than azoospermic and idiopathic infertile men, and a higher conc. of MDA is recorded in asthenoteratozoospermia and oligoasthenoteratozoospermia. These results means that MDA level is related with motility and morphology of spermatozoa, as described by Aziz *et al*., (٢٠٠٤), who observed that ROS production is highest in immature spermatozoa from males with abnormal semen values; Saleh and Agarwal (٢٠٠٢), which demonstrate that ROS can directly damage spermatozoa by inducing peroxidation of the lipid-containing sperm plasma membrane, which decreases its integrity and may also affected sperm motility by damaging the axonemal structure; Weng *et al*., (٢٠٠٢), found that high levels of ROS are important in the impairment of sperm motility and the occurrence of asthenozoospermia.

The results are in contrast with the findings of Nakamura *et al*., (٢٠٠٢), whose investigation showed no significant differences in the seminal plasma level of MDA between asthenozoospermic and normozoospermic fertile males.

The present study is in accordance with the observations of Fraczek *et al*., (٢٠٠١) and Tavilani *et al*., (٢٠٠٥) who showed that MDA levels in asthenozoospermic males is higher than normozoospermic fertile men. Also Keskes-Ammar *et al*., (٢٠٠٣) and Zarghami and Kosrowbeygi, (٢٠٠٥) observed a significant indirect inverse correlation between seminal plasma levels of MDA and sperm motility.

Other results of the present study which recorded in figure (٨) showed that the conc. of MDA in varicocele, leucocytospermic and smokers infertile men is higher than that of non-varicocele, normal leucocytes and non-smokers infertile men. Elevated level of ROS has been detected in infertile patients with varicocele, along with reduced levels of both seminal plasma and blood plasma antioxidants, level of ROS positively correlate with the degree of varicocele and are expected decrease after varicocelectomy (Barbieri *et al*.,١٩٩٩). Infertile men with varicocele have significantly greater spermatozoa DNA damage, which can be related to high levels of ROS in semen (Saleh *et al*., ٢٠٠٢).

Most important leucocytospermia has been associated with occult sperm DNA damage indirectly as a result of pathologic ROS levels, which are frequent in leucocytospermic patients. In one study, levels of the oxidative DNA damage marker ٨-hydroxy ٢-deoxyguanosine (٨-OH-Dg) were significantly elevated in infertile patients (Kodama *et al*., ١٩٩٧).

Arabi and Moshtaghi, (٢٠٠٥), found that exposure of spermatozoa from the non-smoker to the seminal plasma from smoker yielded lipid peroxidation propagation. Addition of pure seminal plasma to the non-smoker spermatozoa (with incubation of ١٨٠ minutes), resulted in a significant elevation in MDA levels as an index of lipid peroxidation damage. However, exposure of spermatozoa from the smoker to the seminal plasma from non-smoker yielded

no significant inhibition in MDA generation from ٢.٨٩ ± ٠.٨٨ to ١.٩٢ ± ٠.٩٦. There is a correlation between cigarette smoking in infertile men and increased leucocyte infiltration into semen (Close *et al*., ١٩٩٠). Moreover smoking can be linked to significantly increased levels of seminal ROS (Saleh *et al*., ٢٠٠٢).

٥.٥. Leucocytospermia:

٥.٥.١. Leucocyte determination by HPF and Endtz methods:

Most Iraqi laboratories depend on the HPF method for determination of leucocytes in semen. This method is not accurate because they didn't distinguish between leucocytes and other round cells present in the semen such as spermatocytes, spermatids, epithelial and prostatic cells. So that the other method which called myeloperoxidase cytochromical (Endtz) test was used to distinguish between leucocytes and other round cells in the semen.

The results which are recorded in (table ١٨) showed a significant difference between the two methods in fertile and all infertile groups. Semen leucocytes with Endtz method are significantly lower compare with the HPF method. The results are in agreement with the findings of Al-Dujaily, (٢٠٠٥) who found that the numbers of leucocytes in semen using Endtz method is significantly lower than them with using the HPF method in normospermic fertile men, azoospermia, oligozoospermia, asthenozospermia, asthenoteratozospermia, teratozoospermia, and oligoasthenozoospermia.

Researchers have shown that increased levels of leucocyte in the ejaculate commonly are found in infertile men, when compared with controls

(El-Demiry, ١٩٨٧), whereas Curtis, (١٩٩٩) found that many of bacteria isolated from infertile and fertile men have considered to be part of normal flora, but some organisms were probably related to infertility. It has been reported that treatment with antibiotic may have negative effect and correlation on certain sperm function parameters e.g. sperm conc., sperm motility and sperm morphology (Hafez *et al*., ١٩٩٧). So that, detecting of the leucocytes in the semen at the time of semen fluid examination using Endtz method may be the proper first step recommended to prevent the random treatment and negative effect of antibiotics (Al-Dujaily, ٢٠٠٥).

The distinction between leucocytes and immature germ cells is made possible because Endtz solution brown intracellularly due to the effect of peroxidase or hydrogen peroxide (Shekarriz *et al*., ١٩٩٥). Endtz test is easy to performed, complete with short time with high accuracy, not cost and can sustain the correct treatment. It can be used in any andrology laboratory in Iraq (Al-Dujaily, ٢٠٠٥).

٥.٥.٢. Leucocytes and semen quality:

Origin and the precise of white blood cells in semen are not fully understood (Fraczek *et al*., ٢٠٠٤). The presence of seminal leucocytes as numbers >١×١٠٦/ ml of semen, defined by the (WHO, ١٩٩٩) as leucocytospermia, are considered to be pathological. Some authors have suggested that there is an association between increased seminal WBC concentrations and poor semen parameters (Yanushpolsky *et al*., ١٩٩٦), or reduced sperm fertilizing capacity (Vogelpoel *et al*., ١٩٩١). Statistical data indicated that leucocytes more occur in semen samples of infertile males when comparing with the fertile ones. Leucocyte concentration in semen can be potentially harmful to spermatozoa through the release of toxins substances

such as proteases or reactive oxygen intermediates often associated with phagocytosis (Fraczek *et al*., ٢٠٠٤), the mechanism by which leucocytes release ROS is mentioned in detail in review of literature.

The results of the present study showed that the leucocyte conc. in semen with both HPF and Endtz methods is lower in fertile men compare with infertile male and different infertile groups. Other results which shown in table ١٩, showed that most semen parameters not affected significantly with the increased leucocyte in semen. Leucocytospermic patients have lower grade activity, the percentage of sperm motility and sperm motility index than that of non-leucocytospermic patients.

The effects of the leucocyte on semen quality is contaversal among different studies, Arata de Belabarba *et al*., (٢٠٠٠) and Diemer *et al*., (٢٠٠٣) observed that there is an association between increased seminal WBC conc. and poor semen parameters, while Tomlinson *et al*., (١٩٩٢) and Rodin *et al*., (٢٠٠٣) found no negative influence between leucocytes and semen parameters. Simbini *et al*., (١٩٩٨) showed an inverse relation is appearing between leucocyte conc. and sperm count. The percentage of abnormal sperm was higher and the sperm motility poorer in leucocytospermic samples. The recent study of Yilmaz *et al*., (٢٠٠٥) found that the progressive motility rate and sperm conc. were lower in the leucocytospermic group compare with the control.

It has been found that prostate and seminal fluid infection may lead to morphology abnormalities, dysfunction of sperms and changes in semen parameters (Giblin *et al*., ١٩٨٨). Cardoso *et al*., (١٩٩٨) documented that the live bacteria found in semen markedly reduced the motility and viability of sperms. Swenson *et al*., (١٩٧٠) and Ismaeel, (٢٠٠١) investigated that certain

microorganisms have been shown to be capable of adhering to human spermatozoa and may in this way decrease the motility of these cells.

٥.٦. Varicocele and semen quality:

Testicular temperature is a function of the heat entering the testis by arterial flow, heat loss from the testis by venous return and heat loss through the scrotal skin by conduction, radiation, convection and evaporation (Sealfon, ١٩٩١). Lund and Nielsen, (١٩٩٦), observed that there were no apparent differences in scrotal skin temperature in men with or without a varicocele. As mentioned in review of literature, the exact mechanism by which the varicocele depress spermatogenesis is unknown but several theories have been advanced to explain the mechanism by which varicocele impairs male infertility. These theories include scrotal hyperthermia , retrograde flow of adrenal or renal metabolities , Leydig cell dysfunction and hypoxia .Varicocele was reported to be associated with elevated ROS production in spermatozoa and diminished seminal plasma antioxidant activities (Mostafa *et al* ., ٢٠٠١).

Micic *et al*., (١٩٨٦) showed a significant elevation of FSH in the seminal plasma of the infertile men with varicocele compared with the seminal plasma of the other infertile and fertile men. Seminal LH and prolactin values were similar in both infertile groups but significantly higher than in fertile men. Testosterone and oestradiol levels in the seminal plasma of infertile men with varicocele were lower than fertile and other infertile males. These findings indicates that both steroids were decreased in infertile men with varicocele could explain disturbed function of spermatozoa in men with varicocele.

Further analysis will elucidate the importance of these hormones findings in the seminal plasma of infertile men with varicocele.

The results of the performed study on varicocele which presented in figure ٨ and table ٢٠, found that the sperm density and motility are significantly lower in infertile patients with varicocele compare with infertile patients without varicocele. Also the seminal plasma MDA is higher in varicocele patients compare with non-varicocele patients. These results are in agreement with some studies and in opposite to others because the studies which explained the relationships between varicocele and semen quality are contaversal. There are a decrease in sperm count, sperm motility and normal sperm morphology, following varicocelectomy, semen quality improves significantly, but the mechanism of action is still unknown (Dubin and Amelar, ١٩٧٧; Hendry, ١٩٩٢). Lund and Nielsen, (١٩٩٦) found that sperm conc., and total sperm count was significantly lower in men with varicocele than that of control subjects, but no differences in the volume of semen and sperm motility are observed. Study of Freiha and Mroueh, (١٩٧٦) showed that there are a significant improvement of sperm count, motility and morphology of patients with varicocele after ligation of spermativ vein. Scrotal cooling at night resulted in a scrotal temperature drop by ٠.٨ ć (median), and a significant increase in sperm conc. and total sperm count (Jung *et al*., ٢٠٠٥).

٥.٧. Smoking and semen quality:

Inhalation of cigarette smoking (CS), leads to absorption of nicotine, carbon monoxide and recognized carcinogens and mutagens such as radioactive polonium, cadmium, benzo (a) pyrene, dimethylene (a) anthracene through vascular and blood circulation (Stillman, ١٩٨٦). In habitual heavy smoking, long-term exposure to CS whose by-products move across the blood-

testis barrier and exist at high concentration in the testes may adversely affect the sperm function (Stedman, ١٩٦٨). Numerous investigations have been conducted on the relationship between CS and male infertility, but the exact molecular mechanism is not fully understood in most the cases and the data are very disputable.

Table ٢١ showed the effects of smoking on semen quality. The volume of semen, sperm conc., motility, viability, and normal sperm morphology are higher in non-smoking infertile men compare with smoker infertile men. These results is in agreement with those of, Vine *et al* ., (١٩٩٤) ; Sofikitis *et al* ., (١٩٩٥) ; Merino *et al* ., (١٩٩٨) ; Zenzes , (٢٠٠٠) ; Zinaman *et al* ., (٢٠٠٠) and Kunzle *et al*., (٢٠٠٣) who observed that there are a significant decrease in semen volume, normal sperm morphology, sperm density, sperm motility and viability of smoker men when compare with non-smoker infertile men.

Infertile men who smoke have higher levels of seminal OS than infertile non-smoker. The link between CS and higher seminal ROS can be attributed in part to the associated increase in seminal leucocyte concentrations by as much as ٤٨ % (Saleh *et al* ., ٢٠٠٢). Arabi and Moshtaghi, (٢٠٠٥), found that when pure semen of smoker men is added to the semen of control non-smoker men (about ٩٢ % motile spermatozoa), the motility will be lost within ١٢٠-١٨٠ minutes of incubation. Also a negative correlation was found between final MDA level and sperm motility, that is indicated the negative impact of pro-oxidant substances exist in the seminal plasma from smoker men. Factors such as the number of cigarette smoked per day, years of smoking and levels of nicotine by-product presents in body fluids have correlated negatively with semen and sperm quantity and quality (Chi *et al*., ١٩٩٤). Smoking has also

been found to affect accessory sex glands (e.g. prostate and seminal vesicles) (Pakrashi and Chatterjee, ١٩٩٥).

٥.٨. Immunological factors and infertility:

The blood-testis barrier and immunoregulatory proteins at the level of Sertoli cells, rete testis and efferent ductules, provide immunological protection of sperm antigens and inhibit lymphocyte proliferation and complement-mediated cell lysis (Furuya *et al*., ١٩٧٨). Disruption of this blood-testis barrier is believed to result in the production of antisperm antibodies. The proposed aetiologies for such disruption include ductal obstruction, testicular torsion, infection/epididymitis, prostatitis, testicular trauma and varicocele (Jarrow and Sanzone, ١٩٩٢). Antisperm antibodies have been shown to cause agglutination and immobilization of spermatozoa, sperm cytotoxicity, impairment of sperm penetration into the cervical mucus, prevention of capacitation or the acrosome reaction in response to zona pellucida, and enhanced phagocytosis of spermatozoa by macrophages (Bronson *et al*., ١٩٨٤; Haas, ١٩٨٦).

Sperm antibodies in semen belong almost exclusively to two immunoglobulin classes, IgA and IgG. IgA antibodies might have greater clinical important than do IgG antibodies. IgM antibodies, because of their large molecular size are rarely found in semen (WHO, ١٩٩٩).

In the present study the ASAs are detected in semen of fertile and infertile males using tray agglutination test (TAT), this test is first performed by Friberge, (١٩٧٤), and it used widely and considered the reference test for detection of ASAs in serum and seminal plasma (Linnet and Suominen, ١٩٨٢). There are other tests which used for detection of ASAs such as, mixed immunoglobulin reaction (MAR) test, immunobead test (IBT), and enzyme linked immunosorbant assay (ELISA), but these tests not used in this study due to the high expensive cost of them. Eggert-Kruse *et al*., (١٩٩٣) found that all MAR positive (semen) patients were negative for circulating ASA when assessed using an ELISA technique. The meaning of this discrepancy is unclear and may be explained by the fact that serum ASA values do not sufficiently reflect the immunological situation in the semen.

Results on immunological factors showed that the agglutination of spermatozoa is higher among infertile men than that of fertile men (١٣.٧٩ % and ٦.٦٦ %) respectively. Also when TAT is used for detection of ASAs, no ASAs are observed in fertile men, while ٦.٠٣٤ % of infertile men are shown positive detection of ASAs. Other results found that the incidence of ASAs among asthenozoospermic group is higher than that of other groups, that is means the presence of negative correlation between ASAs and motility of spermatozoa. Results is in agreement with the results of Rumke and Hellinga, (١٩٥٩), which observed that about ٣ % of infertile men had sperm agglutinating antibodies, whereas it was absent from fertile controls; Ayvaliotis *et al*., (١٩٨٥) and Naz and Menge (١٩٩٤), who had been shown that incidence of ASAs in men suspected of infertility was ٤-٢١ %.

Shibahara *et al*., (٢٠٠٢) found that from ٢٧٥ infertile patients, ٩ samples have positive detection of ASAs, which included IgG (٢.٥ %), IgA (١.٨ %) and

IgM (٠.٤ %). Also from these ٩ samples, ٧ samples of them are in asthenozoospermic patients, while the other ٢ samples are from normozoospermia and oligoasthenozoospermia. It has been shown that the presence of ASAs can reduce the motility (Witken *et al*., ١٩٩٢). When the majority of sperm are coated with ASA over their heads, fertilization is often impaired by reducing the sperm binding to the egg (Yeh *et al*., ١٩٩٥). On the contrary, tail directed ASA tend rather to impair sperm motility, resulting in the reduction of the fertilization rate *in vitro* (Witken *et al*., ١٩٩٢).

Both IUI and IVF yielded unexpectedly high pregnancy rates in the selected group of patients with long-standing infertility due to sperm surface (predominantly IgG) antibodies. Since cost benefit analysis comparing IUI with IVF may favor a course of four IUI cycles, so that the IUI is used as the first line therapy in male immunological infertility. ٦٤.٣ % of patients conceived after a maximum of three IUI cycles, whereas ٤٦.٦ % of patients become pregnant during the first IVF cycles (Ombelet *et al*., ١٩٩٧).

٥.٩. Hormonal evaluation and infertility:

In the current study some hormones evaluated which are strongly related to the male infertility, these hormones are, LH, FSH, testosterone, prolactin, and Estradiol II. Swerdloff *et al*., (١٩٩١) found that any infertile male requires an endocrine evaluation should at least have the following basal hormone measurements; FSH, LH, testosterone, sex-hormone binding globulin (SHBG), and prolactin. These hormones play an important role in the regulation of spermatogenesis and secondary sex characteristics in males which are mentioned in the review of literature. Primary endocrine abnormalities are

rare in patients with sperm conc. of > ٥ × ١٠^{٦} / ml (Butt and Blunt, ١٩٨٨), hence the above hormones in this study is measured only in severe oligozoospermic, and azoospermic infertile males, while selected fertile men is used for statistical comparisons with infertile patients.

No significant differences recorded in serum LH, testosterone, prolactin and Estradiol II between fertile men, severe oligozoospermic and azoospermic infertile males. Except FSH is significantly higher in azoospermia compare with fertile men and severe oligozoospermic patients. Other results which are recorded in table ٢٧, found that the high percentage of hormonal abnormalities is FSH (٣٣ % and ٤٠ % abnormalities is seen in oligozoospermia & azoospermia respectively). The other hormonal abnormalities is LH, one case from ١٥ cases of oligozoospermia and two cases from ٢٠ cases of azoospermia showed an increase in the level of serum LH. Testosterone decrease including ١ patient of oligozoospermia and ٣ patients of azoospermia. One patient of azoospermia has an increase in conc. of prolactin, while ١ patient of oligozoospermia has an increase in Estradiol II conc.

Islam and Trainer, (١٩٩٨) found that a patient with oligo/azoospermia and elevated circulating FSH, but normal LH and testosterone levels has primary germinal tubular failure with no associated Leydig cell damage. Hence the azoospermic men in the present study which has higher FSH and normal LH and testosterone, may have germinal tubular failure. Other azoospermia patients with normal serum testosterone, LH and FSH may has either retrograde ejaculation or obstructive azoospermia, while in some cases a high FSH and LH and low testosterone are recorded, this may be indicates a primary testicular failure, hypergonadotrophic hypogonadism, involving both Leydig and germinal epithelium (Islam and Trainer, ١٩٩٨).

In one patient of azoospermia elevated prolactin was observed but not significant Elevated circulating prolactin conc. are associated with impotence and decreased libido rather than spermatogenesis failure and decreased of fertility (Thorner *et al*., ١٩٧٧; Segal *et al*., ١٩٧٩).

٥.١٠. *In vitro* human sperm activation:

As mentioned in the review of literature, *in vitro* addition of seminal fluid to the ovum directly without activation of the spermatozoa lead to failure of fertilization due to inocurrence of capacitation process , as well as due to the effects of some abnormal seminal components such as leucocytes and phagocytes (Kanwar *et al*., ١٩٧٩). Increasing of the percentage of sperm motility and normal spermatozoa is very important to increases the chance of fertilization and pregnancy rates through the Artificial insemination by husband (AIH) , gametes intrafalopian transfers (GIFT) , *in vitro* fertilization and *in vitro* fertilization – embryo transfers (IVF – ET) (Menkveld *et al* ., ١٩٩٠).

The current results indicate that there is a decrease in sperm conc., spermatozoal agglutination and leucocytes after sperm activated with modified balanced Earle's activation medium, comparing with them before activation. Also a good improvement in percentage of sperm motility, grade activity, and normal sperm morphology is achieved after activation. The improvement of semen quality resulted with both, simple layer and centrifugation- swim up techniques. Moreover the improvement is greater with the latter technique. These results is in agreement with the findings of Al-Hady, (١٩٩٧) who found a significant improvement of sperm functions ($p<٠.٠٠١$) which include; sperm motility %, normal sperm morphology %, sperm agglutination %, leucocytes %, and sperm grade activity after sperm activation compared with the value before activation when used different

techniques and two sperm activation medium Ham's F- ١٠ medium and modified balanced Earle's medium. Ridha-Al barzanchi *et al*., (١٩٩١) and Al-Janabi, (١٩٩٢) observed a significant decrease in sperm conc. after activation. The decrease in sperm conc., may be due to the remains of dead and immobile spermatozoa in the sperm pellet and inability of them to move up of the medium (Cheeck *et al*., ١٩٩٣).

Hoing *et al*., (١٩٨٦), found that washing and centrifugation of semen samples of asthenozoospermic infertile men with ٢٠٠٠ rpm for ٥ minutes and using Earle's medium resulted in a significant improvement of the percentage of sperm motility by ٥٧ %. The increase in sperm motility due to using of activation medium. The activation medium which used in this study contains sodium, potassium, calcium, magnesium, phosphate, and pyruvate ions. These ions are used as nutritive source in addition to stimulate of capacitation process, which result in an increase of sperm motility and activity (Al-Taee, ١٩٩٤). Moreover the increase in motility and grade activity may be due to addition of female serum to the activation medium. Hicks *et al*., (١٩٧٢) found that steroidal components of female serum help the human spermatozoa for adaptation and increasing the activity *in vitro* and *in vivo* through increasing sperm metabolic rate. Al-Hady, (١٩٩٧) found that using of ٣٠ % of female serum to the activation medium resulted in the high proportion of the percentage of motility and grade activity of spermatozoa.

Normal sperm morphology is considered important physiological features of spermatozoa and occurrence of fertilization and the high proportion of abnormal sperm morphology in semen significantly reduced the percentage of fertilization (Franken *et al*., ١٩٨٩). There are other study mentioned that *in vitro* fertilization is depends mainly on the normal head

shape of spermatozoa which bind with ovum. Also the percentage of sperm penetration the ovum is greater in normal head shape than abnormal one (Aitken *et al*., ١٩٨٢; Kruger *et al*., ١٩٨٦).

Most semen samples contain leucocytes. The presence of leucocytes in semen is normal, but when the numbers of them reaches to more than ١× ١٠٦ / ml and according to WHO, (١٩٩٩) is defined leucocytospermia and considered abnormal. Increased conc. of leucocytes in semen provides an important clinical indicator of genital tract infection or inflammation (Comhaire *et al*., ١٩٨٠). Some authors have suggested that there is an association between increased seminal WBC conc. and poor semen parameter (Wolff *et al*., ١٩٩٠; Vicino *et al*., ١٩٩٩; Arata de Belabarba *et al*., ٢٠٠٠; Diemer *et al*., ٢٠٠٣) or reduced sperm fertilization capacity (Vogelpoel *et al*., ١٩٩١).A significant decrease of leucocyte in semen after activation is recorded in this study and this is may be due to addition of antibiotic to the activation medium during preparation, technique of centrifugation and swim up of motile spermatozoa leaving the leucocytes in the lower portion of the medium, and reduction of leucocytes by dilution with activation medium (Al-Hady, ١٩٩٧).

Finally and from a physiological point of view , immunological infertility due to sperm surface antibodies can result from the effect on sperm transport , the destruction of gametes , acrosome reaction abnormalities , by inhibition of sperm zona – pellucida binding or by prevention of embryo cleavage and early development of the embryo (Bronson *et al* ., ١٩٨٤ ; Shushan and Schenker , ١٩٩٢). A study of Abdel-Latif *et al*., (١٩٨٦) found that the presence of ASAs in semen of males resulted in a decrease of sperm motility and the effect of them is remaining inside the female genital tract. In the current study

agglutination of spermatozoa is reduce from ١٠.٣٣ % before activation to ٣.١٣ % and ٠.٧٦ % after activation with both simple layer and centrifugation techniques respectively. The best technique for separation of ASAs from spermatozoa is achieved by washing and centrifugation of spermatozoa which lead to sedimentation of ASAs in the pellet and release and move of motile spermatozoa to the upper portion of the medium (Ayvaliotis *et al*., ١٩٨٥).

Conclusions

١- High viscosity of semen decreased the percentage of motility, grade activity, and sperm motility index in infertile patients.

٢- Seminal plasma of infertile men showed an increase in osmolality compare with fertile men, also there are inverse relationships between semen osmolality and motility of spermatozoa.

٣- Seminal plasma MDA level, the end product of lipid peroxidation is high in infertile men and all infertile groups than that of fertile men.

٤- MDA level increases in patients with varicocele, leucocytospermia and smoking.

٥- Leucocyte determination in semen is lower with using Endtz method compare with HPF method in fertile men and all infertile groups.

٦- The motility of spermatozoa negatively correlated with increasing of leucocytes in semen.

٧- Semen quality is negatively influenced with smoking in population of infertile men.

٨- There is poor semen quality of infertile patients by varicocele.

٩- The agglutination of spermatozoa and the presence of ASAs are higher in infertile men. Also asthenozoospermia showed higher incidence of ASAs.

١٠- Hormonal evaluation of blood plasma resulted in an increase of FSH of azoospermic patients compare with fertile and severe oligozoospermic men.

١١- High improvement of semen quality is observed in patients with asthenozoospermia after activation with modified balanced Earle's medium and using both simple layer and centrifugation-swim up techniques.

Recommendations

١. Evaluation of some enzymes and markers of prostate and seminal vesicles such as fructose, zinc, α glycosidase, and prostate specific antigen which is strongly related to infertility and dysfunction of these accessory sex glands is very important.

٢. Treatment of hyper-viscous samples with proteolytic enzymes which could result in improved sperm motility and render the samples more suitable for IUI.

٣. The infertile patients which suffer from high semen osmolality, we recommended to transfer the semen of them to a medium with lower and within the normal ranges of osmolality to improve the spermatozoal

motility, this is lead to increase the chance of fertilization when IUI or IVF is done to them.

٤. Treatment of patients with high (ROS) , to decrease the conc. of MDA which may be lead to decrease the percentage of spermatozoal DNA damage , hence increases the chance of pregnancy when IUI, IVF, or ICSI are perform.

٥. Endtz method should be done instead of HPF for determination of semen leucocytes to differentiate between true leucocytes and other round cells found in the semen.

٦. We hopping that IVF, ICSI, gametocyte Intrafallopian Transfer (GIFT) to be carried out in this region for unexplained infertility cases, oligozoospermic, asthenozoospermic, and teratozoospermic, also to help the hopeless infertile couples.

References

Abdel-Latif, A., Mathur, S., Rust, P.F., Fredericks, C.M., Abdel-Latif, H. and Williamson, H.O. (١٩٨٦). Cytotoxic sperm antibodies inhibit sperm penetration of zona-free hamster eggs. *Fertil Steril*, ٤٥: ٥٤٢-٥٤٨.

Adeghe, J.H.A. (١٩٩٢). Male subfertility due to sperm antibodies: a clinical overview. *Obstet. Gynecol. Surv.*, ٤٧: ١-٨.

Agarwal, A. and Said. T.M. (٢٠٠٥). Oxidative stress, DNA damage and apoptosis in male infertility: *a clinical approach. BJU International*, ٩٥: ٥٠٣-٥٠٧.

Agarwal, A., Nallella, K.P., Allamaneni, S.S. and Said, T.M. (٢٠٠٤). Role of antioxidant in treatment of male infertility: an overview of the literature. *Reprod. Biomed. Online*, ٨: ٦١٦-٦٢٧.

Agarwal, A., Saleh, R.A. (٢٠٠٢). Role of oxidants in male infertility: rational, significance, and treatment. *Urol. Clin. North Am.*, ٢٩: ٨١٧-٨٢٧.

Agarwal, A., Saleh, R.A. and Bedaiwy, M.A. (٢٠٠٣). Role of reactive oxygen species in the pathophysiology of human reproduction. *Fertil Steril*, ٧٩: ٨٢٩-٨٤٣.

Aitken, R.J. (١٩٩٩). The Amoroso lecture. The human spermatozoa- a cell in crises. *J. Reprod. Fertil*, ١١٥: ١-٧.

Aitken, R.J., Best, F.S.M., and Richardson, D.W. (١٩٨٢). An analysis of semen quality and sperm function in cases of oligospermia. *Fertil Steril*, ٣٧: ٧٠٥-٧١١.

Aitken, R.J., Buckingham, D.W. and West, K.M. (١٩٩٢). Reactive oxygen species and human spermatozoa: analysis of the cellular mechanisms involved in luminal and leuciginin- dependent chemiluminescence. *J. Cell. Physiol.*, ١٥١: ٤٦٦-٤٧٧.

Aitken, R.J., Fisher, H.M., Fulton, N., Gomez, E., Knox, W., Lewis, B. and Irvine, S. (١٩٩٧). Reactive oxygen species generation by human spermatozoa is induced by exogenous NADPH and inhibited by flavoprotein inhibitors diphenylene iodinium and quinacrine. *Mol. Reprod. Dev.*, ٤٧: ٤٦٨-٤٨٢.

Al-Dujaily, S.S. (٢٠٠٥). Detecting leucocytospermia by myeloperoxidase staining method for patients of Baghdad IVF Institute. *Journal of Babylon Medicine*, ٢(٢); ٨-١٢.

Al-Hady, F.N. (١٩٩٧). Use of double layer centrifugation technique for *in vitro* sperm activation of asthenozoospermic patients (Ph.D.Thesis, college of science, University of Baghdad)

Al-Janabi, F.S.D. (١٩٩٢). Male infertility factors: the effect of female serum on sperm activation *in vitro* intrauterine insemination. (M.Sc thesis, college medicine. University of Baghdad, ٦٧-٧٦).

Allen, G.M. and Boune, J. (١٩٧٨). Interaction of immunoglobulin fragments with the mammalian sperm acrosome. *J. Exp. Zool.*, ٢٠٣: ٢٧١-٢٧٦.

Al-Sultani, Y.K. (١٩٩٧). *In vitro* sperm activation of oligospermic and leucocytosperemic patients by using media and gonadotropic hormones. (Ph.D. Thesis, college of Science, University of Baghdad).

Al-Taee, H.A.J. (١٩٩٤). Sperm activation and intrauterine insemination : the effect of serum concentrations and culture media on sperm activation

potential *in vitro*. (MSc. Thesis, college of Medicine, Universityof Al-Mustansuriah ٩٢-٩٥).

Alwachi, S.N. (٢٠٠٢). Human physiology. Daralfiker Publishing Company, Amman, Jordan.

Alwachi, S.N., and Al-Shakarchi, T.K. (١٩٨٧). The effect of different doses of $PGF_{٢}\alpha$ of male reproductive system of mice with different ages. *J.Biol. Sci.Res*, ١٨:٥٧-٦٣.

Alwachi, S.N. and Al-Saadi, H.K.Z. (٢٠٠٢). Effect of $PGF_{٢}\alpha$ on spermatogenesis in Albino mice. *Journal of Babylon Univ.*, ٢:٦٨٦-٦٩١.

Alwachi, S.N., Al-Kobaisi, M.N., Mahmoud, F.A. and Zhaid, Z.R. (١٩٨٦). Possible effect of nicotine on the spermatogenesis and testicular activity of the mature Albino mice. *J.Biol.Sci.Res.* ١٧:١٨٥-١٩٥.

Amelar, R.D., Dubin, L. and Schoenfeld, C.Y. (١٩٨٠). Sperm motility. *Fertil Steril*, ٣٤: ١٩٧-٢١٥.

Arabi, M. and Moshtagi, H. (٢٠٠٥). Influence of cigarette smoking on spermatozoa via seminal plasma. *Andrologia*, ٣٧:١١٩-١٢٤.

Arata de Bellabarba, G., Tortolero, I, Villarroel, V., Molina, C.Z., Belabarba, C. and Velazqueze, E. (٢٠٠٠). Nonsperm cells in human semen and their relationship with semen parameters. *Archives of Andrology*, ٤٥: ١٣١-١٣٦.

Ayvaliotis, B., Bronson, R., Rosenfeld, D. and Cooper, B. (١٩٨٥). Conception rates in couples where autoimmunity of sperm is detected . *Fertil Steril*, ٤٣: ٧٣٩-٧٤٢.

Aziz, N., Saleh, R., Sharma, R. Lewis-Jones, I., Esfandiari, N. Thomas, A.J. and Agarwal, A. (٢٠٠٤). Novel association between sperm reactive oxygen species production, sperm morphological defects, and the sperm deformity index. *Fertil Steril*, ٨١: ٣٤٩-٣٥٤.

Barbieri, E.R., Hidalgo, M.E., Venegas, A., Smith, R. and Lissi, E.A.(١٩٩٩). Varicocele-associated decrease in antioxidant defenses. *Journal of Andrlogy*, ٢٠: ٧١٣-٧١٧.

Barratt, C., Tomlinson, M.J., and Cooke, I.D. (١٩٩٣). Prognostic significance of computerized motility analysis for *in vivo* fertility.Fertil Steril, ٦٠:٥٢٠-٥٢٥.

Beer, A.E. and Neaves, W.B. (١٩٧٨). Antigenic status of semen from the viewpoints of the female and male. *Fertil Steril*, ٢٩: ١٠٧-١١٠.

Benoff, S., Cooper, G.W., Hurley, I., Mandel, F.S. and Rosenfeld, D.L. (١٩٩٣). Antisperm antibody binding to human sperm inhibits capacitation induced changes in the levels of plasma membrane sterols. *Am. J. Reprod. Immunol.*, ٣٠: ١١٣-١٣٠.

Blake, D.R., Allen, R.E. and Lunec, J. (١٩٨٧). Free radical in biological systems-a review oriented to inflammatory processes. Br. Med. Bull., ٤٣: ٣٧١-٣٨٥.

Braedel, H.U., Steffens, J., Ziegler, M., Polsky, M.S., and Platt, M.L. (١٩٩٤). A possible ontogenic etiology for idiopathic left varicocele. *J. Urol.*, ١٥١: ٦٢-٦٦.

Bronson, R., Cooper, G. and Rosenfeld, D. (١٩٨٤). Sperm antibodies: their role in infertility. *Fertil Steril*, ٤٢: ١٧١-١٨٣.

Bunge, R.G. (١٩٧٠). Some observations on the male ejaculate. *Fertility and Sterility*, ٢١: ٦٣٩-٦٤٤.

Butt, W.R. and Blunt, S.M. (١٩٨٨). The role of laboratory in the investigation of infertility. *Ann. Clin. Biochem.*, ٢٥: ٦٠١-٦٠٩.

Calamera, J., Buffone, M., Ollero, M., Alvarez, J., Doncel, G.F. (٢٠٠٣). Superoxide dismutase content and fatty acid composition in subsets of human spermatozoa from normozoospermic, asthenozoospermic, and polyzoospermic semen samples. *Mol. Reprod. Dev.*, ٦٦:٤٢٢-٤٣٠.

Campana, A., deAgostini, A., Bischof, P., Tawfig, E. and Mastroilli, A. (١٩٩٥). Evaluation of infertility. *Hum. Reprod. Update*, ١: ٥٨٦-٦٠٦.

Cardoso, E.M., Santoianni, J.E., and De-Paulis, A.N. (١٩٩٨). Improvement of semen quality in infected asymptomatic infertile male after bacteriological cure. *Medicina-B-Aires*, ٥٨(٢): ١٦٠-١٦٤.

Carlsen, E., Giwercman, A., Keiding, N. and Shakkebaek, N.E. (١٩٩٢). Evidence for decreasing quality of semen during past ٥٠ years. *Brit. Med. J.*, ٣٠٥: ٦٠٩-٦١٣.

Carpino, A. and Siciliano, L. (١٩٩٨). Unaltered protein pattern/genital tract secretion marker levels in seminal plasma of highly viscous human ejaculates. *Archives of Andrology*, ٤١:٣١-٣٥.

Carrell, D., Wilcox, A., Lowy, L., Peterson, C.M., Jones, K.P., Erickson, L., Campbell, B., Branch, D.W. and Hatasaka, H.H. (٢٠٠٣). Elevated sperm chromosome aneuploidy and apoptosis in patients with unexplained recurrent pregnancy loss. *Obstet. Gynecol.*, ١٠١: ١٢٢٩-١٢٣٥.

Casslen, B. and Nilsson, B. (١٩٨٤). Human uterine fluid, examined in undiluted samples for osmolarity and the concentrations of inorganic ions, albumin, glucose and urea. *Am. J. Obstet. Gynecol.*, ١٥٠: ٨٧٧-٨٨١.

Cheeck, J.H., Zavos, P.M., Katsoff, D., and Kiefer, D. (١٩٩٣). Effects of percoll discontinuous density gradients vs. saphedeax G-٥٠ gel filtration on sperm parameters. *J.Exp.Mead.*, ١٦٩:٢٢٥-٢٣١.

Chi, S.E., Xu, B., Ong, C.N., Tsakok, F.M. and Lee, S.T. (١٩٩٤). Effect of cadmium and cigarette smoking on human sperm quality. *Int. J. Fertil Menopausal Stud.*, ٣٩: ٢٩٢-٢٩٨.

Chia, S-E., Lim, S-T. A., Tay, S-K., and Lim, S-T. (٢٠٠٠). Factors associated with male infertility: a case-control study of ٢١٨ infertile and ٢٤٠ fertile men. *BJOG.* ١٠١,*(١)*: ٥٥-٦١.

Close, C.E., Roberts, P.L., and Berger, R.E. (١٩٩٠). Cigarettes, alcohol and marijuana are related to pyospermia in infertile men. *Journal Urology*, ١٤٤:٩٠٠-٩٠٣.

Cockett, A.T.K., Takihara, H.; and Constentino, M.J. (١٩٨٤). The varicocele. *Fertil Steril*, ٤١: ٥-١١.

Comhaire, F. and Vermeulen, L. (١٩٩٥). Human semen analysis. *Hum. Reprod. Update*, ٤: ٣٤٢-٣٦٢.

Comhaire, F., Verschraegen, G. and Vermeulen, L. (١٩٨٠). Diagnosis of accessory gland infection and its possible role in male infertility. *Int.J. Androl.*, ٣:٣٢-٤٥.

Confino, J.M., Friberge, J., Dudkiewicz, A.B. and Gleicher, N. (١٩٨٦). Intrauterine insemination with washed human spermatozoa. *Fertil Steril*, ٤٦: ٥٥-٦٠.

Cooper, T.G., and Yeung, C.H. (٢٠٠٣). Acquisition of volume regulatory response of sperm upon maturation in the epididymis and the role of the cytoplasmic droplet. *Microsc. Res. Techn.*, ٦٠.

Curtis, J. (١٩٩٩). Textbook of prostatitis. Isis medical media, UK.

Daitch, J.A., Bedaiwy, M.A., Pasqualotto, E.B., Hendin, B.N., Hallak, J., Falcone, T., Thomas, A.J., Nelson, D.R., and Agarwal, A. (٢٠٠٠). Varicocelectomy improves intrauterine insemination success rates among men with varicoceles. *J. Urol.*, ١٦٥: ١٥١٠-١٥١٣.

Dale-McClure, R. (١٩٨٦). Topics in primary care medicine. *West. J. Med.*, ١٤٤: ٣٦٥-٣٦٨.

Dandekar, S.P., Nadkarni, G.D., Kulkarni, V.S. and Punekar, S. (٢٠٠٢). Lipid peroxidation and antioxidant enzymes in male infertility. *J.Postgrade Med.*, ٤٨: ١٨٦-١٨٩.

Darley-Usmer, V., Wiseman, H. and Halliwell. (١٩٩٥). Nitric oxide and oxygen radicals: a question of balance. *FEBS. Letters*, ٣٦٩: ١٣١-١٣٥

De Almeida, M., Zouari, R., Jouannet, P. and Feneux, D. (١٩٩١). In vitro effects of antisperm antibodies on human sperm movement. *Hum. Reprod.*, ٦: ٤٠٥-٤١٠.

de Lamirande, E. and Gagnon, C. (١٩٩٥). Impact of reactive oxygen species on spermatozoa: a balancing act between beneficial and detrimental effects. *Hum. Reprod.*, ١٠: ١٥-٢١.

Diemer, T., Huwe, P., Ludwig, M., Schroeder-Printzen, I., Michelmann, H., Schiefer, H., and Weidner, W. (٢٠٠٣). Influence of autogenous leucocytes and *Escherichia coli* on sperm motility parameters *in vitro*. *Andrlogia*, ٣٥: ١٠٠-١٠٥.

Dubin, L. and Amelar, R.D. (١٩٧٧). Varicocelectomy: ٩٨٦ cases in a edition. A Lang medical book.

Edwards, R.G. (١٩٧٤). Follicular fluid. *Journal of Reproduction and Fertility*, ٣٧: ١٨٩-٢١٩.

Eggert-Kruse, W., Huber, K., Rohr, G., and Runnebaum, B. (١٩٩٣). Determination of antisperm antibodies in serum samples be means of enzyme-linked immunosorbant assay- a procedure to be recommended during infertility investigation. *Hum. Reprod.*, ٩ :١٤٠٥-١٤١٣.

El-Demiry, M.I., Hargreave, T.B., Busuttil, A., Elton, R., James, K. and Chrisholm, G. (١٩٨٧). Immunocompetent cells in human testis in health and disease. *Fertil Steril*, ٤٨:٤٧٠-٤٧٩.

Eliasson, R. (١٩٧٠). Correlation between the sperm density, morphology and motility and secretory function of the accessory genital glands. *Andrologia*, ٢: ١٦٥-١٧٠.

Eliasson, R. and Lindholmer, C. (١٩٧٢). Distribution and properties of spermatozoal in different fractions of split ejaculate. *Fertility and Sterility*, ٢٣: ٢٥٢-٢٥٧.

Elzanaty, S., Malm, J. and Giwercman, A. (٢٠٠٤). Visco-elasticity of seminal fluid in relation to the epididymal and accessory sex gland function and its impact on sperm motility. *International Journal of Andrology*, ٢٧: ٩٤-١٠٠.

Endtz, A.W. (١٩٧٤). A rapid staining method for differentiating granulocyte from germinal cells in papanicolaou-stained semen. *Acta Cytologica*, ١٨ : ٢-٧.

Fedder, J., Askjaer, S.A., and Hjort, T. (١٩٩٣). Non-spermatozoal cells in semen: relationship to other semen parameters and fertility status of the couple. *Arch. Androl.*, ٣١: ٩٥-١٠٣.

Fraczek, M., Sanocka, D. and Kurpisz, M. (٢٠٠٤). Interaction between leucocytes and human spermatozoa influencing reactive oxygen intermediates release. *International Journal of Andrology*, ٢٧:٦٩-٧٥.

Fraczek, M.; Szkutnik, D.; Sanocka, D. and Kurpisz, M. (٢٠٠١). Peroxidation components of sperm lipid membranes in male infertility. *Ginekologia Polska*, ٧٢: ٧٣-٧٩.

Franken, D.R., Oehninger, S., Burkman, L.J., Coddington, C.C., Kruger, T.F., Rosenwaks, Z., Acosta, A.A., and Hodgen, G.D. (١٩٨٩). The Hemizona Assay (HZA): A predictor of human sperm fertilizing potential in *in vitro* fertilization (IVF) treatment. *J. Vitro Fert. Embryo Trensfer*, ٦:٤٤-٥٦.

Franks, S., Jacobs, H.S., Martin, N., Nabarro, J.D. (١٩٧٨). Hyperprolactinemia and impotence. *Clin. Endocrinol.*, ٨: ٢٧٧- ٢٨٧.

Freiha, F. and Mroueh, A. (١٩٧٦). Varicocele and infertility in men. *West J. Med.*, ١٢٥: ٤٣١- ٤٣٣.

Freund, J.E. (١٩٨١). Statistics: A first course. ٣rd edition. Prentice-Hall. INC, New Jersey.

Friberge, J. (١٩٧٤). A Simple and sensitive micr-omethod for determination of sperm agglutinating-activity in serum from infertile men and women. *Acta. Obstet. Gynecol. Scan. Suppl.*, ٣٦:٢١-٢٩.

Fujii, J., Iuchi, Y., Matsuki, S. and Ishii, T. (٢٠٠٣). Cooperative function of antioxidant and redox systems against oxidative stress in male reproductive tissues. *Asian J. Androl.*, ٥: ٢٣١-٢٤٢.

Furuya, S., Kumamoto, Y., and Sugiyama, S. (١٩٧٨). Fine structure and development of Sertoli junction in human testis. *Arch. Androl.*, ١ : ٢١١-٢١٩.

Ganong, W.F. (١٩٩٥). Review of medical physiology. Seventeenth edition. a Lanch medical book .

Garrido, N., Meseguer, M., Simon, C. Pellicer, A. and Remohi, J. (٢٠٠٤). Pro-oxidative and anti-oxidative imbalance in human semen and its relation with male infertility. *Asian J. Androl.*, ٦: ٥٩-٦٥.

Gersh, I. (١٩٧٠). Pancreatic dornase for liquefaction of viscid human semen. *Fertility and Sterility*, ٢١: ١٤٧-١٥٠.

Giblin, P.T., Ager, J.W., Poland, M.L., Olsen, J.M. and Moghsji, I.S. (١٩٨٨). Effect of stress and characteristics adaptability on semen quality in healthy men. *Fertil Steril*, ٤٩:١٢٧-١٣٢.

Gil-Guzman, E., Ollero, M., Lopez, M.C., Sharma, R.K., Alvarez, J.G., Thomas, A.J., and Agarwal, A. (٢٠٠١). Differential production of reactive oxygen species by subsets of human spermatozoa at different stages of maturation. *Hum. Reprod.*, ١٦: ١٩٢٢-١٩٣٠.

Glass, R.H. and Mrouch, A. (١٩٦٧). The post-coital test and semen analysis. *Fertility and Sterility*, ٢١: ٣١٤-٣١٧.

Gomez, E., Irvine, D.S. and Aitken, R.J. (١٩٩٨). Evaluation of a spectrophotometric assay for the measurement of malondialdehyde and ٤-hydroxyalkenals in human spermatozoa: relationships with semen quality and sperm function. *Int. J. Androl.*, ٢١: ٨١-٩٤.

Gonzales, G.F., Kortebani, G. and Mazzoli, A.B. (١٩٩٣). Hyper-viscosity and hypo-function of the seminal vesicles. *Archives of Andrology*, ٣٠: ٦٣-٦٨.

Gopalkrishnan, K., Hinduja, I.N. and Kumar, T.C. (١٩٩٢). Volume of semen as a parameter of its quality. *Indian J. Med. Res.*, ٩٢: ٣٦١ – ٣٦٥ (Abstract).

Gopalkrishnan, K., Padwal, V. and Balaiah, D. (٢٠٠٠). Does seminal fluid viscosity influence sperm chromatin integrity. *Archives of Andrology*, ٤٥: ٩٩-١٠٣.

Griveau, J.F. and Le Lannou, D. (١٩٩٧). Reactive oxygen species and human spermatozoa: Physiology and pathology. *Int. J. Androl.*, ٢٠: ٦١-٦٩.

Guidet, B. and Shah, S.V. (١٩٨٩). Enhanced in vivo H_2O_2 generation by kidney in glycerol-induced renal failure. *Am. J. Physiol.*, ٢٥٧(٢٦): ٤٤٠-٤٥٠.

Haas, G.G. (١٩٨٦). The inhibitory effect of sperm associated immunoglobulin on cervical mucus penetration. *Fertil Steril*, ٤٦:٣٣٤-٣٣٧.

Haas, G.G.; Schreiber, A.D. and Blasco, L. (١٩٨٣). The incidence of sperm associated immunoglobulin and C٣, the third component of complement, in inferile men. *Fertil Steril*, ٣٩: ٥٤٢-٥٤٧.

Hafez, B., Goff, L., and Hafez, S. (١٩٩٧). Recent advanced in andrology research:physiology and clinical application to fertility and infertility. *Arch. Androl*, ٣٩(٣): ١٧٣-١٧٩.

Hendin, B., Kolettis, P., Sharma, R.K., Thomas, A.J. and Agarwal, A. (١٩٩٩). Varicocele is associated with elevated spermatozoal reactive oxygen species production and diminished seminal plasma antioxidant capacity. *J. Urol.*, ١٦١: ١٨٣١-١٨٣٤.

Hendry, W.F. (١٩٩٢). Effects of left varicocele ligation in subfertile males with absent or atrophic right testes. *Fertil Steril*, ٥٧: ١٣٤٢- ١٣٤٣.

Hicks, J.J., Perdon, N., and Rosado, A. (١٩٧٢). Modification of human spermatozoa glycolysis by cyclic adenosine monophosphate (Camp), estrogen and follicular fluid. *Fertil Steril*, ٢٣:٨٨٦-٨٩٠.

Hinting, A. (١٩٨٩). Mechanism of male immunity to spermatozoa. Thesis submitted in fulfillment of the requirement for the degree of special doctor.

Hinton, B.T., Pryor, J.P., Hirsh, A.V. and Setchell, B.P. (١٩٨١). The concentration of some inorganic ions and organic compounds in the luminal fluid of the human ductus deferens. *Int. J. Androl.*, ٤: ٤٥٧-٤٦١.

Hoing, L.M., Devroey. P., and Van-Steirtegham, A.C. (١٩٨٦). Treatment of infertility because of oligoasthenoteratozoospermia by transcervical intrauterine insemination of motile spermatozoa. *Fertil Steril*, ٤٥:٣٨٨-٣٩١.

Hunter, J., Logan, H., and Greer, G. (١٩٧٦). Incidence of sperm antibodies before and after vasectomy. *J. Clin. Path.*, ٢٩:١١٢٧-١١٢٩.

Huszar, G., Sbracia, M., Vigue, L., Miller, D. and Shur, B. (١٩٩٧). Sperm plasma membrane remodeling during spermiogenic maturation in men: relationship among plasma membrane beta ١, ٤-galactosyltransferase, cytoplasmic creatine phosphokinase and creatine phosphokinase isoform ratios. *Biol. Reprod.*, ٥٦: ١٠٢٠-١٠٢٤.

Islam, N. and Trainer P.J. (١٩٩٨). The hormonal assessment of the infertile male. *British Journal of Urology*, ٨٢: ٦٩-٧٥.

Ismaeel, H.O. (٢٠٠١). A microbiological and serological study on some male infertility causes in Arbil city. (M.Sc.Thesis, college of science, University of Salahaddin).

Isojima, S. (١٩٨٨). Recent advances in definding human seminal plasma antigens using monoclonal antibodies. *Am. J. Reprod. Immunol.*, ١٢: ١٥٠-١٥٥.

Jarrow, J.P., and Sanzone, J.J. (١٩٩٢). Risk factors for male partner antisperm antibodies. *J. Urol.*, ١٤٨: ١٨٠٥-١٨٠٧.

Jequier, A., and Crich, J. (١٩٨٦). Semen analysis, a practical guide. ١st edit. Blackwell Scientific Pub, London, pp: ١٠-٩٠.

Jones, W.R. (١٩٨٠). Immunologic infertility-fact or fiction. *Fertil Steril*, ٣٣: ٥٧٧-٥٨٦.

Joyce, D.A. (١٩٨٧). Oxygen radicals in disease. *Advers Drug Reaction Bull*, ١٢٢: ٤٧٦-٤٧٩.

Jung, A.; Schill, W. and Schuppe, H. (٢٠٠٥). Improvement of semen quality by nocturnal scrotal cooling in oligozoospermic men with a history of testicular maldescent. *International Journal of Andrology*, ٢٨: ٩٣-٩٨.

Kanwar, K.C.; Yanagimachi, R.; and Lopata, A. (١٩٧٩). Effects of human seminal plasma on fertilizing capacity of human spermatozoa. *Fertil Steril*, ٣١: ٣٢١-٣٢٧.

Katz, D.F., Overstreet, J.W., Samuels, S.J., Niswander, P.W., Bloom, T.D., and Lewis, E.L. (١٩٨٦). Morphologic analysis of spermatozoa in the assessment of human male infertility. *J. Androl.*, ٧:٢٠٣-٢١٠.

Keskes-Ammar, L., Feki-Chakroun, N., Rebai, T., Sahnoun, Z., Ghozzi, H., Hammami, B., Zghal, K., Fki, H., Damak,J., and Bahloul, A. (٢٠٠٣). Sperm oxidative stress and the effect of an oral vitamin E and selenium supplement on semen quality in infertile men. *Arch. Androl.*, ٤٩: ٨٣-٩٤.

Kessopoulou, E., Tomlinson, M.J. , Barrat, C.L., Bolton, A.E., and Cook, I.D. (١٩٩٢). Origin of reactive oxygen species in human semen spermatozoa or leucocytes. *J. Reprod. Fertil.*, ٩٤: ٤٦٣-٤٧٠.

Khosrowbeygi, A., Zarghami, N. and Delder, Y. (۲۰۰٤). Correlation between sperm quality parameters and seminal plasma antioxidant status. *Iranian Journal of Reproductive Medicine*, ۲(۲): ٥۸-٦٤.

Kiessling, A., Lamparelli, N., Yin, H., Seibel, M. and Eyre, R. (۱۹۹٥). Semen leukocytes : friends or foes. *Fertility and Sterility*, ٦٤: ۱۹٦-۱۹۸.

Kodama, H., Yamaguchi, R., Fukuda, J., Kasai, H. and Tanaka, T. (۱۹۹۷). Increased oxidative deoxyribonucleic acid damage in the spermatozoa of infertile male patients. *Fertil Steril*, ٦۸: ٥۱۹-٥۲٤.

Kondoh, N., Fujimoto, M., Takeyama, M., Nakamura, Y., Kitamura, M., Matsumiya, K., and Okuyama, A. (۱۹۹۹). Treatment of azoospermic patient with genitourinary tuberculosis: a case report. *Hinyokika-Kiyo*, ٤٥(۳): ۱۹۹-۲۰۱.

Kruger, T.F., Menkveld, R., Stander, F.S.H., Lombard, C.J., Van der Merwe, J.P., Van Zyl, J.A., and Smith, K. (۱۹۸٦). Sperm morphologic features as a prognostic factor in *in vitro* fertilization. *Fertility and Sterility*, ٤٦: ۱۱۱۸-۱۱۲۳.

Kunathikom, S., Worasatit, C. and Toongsuwan, S. (۱۹۹٥). Relationship between the direct mixed antiglobulin reaction (MAR) test and spontaneous sperm agglutination in men from infertile couples. *J. Med. Assoc. Thai.*, ۷۸(۲): ۸۹-۹۳.

Kunzle, R., Mueller, M.D., Hanggi, W., Birkhauser, M.H., Drescher, H. and Bersinger, N.A. (۲۰۰۳). Semen quality of male smokers and non-smokers in infertile couples. *Fertil Steril*, ۷۹: ۲۸۷-۲۹۱.

Lansford, B., Haas, G.G., DeBault, L.E. and Wolf, D.P. (۱۹۹۰). Effect of sperm associated antibodies on the acrosomal status of human sperm. *J. Androl.*, ۱۱: ٥۳۲-٥۳۸.

Leon, S., Glass, R.H. and Kase, N.G. (۱۹۹۹). Clinical gynecologic endocrinology and infertility. ٦th ed. Lippincott Williams and Witkins, Maryland, pp: ۱۰۷٥-۱۰۹۰.

Levin, R.M., Brown, M.H., Bell, M., Shue, F., Grenberg, G.N., and Bordson, B.L. (۱۹۹۲). Air-conditional environments do not prevent deterioration of human semen quality during the summer. *Fertil Steril*, ٥۷: ۱۰۷٥-۱۰۸۳.

Lilja, H. (۱۹۸٥). A kallikrein-like serine protease in prostatic fluid cleaves the predominant seminal vesicle protein. *Journal of Clinical Investigation*, ۷٦: ۱۸۹۹-۱۹۰۳.

Linnet, L. and Suominen, J.J. (۱۹۸۲). A comparison of eight techniques for the evaluation of the auto-immune response to spermatozoa after vasectomy. *Journal of Reproductive Immunology*, ٤(۳): ۱۳۳-۱٤٤.

Lund, L. and Nielsen, K.T. (١٩٩٦). Varicocele testis and testicular temperature. *British Journal of Urology*, ٧٨: ١١٣-١١٥.

Lundwall, A., Bjartell, A., Olsson, A.Y. and Malm, J. (٢٠٠٢). Semenogelin I and II, the predominant human seminal plasma proteins, are also expressed in non-genital tissues. *Molecular Human Reproduction*, ٨: ٨٠٥-٨١٠.

Lunec, J. (١٩٩٠). Review Article. *Ann. Clin. Biochem.*, ٢٧: ١٧٣.

Madger, I., Weissenberg, R., Lunenfield, B., Karasik, A., and Goldwasser, B. (١٩٩٥). Controlled trial of high spermatic vein ligation for varicocele in infertile men. *Fertil Steril*, ٦٣: ١٢٠-١٤٢.

Mahandevan, M.M., and Trounson, A.O. (١٩٨٤). The influence of seminal characteristics on the success rate of human *in vitro* fertilization. *Fertile Sterile* ٤٣:٤٠٠-٤٠٥.

Makler, A., Itskovitz, J., Brandes, J.M. and Paldi, E. (١٩٧٩). Sperm velocity and percentage of motility in ١٠٠ normospermic specimens analyzed by the MEP method. *Fertil Steril*, ٣٥: ١٥٥-١٦١.

Marks, J.L.; McMahon, R. and Lipshultz, L.I. (١٩٨٦). Predective parameters of successful varicocele repair. *J. Urol.*, ١٣٦: ٦٠٩-٦١٢.

Martin-Du Pan, R.C. (١٩٩٧). Etiology of male infertility and Oligo-Astheno-Teratospermia (OAT). *Arch. Androl.*, ٣٩: ١٩٧.

Mendeluk, G.R., Munuce, M.J., Carriza, C., and Bregni, C. (١٩٩٧). Sperm motility and ATP content in seminal hyperviscosity. *Archives of Andrology*, ٣٩: ٢٣٣-٢٧٧.

Menezo, Y., Testart, J., Thebault, A., Frydman, R. and Khatchadourian, C. (١٩٨٢). The preovulatory follicular fluid in the human; influence of hormonal pretreatment (clomiphene- hCG) on some biochemical and biophysical variables, *International Journal of Infertility*, ٢٧: ٤٧-٥١.

Menkveld, R., Stander, F.S.H., Kotz, T.J.V., Kruger, T.F. and Van Zyl, J.A. (١٩٩٠). The evaluation of morphological characteristics of human spermatozoa according to stricter criteria. *Human Reproduction*, ٥:٥٨٦-٥٩٢.

Menkveld, R., Swanson, R.J., Kotze, T.J., and Kruger, T.F. (١٩٩٠). Comparison of a discontinuous percoll gradient method versus a swim-up method: effects on sperm morphology and other semen parameters. *Andrologia*, ٢٢: ١٥٢-١٥٨.

Merino,G. , Lira, S.C. , and Mareinez-Chequer, J.C. (١٩٩٨). Effects of cigarette smoking on semen characteristics of a population in Mexico. *Arch. Androl.*, ٤١: ١١-١٥.

Meucci, E., Milardo, D., Mordente, A., Martorana, G.E., Giacchi, E., Marinis, L., and Mancini, A. (٢٠٠٣). Total antioxidant capacity in patients with varicoceles. *Fertil Steril*, ٣: ١٥٧٧-١٥٨٣.

Micic, S., Dotlic,R. ; Ilic,V. and Genbacev, O. (١٩٨٦). Seminal plasma hormone profile in infertile men with and without varicocele. *Arch. Androl.*, ١٧ (٣): ١٧٣ – ١٧٨.

Ming-Chung, L., Tsong-Chang, T. and Yu-Shih, Y. (١٩٩٢). Measurement of viscosity of the human semen with a rotational viscometer. *Journal of Formosan Medical Association*, ٩١: ٤١٩-٤٢٣.

Montagnon, D., Clavert, A., and Cranz, C. (١٩٨٢). Fructose, proteins and coagulation in human seminal plasma. *Andrologia*, ١٤:٤٣٤-٤٣٩.

Mostafa, T., Anis, T.H., El-Nashar, A., Imam, H., Othman, I.A. (٢٠٠١). Varicocelectomy reduces reactive oxygen species levels and increases antioxidant activity of seminal plasma from infertile men with varicocele. *International Journal of Andrology*, ٢٤: ٢٦١-٢٦٥.

Mruk, D.D., Silvestrini, B., Mo, M.Y. and Cheng, C.Y. (٢٠٠٢). Antioxidant superoxide dismutase – a review: its function, regulation in the testes, and role in male fertility. *Contraception*, ٦٥: ٣٠٥-٣١١.

Muslih, R.D., Al-Nimer, M.A. and Al-Zamely, O.M. (٢٠٠٢). The level of malondialdehyde after activation with (H_2O_2 and $CuSO_4$) and inhibited by Desferoxamine and Molsidomine in the serum of patients with acute myocardial infarction . *National Journal of Chemistry*, ٥: ١٤٩-١٤٨.

Nakamura, H., Kimura, T., Nakajima, A., Shimoya, K., Takemura, M., and Hashimoto, K. (٢٠٠٢). Detection of oxidative stress in seminal plasma and fractionated sperm from subfertile male patients. *Eur. J. Obstet. Gynecool. Reprod. Biol.*, ١٠٥: ١٥٥-١٦٠.

Naughton,C.K., Nangia, A.K. and Agarwal, A. (٢٠٠١). Pathophysiology of varicoceles in male infertility. *Human Reproduction Update*, ٧(٥):٤٧٣- ٤٨١.

Naz, R.K. and Menge, A.C. (١٩٩٤). Antisperm antibodies: origin, regulation, and sperm reactivity in human infertility. *Fertil Steril*, ٦١: ١٠٠١-١٠١٣.

Nunez-Calonge, R., Caballero, P. and Redondo, C. (١٩٩٨). Ureaplasma uralyticum reduces motility and induces membrane alterations in human spermatozoa. *Hum. Reprod.*, ١٣: ٢٧٥٦-٢٧٥٨.

Ochsendorf, R.F. (١٩٩٩). Infections in the male genital tract and reactive oxygen species. *Hum. Reprod.*, ٥: ٣٩٩-٤٢٠.

Oeda, T., Henkel, R., Ohmori, H. and Scill, W.B. (١٩٩٧). Scavenger effect of N-acetyl-L-cysteine against reactive oxygen species in human semen: a

possible therapeutic modality for male factor infertility. *Andrologia*, ٢٩: ١٢٥-١٣١.

Okonofua, F., Menakaya, U., Onemu, S.O., Omo-Aghoja, L.O., and Bergstrom, S. (٢٠٠٥). A case-control study of risk factors for male infertility in Nigeria. *Asian J. Andrology*, ٧(٤): ٣٥١-٣٦١.

Ombelet, w., Vandeput, H., Janssen, M., Cox, A., Vossen, C., Pollet, H., Steeno, O. and Bosmans, E. (١٩٩٧). Treatment of male infertility due to sperm surface antibodies: IUI or IVF. *Human Reproduction*, ١٢ (٦): ١١٦٥-١١٧٠.

Padron, O.F., Brackett, N.L., Sharma, R.K., Lynnc, C.M., Thomas, A.J., and Agarwal, A. (١٩٩٧). Seminal reactive oxygen species, sperm motility and morphology in men with spinal cord injury. *Fertil Steril*, ٦٧: ١١١٥-١١٢٠.

Pakrashi, A. and Chatterjee, S. (١٩٩٥). Effect of tobacco consumption on the function of male accessory sex glands. *Int.J. Androl.*, ١٨: ٢٣٢-٢٣٦.

Pal, P.C., Rajalakshmi, M., Manocha, M., Sharma, R.S., Mittal, S., and Rao, O.N. (٢٠٠٦). Semen quality and sperm functional parameters in fertile Indian men . *Andrologia*, ٣٨: ٢٠-٢٥.

Pasqualotto, F., Sharma, R., Kobayashi, H., Thomas, A., and Agarwal, A. (٢٠٠١). Oxidative stress in normozoospermic men undergoing infertility evaluation. *J. Androl.*, ٧٢: ٤٥٩-٤٦٤.

Pasqualotto, F.F., Sobreiro, B.P., Hallak, J., Eleonora, B., Pasqualotto, F.F. and Leucon, A. (٢٠٠٤). Cigarette smoking is related to a decrease in semen volume in a population of fertile men. *BJU. International*, ٩٧: ٣٢٤-٣٢٦.

Peeker, R., Abramsson, L. and Maklind, S.L. (١٩٩٧). Superoxide dismutase isoenzymes in human seminal plasma and spermatozoa. *Mol. Hum. Reprod.*, ٣: ١٠٦١-١٠٦٦.

Peters, A.J. and Coulam, C.B. (١٩٩٢). Review: sperm antibodies. *Am. J. Reprod. Immunol.*, ٢٧: ١٥٦-١٦٢.

Plante, M., de Lamirande, E. and Gagnon, C. (١٩٩٤). Reactive oxygen species released by activated neutrophils, but not by deficient spermatozoa, are sufficient to affect normal sperm motility. *Fertil Steril*, ٦٢: ٣٨٧-٣٩٣.

Polak, B. and Daunter, B. (١٩٨٤). Osmolarity of human seminal plasma. *Andrologia*, ١٦: ٢٢٤-٢٢٧.

Pollanen, P. and Cooper, T.G. (١٩٩٤). Immunology of the testicular excurrent ducts. *J. Reprod. Immunol.*, ٢٦: ١٦٧-٢١٦.

Potts, R., Newbury, C., Smith, G., Notarianni, L.J. and Jefferies, T.M. (١٩٩٩). Sperm chromatin damage associated with male smoking. *Mutation Res.*, ٤٢٣:١٠٣-١١١.

Pundey, J.A. and Anderson, D.J. (١٩٩٣). Organization of immunocompetent cells and their function in the male reproductive tract. In Griffin PD, Johnson PM eds, Local Immunity in the Reproductive Tract Tissues. New Delhi: Oxford University Press, ١٣١-١٤٥.

Redmon, J.B., Carey, P. and Pryor, J.L. (٢٠٠٢). Varicocele – the most common cause of male factor infertility. *Human Reproduction Update*, ٨ (١): ٥٣-٥٨.

Ridha-Albarzanchi, M.T., Alnaeib, Z.T.H., Alnassari, S.A.M., and Jassim, M.M. (١٩٩١). Successful live birth following human ovarian stimulation and in vitro sperm cells hyperactivation and application of sperm intrauterine transfer technique. J.*Fac.Med.Baghdad*, ٣٣:٣٢٧-٣٣٤.

Rodin, D.M., Larone, D., and Goldstein, M. (٢٠٠٣). Relationship between semen cultures, leucocytospermia, and semen analysis in men undergoing fertility evaluation. *Fertility and Sterility*, ٧٩: ١٥٥٥-١٥٥٨.

Ross, L.S. and Niederberger, C.S. (١٩٩٥). Male infertility: diagnosis and treatment. Comp. *Ther.*, ٢١: ٢٧٦-٢٨٢.

Rossato, M., Balercia, G., Lucarelli, G., Foresta, C. and Mantero, F. (٢٠٠٢). Role of seminal osmolarity in the regulation of human sperm motility. *International Journal of Andrology*, ٢٥: ٢٣٠-٢٣٥.

Rossato, M., Balercia, G., Lucarelli, G., Foresta, C. and Mantero, F. (٢٠٠٢). Role of seminal osmolarity in the reduction of human sperm motility. *Int. J. Androl.*, ٢٥: ٢٣٠-٢٣٥.

Rossato, M., Di Virgilio, F. and Foresta, C. (١٩٩٦). Involvement of osmosensitive calcium influx in human sperm activation. *Mol. Hum. Reprod.*, ٢: ٩٠٣-٩٠٩.

Rrumbullaku, L, Boci, R.; Dedja, A. and Dautaj, K. (١٩٩٨). Sperm morphology in infertile men with varicocele. ١st Balkan Symposium of Andrology, ١٢-١٤.

Rrumbullaku, L. (٢٠٠٣). Semen analysis. Geneva Foundation for Medical Education and Research.

Rumke, P. (١٩٥٤). The presence of sperm antibodies in the serum of two patients with oligozoospermia. *Vox Sang*, ٤: ١٣٥-١٤٠.

Rumke, P. and Hellinga, G. (١٩٥٩). Autoantibodies against spermatozoa in sterile men. *Am. J. Clin. Pathol.*, ٣٢: ٣٥٧-٣٦٣.

Said, T., Paasch, U., Glander, H. and Agarwal, A. (٢٠٠٤). Role of caspases in male infertility. *Hum Reprod Update* , ١٠:٣٩-٥١.

Saladin, K.S. (١٩٩٨). Anatomy and physiology. WCB McGraw- Hill.

Saleh, A. and Agarwal, A. (٢٠٠٢). Oxidative stress and male infertility: from research bench to clinical practice. *J. Androl.*, ٢٣; ٧٣٧-٧٥٢.

Saleh, R., Agarwal A., Sharma, R., Nelson, D. and Thomas, A. (٢٠٠٢). Effect of cigarette smoking on levels of seminal oxidative stress in infertile men: a prospective study. *Fertil Steril*, ٧٨: ٤٩١-٤٩٩.

Saleh, R., Agarwal, A., Sharma, R., Said, T., Sikka, S. and Thomas, A. (٢٠٠٣). Evaluation of nuclear DNA damage in spermatozoa from infertile men with varicocele. *Fertile Steril*, ٨٠: ١٤٣١-١٤٣٦.

Sanocka, D. and Kurpisz, M. (٢٠٠٤). Reactive oxygen species and sperm cells. *Reprod. Biol. Endocrinol*., (Abstract).

Saran, M., Beck-Speier, I., Fellerhoff, B., and Bauer, G. (١٩٩٩). Phagocytic killing of microorganisms by radical processes: consequences of the reaction of hydroxyl radicals with chloride yielding chlorine atoms. *Free Radic. Biol. Med.*, ٢٦: ٤٨٢-٤٩٠.

Saypol, D.C., Lipshultz, L.I. and Howards, S.S. (١٩٨٣). Varicocele. In Lipshultz, L.I. and Howards, S.S. (eds). Infertility in the male. Churchill Livingstone, New York, pp ٢٩٩- ٢١٣.

Sealfon, A.I. (١٩٩١). Theoretical and practical consideration in scrotal temperature measurement. New York: Plenum press,٩٩-١١٠.

Seaman, E., Bar-Chama, N. and Fisch, H. (١٩٩٤). Semen analysis in the clinical evaluation of infertility. *Mediguide to Urology*, ٧: ١-٨.

Seely,R.R., Stephens, T.D. and Tate, P. (١٩٩٦). Essentials of anatomy and physiology. Second edition, WCB McGraw – Hill.

Seely,R.R., Stephens, T.D. and Tate, P. (١٩٩٨).Anatomy and physiology. Fourth edition, WCB McGraw – Hill.

Segal, S., Yaffe, H., Laufer,N. and Bendavid, M. (١٩٧٩). Male hyperprolactinemia, effects on male infertility. *Fertil Steril*, ٣٢: ٥٥٦-٥٦١.

Seibel, M.M. and Zilberstain, M. (١٩٩٥). The diagnosis of male infertility by semen quality .The shape of sperm morphology. *Hum. Reprod.*, ١٠: ٢٤٧-٢٥٢.

Sergerie, M., Ouhilal, S., Bissonnette, F., Brodeur, J. and Bleau, G. (٢٠٠٠). Lack of association between smoking and DNA fragmentation in spermatozoa of normal men. *Hum. Reprod.*, ١٥: ١٣١٤-١٣٢١.

Sharma, R.K. and Agarwal, A. (١٩٩٦). Role of reactive oxygen species in male infertility. *Urology*, ٤٨: ٨٣٥-٨٥٠.

Shekarriz, M., Sharma, R.K., Thomas, A.J., and Agarwal, A. (١٩٩٥). Positive myloperoxidase staining (Endtz test) as an indicator of excessive reactive oxygen species formation in semen. *J. Assist. Reprod. Genet.*, ١٢: ٧٠-٧٤.

Shen, H-M., Chia, S-E., and Ong, C-N. (١٩٩٩). Evaluation of oxidative DNA damage in human sperm and its association with male infertility. *Journal of Andrology*, ٢(٦):٧١٨-٧٢٣.

Shibahara, H., Burkman, L.j., Isojima, S. and Alexander, N.J. (١٩٩٣). Effect of sperm-immobilizing antibodies on sperm-zona pellucida tight binding. *Fertil Steril*, ٦٠: ٥٣٣-٥٣٩.

Shibahara, H., Tsunoda, T., Taneichi, A., Hirano, Y., Ohno, A. Takamizawa, S., Yamaguchi, C., Tsunoda, H. and Sato, I. (٢٠٠٢). Diversity of antisperm antibodies bound to sperm surface in male immunological infertility. *A.J.R.I.*, ٤٧: ١٤٦-١٥٠.

Shushan, A. and Schenker, J.G. (١٩٩٢). Immunological factors in infertility. *Am. J. Reprod. Immunol.*, ٢٨: ٢٨٥-٢٨٧.

Siciliano, L., Tarantino, P., Longobardi, F., Rago, V., De Stenfano, C. and Corpino, A. (٢٠٠١). Impaired seminal antioxidant capacity in human semen with hyperviscosity or oligoasthenozoospermia. *Journal of Andrology*, ٢٢: ٧٩٨-٨٠٣.

Siegel, M.S. (١٩٩٣). The male infertility investigation and the role of the andrology laboratory. *J. Reprod. Med.*, ٣٨ (٥): ٣١٧-٣٣٤.

Sies, H. (١٩٩٣). Stratigies of antioxidany defence. *Eur J Biochem* ٢١٥: ٢١٣-٢١٩.

Sikka, S.C. (٢٠٠١). Relative impact of oxidative stress on male reproductive function. *Curr. Med. Chem.*, ٨: ٨٥١-٨٦٢.

Sikka, S.C., Rajasekaran, M. and Hellstrom, W, J. (١٩٩٥). Role of oxidative stress and antioxidant in male infertility. *Journal of Andrology*, ١٦: ٤٦٤-٤٦٨.

Simbini, T., Umapathy, E., Jacobus, E., Tendaupenyu, G. and Mbizro, M.T. (١٩٩٨). Study on the origin of seminal leucocytes using split ejaculate technique and the effect of leucocytospermia on sperm characteristics. *Urologia Internationalis*, ٦١(٢). (Abstract).

Sofikitis, N., Miyagawa, I., Dimitriadis, D., Zavos, P., Sikka, S. and Hellstrom, W. (١٩٩٥). Effects of smoking on testicular function, semen quality and sperm fertilizing capacity. *J. Urology*, ١٥٤: ١٠٣٠-١٠٣٤.

Spitteler, G. (١٩٩٣). Review: on the chemistry of oxidative stress. *J. Lipid Mediat.*, ٧: ٧٧-٨٢.

Stedman, R.L. (١٩٦٨). The chemical composition of tobacco smoke. *Chem. Rev.*, ٦٨:١٥٣-٢٠٧.

Stillman, J.R. (١٩٨٦). Smoking and reproduction. *Fertil Steril*, ٤٦: ٥٤٥-٥٦٦.

Suleiman, S. A., Ali, M.E., Saki,Z.M., el-Malik, E.M. and Nasr, M.A. (١٩٩٦). Lipid peroxidation and human sperm motility: protective role of vitamin E. *Jouranl of Andrology*, ١٧(٥): ٥٣٠-٥٣٧.

Sweeney, T.E. and Beuchat, C.A. (١٩٩٣). Limitation methods of osmometry: measuring the osmolality of biologic fluids. *American Journal of Physiology*, ٢٦٤: ٤٦٩-٤٨٠.

Swenson, A., Toth, C., Toth, L. and Oleary, W.M. (١٩٨٠). Asymptomatic bacteriospermia in infertile men. *Andrologia*, ١٢(١): ٧-١١.

Swerdloff, R.S. Wang, C. and Sokol, R.Z. (١٩٩١). Endocrine evaluation of the infertile male. In Lipshultz, L.I.; Howards, S.S. eds, Infertility in the male. St Louis: Mosby Year Book, ١٧٩-٢١٠.

Taourel,D.B., Guerin, M.C. and Torreilles, J. (١٩٩٢). Is malondialdehyde a valuable indicator of lipid peroxidation. *Biochem. Phamacol.*, ٤٤: ٩٨٥-٩٨٨.

Tavilani, H., Doosti, M., and Saeidi, H. (٢٠٠٥). Malondialdehyde levels in sperm and seminal plasma of asthenozoospermic and its relationship with semen parameters. *Clinica Chimica Acta*, ٣٥٦: ١٩٩-٢٠٣.

Tesarik, J., Greco, E., Cohen-Bacrie, P. and Mendoza, C. (١٩٩٨). Germ cell apoptosis in men with complete and incomplete spermiogenesis failure. *Mol Hum Reprod* , ٤ :٧٥٧-٧٦٢.

Thomas, L. (١٩٩٨). Clinical Laboratory Diagnostics. ١st edi, Franklen: TH-Books Verlagsesellschaft; pp ١٣١-١٣٧.

Thorner, M.O., Edward, C.R., Hanker, J.P., Abraham, G. and Besser, G.M. (١٩٧٧). The testis in normal and infertile men. New York, Raven Press, ٣٥١-٣٥٦.

Tomlinson, M.J., Barratt, C. and Cooke, I.D. (١٩٩٢). Prospective study of leucocytes and leucocyte subpopulation in semen suggests they are not a cause of male infertility. *Fertility and Sterility*, ٦٠: ١٠٦٩-١٠٧٥.

Tortora, G.J. (١٩٩٢). Principles of human anatomy. Sixth edition. Harper Collins Publishers.

Trainer, P.J. and Besser, G.M. (١٩٩٥). In Barts Protocols, Churchill Livingstone.New York.

Tuck, R.R., Setchell, B.P., Waites, G.M. and Young, J.A. (١٩٧٠). The composition of fluid collected by micropuncture and catheterization from the seminiferous tubules and rete testis of rats. *Pfluges Archives*, ٣١٨:٢٢٥-٢٤٣.

Turek, P.J. and Lipshultz, L.I. (١٩٩٤). Immunologic infertility. *Urol. Clin. N. Am.*, ٢١: ٤٤٧-٤٦٨.

Velazquez, A., Padron, N., Delgado, N.M. and Rosado, A. (١٩٧٧). Osmolarity and conductance of normal and abnormal human seminal plasma. *Int. J. Fertil.*, ٢٢: ٩٢-٩٧.

Vicino, M., Loverro, G., Simonetti, S., Mei, L. and Selvaggi, L. (١٩٩٩). The correlation between idiopathic leucocytospermia, embryo quality and outcome in the FIVET and ICSI procedures. *Minerva Gynecologia*, ٥١ : ٤١٣-٤٢٠ .

Vine, M.F. (١٩٩٦). Smoking and male reproduction: a review. *Int. J. Androl.*, ١٩: ٣٢٣-٣٣٧.

Vine, M.F., Margolin, B.H., Morrison, H.I. and Hulka, B.S. (١٩٩٤). Cigarette smoking and sperm density: a meta-analysis. *Fertil Steril*, ٦١: ٣٥-٤٣.

Vogelpoel, F.R., Van-Kooij, R.J., Velde, E.R. and Verhoef, J. (١٩٩١). Influence of polymorphonuclear granulocytes on the zona-free hamster oocyte assay. *Human Reproduction*, ٦: ١١٠٤-١١٠٧.

Vogt,H.J., Heller, W.D. and Borelli, S. (١٩٨٦). Sperm quality of healthy smokers, ex-smokers, and never-smokers. *Fertil Steril*, ٤٥: ١٠٦-١١٠.

Weese, D.L., Peaster, M.L., and Himsl, K.K. (١٩٩٣). Stimulated reactive oxygen species generation in the spermatozoa of infertile men . *J. Urol.*, ١٤٩:٦٤-٦٧.

Weng, S., Taylor, S., Morshedi, M., Schuffner, A., Duran, E.H., Beebe, S. and Oehninger, S. (٢٠٠٢). Caspase activity and apoptotic markers in ejaculated human sperm. *Mol. Hum. Reprod.*, ٨(١١) : ٩٨٤-٩٩١.

Witken, S.S., Viti, D., David, S.S., Stangel, G., and Rosenwaks, Z. (١٩٩٢). Relation between antisperm antibodies and the rate of fertilization of human oocytes *in vitro*. *J. Assist. Reprod. Genet.*, ٩:٩-١٣.

Wolff, H. (١٩٩٥). The biologic significance of white blood cells in semen. *Fertil Steril*, ٦٣: ١١٤٣-١١٥٧.

Wolff, H., Politch, J.A., Martinez, A., Hiamovici, F., Hill, J.A. and Anderson, D.J. (١٩٩٠). Leucocytospermia is associated with poor semen quality. *Fertility and Sterility*, ٥٣: ٥٢٨-٥٣٦.

World Health Organization. (١٩٨٧). WHO Laboratory Manual for the Examination of Human Semen and sperm-cervical mucus interaction, ٢nd edition. Cambridge University Press, Cambridge.

World Health Organization. (١٩٩٢). The influence of varicocele on parameters of fertility in a large group of men presenting to infertility clinics. *Fertil Steril*, ٥٧: ١٢٨٩-١٢٩٢ .

World Health Organization. (١٩٩٢). WHO Laboratory Manual for the Examination of Human Semen and sperm-cervical mucus interaction, ٣rd edition. Cambridge University Press, Cambridge.

World Health Organization. (١٩٩٩). WHO Laboratory Manual for the Examination of Human Semen and sperm-cervical mucus interaction, ٩th edition. Cambridge University Press, Cambridge.

Yanushpolsky, E. H., Politch, J.A., Hill, J.A. and Anderson, D.J. (١٩٩٦). Is leucocytospermia clinically relevant. *Fertility and Sterility*, ٦٦:٨٢٢-٨٢٥.

Yaseen, Z. A. (٢٠٠٤). Sero - bacterial assessment of asymptomatic infertile males in Erbil. (Ph.D.Thesis, college of Science, Uniersity of Salahaddin).

Yeh, W.R., Acosta, A., Seltman, H.J. and Doncel, G. (١٩٩٥). Impact of immunoglobulin isotope and sperm surface location of antisperm antibodies of fertilization *in vivo* in the human. *Fertil Steril*, ٦٣:١٢٨٧-١٢٩٢.

Yenilmez, E. Yildirmis, S. Yulug, E., Aydin, S., Tekelioylu, Y., Erdem, E., Topbas, M. and Arvas, H. (٢٠٠٦). Hams F-١٠ medium and Hams F-١٠ medium plus vitamin E have protective effect against oxidative stress in human semen. *Urology*, ٦٧(٢): ٣٨٤-٣٨٧. (Abstract).

Yilmaz, S., Koyuturk, M., Kilic, G., Alpak, O. and Aytoz, A. (٢٠٠٥). Effects of leucocytospermia on semen parameters and outcomes of intracytoplasmic sperm injection. *International Journal of Andrology*, ٢٨: ٣٣٧-٣٤٢.

Yueng, C.H., Anapolsky, M., Depenbusch, M., Zitzmann, M. and Cooper, T.G. (٢٠٠٣). Human sperm volume regulation. Response to physiological changes in osmolality, channel blokers and potential sperm osmolytes. *Human Reproduction*, ١٨(٥) : ١٠٢٩-١٠٣٦.

Yule, T., Montoya, G.D., Russell, L.D., Williams, T.M. and Tung, K.S. (١٩٨٨). Autoantigenic germ cells exist outside the blood-testes barrier. *J. Immunol.*, ١٤١: ١١٦١-١١٦٧.

Zarghami, N. and Khosrowbeygi, A. (٢٠٠٤). Evaluation of lipid peroxidation as indirect measures of oxidative stress in seminal plasma. *Iranian Journal of Reproductive Medicine*, ٢: ٣٤-٣٩.

Zarghami, N. and Khosrowbeygi, A. (٢٠٠٥). Seminal plasma levels of ١٠-f٢α-isoprostane, malondialdehyde and total homocysteine in normozoospermic and asthenozoospermic males. *Indian Journal of Clinical Biochemistry*, ٢٠ (٢): ٨٦-٩١.

Zavos, P.M. (١٩٨٩). Cigarette smoking and human reproduction: effects of male and female fecundity. *Infertility*, ١٢: ٣٥-٤٦.

Zavos, P.M., Correa, J.R., Antypas, S., Zarmakoupis-Zavos, P.N. and Zarmakoupis, C.N. (١٩٩٨). Effects of seminal plasma from cigarette smokers on sperm viability and longevity. *Fertility and Sterility*, ٦٩: ٤٢٥-٤٢٩.

Zenzes, M.T. (٢٠٠٠). Smoking and reproduction: gene damage to human gametes and embryos. *Human Reproduction Update*, ٦: ١٢٢-١٣١.

Zinaman, M.J., Brown, C.C., Selevan, S.G. and Clegg, E.D. (٢٠٠٠). Semen quality and human fertility: a prospective study with healthy couples. *J. Andrology*, ٢١: ١٤٥-١٥٣.

Zini, A., Garrels, K. and Phang, D. (٢٠٠٠). Antioxidant activity in the semen of fertile and infertile men. *Urology*, ٥٥: ٩٢٢-٩٢٦.

Zouari, R., De Almeida, M., Rodrigues, D. and Jouannet, P. (١٩٩٣). Localization of antibodies on spermatozoa and sperm movement characteristics is good

Semen quality	Fertile men (control)	Oligoasthenozoospe-rmic men	Oligoasthenoterato-zoospermic men	L.S.D.
١- Volume (ml)	٤.١٣٣ ± ٠.١٤٧	٢.٥٠٠ ± ٠.١٨٦ ***	٣.٢٦٤ ± ٠.٤٣٧ **	٠.٧٨٩
٢-Sperm conc. (× ١٠^{٦} / ml)	٩٧.٠٠ ± ١.٦٠١	٧.٧٩١ ± ٠.٧٦٣ ***	٦.٣٧٥ ± ١.٤٧٥ ***	٨.٩٥٩
٣- Total sperm count (× ١٠^{٦} / Ejaculate)	٤٠٠.٣٣ ± ١٥.١٧	١٩.٨١٠ ± ٢.٧٩١***	١٩.٣٢١ ± ٤.٩١١***	٦٩.١٢٣
٤- Grade activity	٣.٨٠٠ ± ٠.٠٧٤	٠.٨٨١ ± ٠.٠٦٠ ***	٠.٥٠٠ ± ٠.١٣٨ ***	٠.٥٦٧
٥- Motility (%)	٥٨.٠٠ ± ٠.٧٤٢	١٤.٢٦١ ± ١.٠١٤ ***	٠.٥٠٠ ± ٠.١٣٨ ***	٧.٠٣٠
٦-Sperm motility index	٢٢٢.٠٠ ± ٦.٦٨٥	١٥.٠٧١ ± ١.٥٦٩***	٨.٢١٤ ± ٢.٢٨٥***	٥٦.٧٤٨
٧- Viability (%)	٧٣.١٦٦ ± ٠.٦٩٨	٢٨.٥٧١ ± ١.٥٨٢ ***	٢٠.٣٥٧ ± ٣.٩٣٨ ***	٧.٥٢٥
٨- Normal morphology (%)	٧١.٥٠٠ ± ٠.٨٣٥	٥٠.١١٩ ± ١.٥٠٩ ***	١٦.٣٥٧ ± ٣.٠٨٤ ***	١١.٧١٦
٩- Leucocytes (× ١٠^{٦} / ml) ; HPF :	١.٨٠٠ ± ٠.٤٨٠	٧.٥٩٥ ± ١.٢٦٢ **	١٠.٢١٤ ± ٢.٦١٢ ***	٥.٢٣٢
Endtz method :	٠.٤٣٣ ± ٠.١٤٩	٢.١٠٧ ± ٠.٥٠٤ *	٢.٨٧١ ± ١.٠٨٥*	٢.٦٧٤

predictors of in vitro fertilization success in cases of male autoimmune infertility. *Fertil Steril*, ٥٩: ٦٠٦-٦١٢.

Table (١١): Semen quality of fertile men, oligoasthenozoospermic and oligoasthenoteratozoospermic

١٠-Osmolality (m OSm/ kg)	٣٠٣.٧٠ ± ٢.٤٤٢	٣٦٠.٦٤٢ ± ١٠.٥٩٨ ***	٣٤٦.٤٢٨ ± ٦.٤٣١*	٤٠.٢٣٢
	N= ٣٠	N= ٤٢	N=١٤	

men (Means ± S.E) .

*** P < ٠.٠٥ ** P < ٠.٠١ *** P < ٠.٠٠١ LSD was used for multiple comparisons**

Table (١٢): Semen quality of fertile men , asthenozoospermic and asthenoteratozoospermic men (Means ± S.E) .

Semen quality	Fertile men (control)	Asthenozoospermic-men	Asthenoteratozoo-spermic men
١- Volume (ml)	٤.١٣٣ ± ٠.١٤٧	٢.٨٥٢ ± ٠.١٦٣ ***	٣.٤٢٣ ± ٠.٣٤٣ *
٢-Sperm conc. (× ١٠^{٦} / ml)	٩٧.٠٠ ± ١.٦٠١	٥٤.٩٨٤ ± ٢.٢٨٢ ***	٤٦.٠٠٠ ± ٣.٥٨٥ ***
٣- Total sperm count (× ١٠^{٦} / Ejaculate)	٤٠٠.٣٣ ± ١٥.١	١٥٣.١٧ ± ٩.٧٧ ***	١٥٤.٨٨ ± ١٧.٢٣ ***
٤- Grade activity	٣.٨٠٠ ± ٠.٠٧٤	١.٥٣٨ ± ٠.٠٦٦ ***	١.٠٠٠ ± ٠.٢٢٦ ***
٥- Motility (%)	٥٨.٠٠ ± ٠.٧٤٢	٢٦.٠٧٦ ± ١.١٨١***	١٧.٠٠٠ ± ٣.٩٤٠ ***
٦-Sperm motility index	٢٢٢.٠٠ ± ٦.٦٨٥	٤٥.٠٩٢ ± ٣.٢٩٦ ***	٢٧.٦٩٢ ± ٨.٢٨٢ ***
٧- Viability (%)	٧٣.١٦٦ ± ٠.٦٩٨	٤٤.٠٠٠ ± ١.٤٦٣ ***	٣٥.٣٨٤ ± ٣.٧٣٢ ***
٨- Normal morphology (%)	٧١.٥٠٠ ± ٠.٨٣٥	٦٠.٨٤٦ ± ١.٢٥٧ ***	٢١.١٥٣ ± ١.١٥٣ ***
١٠- Leucocytes (× ١٠^{٦} / ml) ; HPF : Endtz method :	 ١.٨٠٠ ± ٠.٤٨٠ ٠.٤٣٣ ± ٠.١٤٩	 ١٠.٤٩٢ ± ١.٣٤٨*** ٢.٩٦٩ ± ٠.٥٠٩ *	 ٩.١٥٣ ± ٢.٣٩٠ * ٢.٥٣٨ ± ٠.٩٨٤
١١-Osmolality (m OSm/ kg)	٣٠٣.٧٠ ± ٢.٤٤٢	٣٤٣.٩٦٩ ± ٧.٦٩٧ **	٣٣٦.٣٨٤ ± ١٥.٢٨٦ *
	N= ٣٠	N= ٦٥	N=١٣

*** P < ٠.٠٥ ** P < ٠.٠١ *** P < ٠.٠٠١ LSD test was used for multiple comparisons**