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نصير جواد حمد المختار

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Physiological and Immunological Studies for Human Infertile Couples

A Thesis

Submitted to the Council of the College of Science University of Babylon

In Partial Fulfillment of the Requirements for the Degree of

Ph.D. In Biology / Zoology

By

Naseer Jawad Hamad Al- Mukhatar

March - ٢٠٠٦ **Safar - ١٤٢٧**

Dedication

To:

The Memory Of My Father : May He Rest in Heavenly Peace;

My Dear Mother ; The Source Of All Love,Tenderness and Patience ;

My Dear Wife ; My Soulmate Who Has Suffered A Lot For me ;

My Two Sons: Tammar And Nouran Besides The New one to Knock At My Heart's Gatee ;

My Brothers And Sisters : Parts Of Me And My Support In Life ; And

All Loving Friends, And All Faithful Fellows .

Naseer

بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

((وأنَزَل اللهَ عليَك الِكَتابَ والِحكَمَة وَعَّلَمَك ماَلم

تَكُن َتعَلم وكاَن َفضلُ اللهَ عليكَ َعظمياً))

صدق الله العليّ العظيم

النساء(١١٣)

Examination Committee

We, the examination committee, after reading thesis entitled "**Physiological and Immunological Studies of Human Infertile Couples**", and examining the student **Naseer Jawad Hamad Al-Mukhatar** in its content, have found that it meets standard requirement as a thesis in fulfillment for the degree of Ph.D. in Immunophysiology, and the thesis has been accepted with (**Excellent**) estimation.

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Summary

The present work has been undertaken to investigate the cellular and humoral immune responses as well as the seminological features of human infertile couples, beside making up a lapin

laboratory model for detection of sperm specific immunity. One hundred and twenty five clinically and seminoligically proven infertile couples and twenty clinically normal subjects were considered as a test and control groups respectively. These groups were the attendance of Babylon Maternity and Child Care Hospital during October ٢٠٠٣ to July ٢٠٠٥.

From infertile and control males; semen and blood were collected (with and without anticoagulant). Meantime, blood samples (with and without anticoagulant) and cervical mucus were obtained from the infertile and control females. In both instances, standardized sampling techniques were followed.

Sera from blood sample without anticoagulant were separated. Likewise, mucosal immunoglobulins were separated from both semen plasma and cervical mucus samples and kept frozen till use.

Whole sperm, sperm sonicate, sperm sonicated coated ovine erythrocyte and rabbit antihuman sperm antiserum coated ovine erythrocyte were the laboratory prepared test antigens.

The determination of cell mediate immune function were tested by counting leucocytospermia in the semen and by using leucocyte inhibitory test with capillary method. However, the humoral immune function tests were concluded by the following: ١) Semiquantitative slide agglutination. ٢) Microhaemagglutination for mucosal and systemic agglutinins and haemagglutinins. ٣) Rabbit antisperm antibody coated reverse haemagglutination test for detection of mucosal and systemic antigenemia.

It was found that leucocytospermia levels range from ٢.٣ to ٦.١×١٠^{٦} ± ٠.٩٢ to ١.١٠ cell per ml in infertile subjects as compared to ٠.٦×١٠^{٦} ± ٠.١٠ in normal control subject. Such levels may indicate nonspecific cellular immune surveillance and/or infection state in those infertile men.

Leucocyte inhibitory factors (LIF) was found to be range from ٠.٢٪ – ٠.٧٪ in those of infertile couples compared to those of control subjects ranges(٠.٧٥ – ١.٠٠٪). In other words, the significant difference of LIF mostly present among infertile couples may give an indication for involvement of hypersensitivity state of delayed type reaction among infertile couples, and sperm antigen contained an epitope that activate Th١ T_{dth} subsets of the T lymphocytes.

For the detection of sperm specific humoral immune responses, two approaches were followed. First, sperm antigens were used to detect sperm specific antibodies (SSA). And the second, was by using SSA for detection of sperm specific antigens to react with sera and mucosal immunoglobulin solution of infertile and normal subjects.

Sperm specific mucosal and circulating agglutinins and haemagglutinins titer were range from(٤٠-٣٢٠) for mucosal and (٤٠٠ – ٣٢٠٠) for systemic agglutinins. While they were (٣٢ – ٢٠٤٨)for mucosal and (٣٢٠ – ١٠٢٤٠) for circulating haemagglutinins among infertile couples respectively.

Fifteen out of ٣٥ infertile couples were showed mucosal and systemic antigenemia. Such antigenemia levels are ranging from (٨ – ١٢٨) and (٤٠ – ٦٤٠) for mucosal and circulating accordingly. Such finding, may be reported for first instance in this area.

From these seroprofile studies, it can be suggested that titer levels are pathognomonic with clinical state of the immune infertile couples. In these titer leveling: baseline, suspect and clinical titer limits were affixed.

The seroprofile and immune status of the infertile couples were mapped. From these mapping efforts, a supposed immunologic classification of infertility was made, in which the author hopes to be clinical relevance and/or of academic interest.

The immunologic test battery applied in this study which include leucocytospermia, LIF, agglutinins, haemagglutinins and antigenemia levels was found statically feasible for diagnosis and follow up of immune infertility. Lapin model for sperm specific immunity in male and female rabbit supported these views.

The sperm specific antigenic epitopes in infertility couples may be ١) B cell or T cell independent, ٢) T dependent, ٣) T dependent and allergenic potential and ٤) a combination of any them, as indicated from the results of this study.

Seminal fluid analysis was performed for the infertile and control groups to detect sperm concentration, direct sperm agglutination, motility percent, shaky head motion and abnormal sperm morphology. From these parameters, patients were assigned as asthenozoospermic, oligozoospermic and azoospermic. These infertile couples were found that they were of $\overset{o}{1}$ and $\overset{o}{2}$ infertility type of ٨٩.٦٠ vs ١٠.٤٠ and ٨٨.٨٩ vs ١١.١١ of asthenozoospermia and oligozoospermia consequentely. An attempt was made to correlate sperm motility index and SSA as well as Shaky head motion and SSA. It was found with significant correlation coefficient. Non-significant correlation coefficient was found between direct sperm agglutination and SSA.

الخلاصة

تمت هذه الدراسة بقصد التقصي عن وجود الاستجابة المناعية الخلوية Cellular Immunity والاستجابة المناعية الخلطية Humoral Immunity ، فضلاً عن دراسة صفات وخواص المني في الإنسان لدى الأزواج العقم (Infertile Couples) وقد تماشت هذه الدراسة مع دراسة محاكية أخرى

من خلال استعمال أزواج من الحيوانات المختبرية والمتمثلة هنا بأزواج من ذكور وإناث الأرانب Male and Female Rabbits وذلك بقصد التحري (الكشف) عن المناعة الخاصة (المتخصصة) تجاه الحيوانات المنوية البشرية (Sperm Specific Immunity).

شملت الدراسة ١٢٥ مريضاً (٧٩ ذكراً و ٤٦ أنثى) واللذين كانوا من الناحية السريرية والمختبرية (Clinical, Seminal Fluid Analysis and Cervical Mucus investigation) قد أظهروا عقماً وعدم إنجاب واضحين لا لبس فيه، كذلك تم استعمال (٢٠ شخصاً سليماً) ١٠ ذكر و١٠ أنثى (كمجموعة مقارنة أو كمجموعة سيطرة) مع بقية المجاميع المدروسة، علماً أن المجاميع المدروسة قد تم دراستها خلال قدومها إلى المستشفى التعليمي للولادة والأطفال في بابل وخلال مدة استمرت من شهر تشرين الأول لسنة ٢٠٠٣ ولغاية شهر تموز سنة٢٠٠٥.

تم إجراء جمع عينات السائل المنوي والدم بوجود مانع تخثر وبعدم وجود مانع تخثر Blood with and without anticoagulant في كل من الذكور المصابين والأسوياء، وبنفس الوقت تم جمع عينات مخاط عنق الرحم (Cervical Mucus Secretion) والدم بوجود وعدم وجود مانع تخثر في كل من النساء المصابات والأسوياء. وقد أعتمد منهج عمل ثابت Standarized Techniques للعينات المدروسة ولكلا الجنسين.

تم فصل المصل (Serum) من عينات الدم بدون إضافة مانع التخثر لغرض الحصول على المصل في دم المصابين ومجموعة السيطرة، كذلك تم فصل نماذج المناعة المخاطية Mucosal Immunology في كل من البلازما المنوية Seminal Plasma وفي مخاط عنق الرحم (Cervical Mucus Secretion) وحفظت تحت درجة ^{o}C ١٨ – حين الاستعمال.

وقد استعملت كامل تركيبة النطف بقصد تكسيرها (Sonicate Sperms) بوساطة جهاز الـ(Sonicater Probe) وتم تحميل عينات النطف المعالجة المكسرة إلى كريات دم الخراف الحمر (Sheep Red Blood Corpuscles " $SPBC_S$ "). كذلك تم استعمال معالجات النطف المكسرة كجرع حقن في الأرانب بقصد تحضير الأضداد Antiserum الخاصة بانتيجينات النطفة Sperm Antigens.

تم اختبار (وتحديد) المناعة الوسيطة الخلوية Mediate Immunity من خلال ظهور الخلايا البيض في السائل المنوي Leucocytospermia وخلال إجراء فحص طريقة هجرة الخلايا البيض المثبطة بطريق الأنابيب الشعرية.

بينما تم اختبار (تحديد) فحص وظيفة المناعة الخلطية من خلال ما يأتي

١- اختبار فحص التلازن شبه الكمي على الشريحة الزجاجية.

٢ - اختبار فحص التلازن الدموي الدقيق لكل من الأضداد المخاطية والجهازية.

٣ - اختبار فحص التلازن الدموي المعكوس للكشف عن وجود الأنتيجينات في الدم بعد تحميل الـ Sperm Antisera لكريات دم الخراف الحمر ومعرفة وجود أو عدم جود الـ Antigenemia. خلال هذه الدراسة تراوح المدى في ابيضاض السائل المنوي Leucocytospermia من ٢.٣ –٦.١ × $١٠^{٦}$ ٠.٩٢ ± ٠.١٠ – خلية بيضاء /مل في الاشخاص العقيمين (العقم) مقارنة بـ ٠.٦× $١٠^{٦}$ ± ١٠.٠ خلية بيضاء /مل في مجموعة السيطرة. وقد أوحت بعض المديات (Ranges) أو أعطت مؤشراً بوجود استجابة مناعية غير تخصصية و/أو وجود حالة التهاب لدى الأشخاص العقم من الرجال.

وأوضحت الدراسة فيما يخص عامل الهجرة المثبط لكريات الدم البيض (LIF) Leucocytes Migration Inhibitory Factor مديات تراوحت بين ٠.٢– ٠.٧ % لدى الأزواج العقم مقارنة بمدى ٠.٧٥ –١.٠٠% لدى الأزواج الخصبين (السيطرة).

وبعبارة أخرى فإن عامل (LIF) كان بمستوى معنوية لدى أغلب الازواج العقم والذي أعطى مؤشراً أضافياً ينم عن حالة فرط الحساسية المتأخرة (Delayed Type Hypersensitivity) بين الأزواج العقم واحتواء الأنتيجينات في النطف على مواقع تنشيط الخلايا T المساعدة نوع ١ وخلايا T نوع T dth من حث سلائل خلايا T اللمفاوية، ولغرض التحري عن وجود الاستجابة المناعية الخلطية المتخصصة بالحيمن فقد أتبع سبيلان لأجل ذلك التحري إذ تمثل الأول باستعمال مستضدات النطف (Sperm Antigens). أما الثاني فكان باستعمال أضداد النطف المتخصصة (SSA التخصصية) للتحري أو الكشف عن وجود الأنتيجينات التي تتفاعل مع الكلوبيولينات المصلية أو المخاطية للأشخاص العقم والأصحاء.

أن أضداد النطف الجهازية والمخاطية التخصصية وتلازنها من خلال فحص تلازن الأضداد Agglutinins و/أو التلازن الدموي Haemagglutinins كانت بمعيار تراوح ٤٠-٣٢٠ في التلازن المخاطي، ٤٠٠ - ٣٢٠٠ للتلازن الدموي (الجهازي)، في حين تراوح بين ٣٢ –٢٠٤٨ للتلازن المخاطي و ٣٢٠- ١٠٢٤٠ في التلازن الجهازي فيما يخص التلازن الدموي Haemagglutinins بين الازواج العقم على التعاقب.

وقد ظهر أن ١٥ من أصل ٣٥ من الازواج العقم يحملون أستجابة مناعية خاصة بظهورالـ Antigenemia وبكلا المستويين الجهازي والمخاطي إذ ظهر مستوى هذه الأستجابة يتراوح بمعيار ٨ –١٢٨و ٤٠ –٦٤٠ في كل من فحص التلازن المخاطي والجهازي على التوالي.

أن هذه العلامة السريرية (Antigenemia) ربما سجلت لاول مرة في هذه الدراسة. ومن خلال دراسة الصورة المصلية (Seroprofile) أصبح من الممكن اقتراح مستوى معياري يمكن اعتماده كواسم للمرض (العقم المناعي)، إضافة إلى الوضع السريري في تشخيص العقم المناعي في الأزواج. أن القوى المناعية المطبقة في اختبارات هذه الدراسة تضمنت فحص وجود خلايا الدم البيض في المني

Leucocytospermia وعامل تثبيط هجرة الخلايا البيض (LIF) واختبار تلازن الاضداد Agglutinins وأختبار التلازن الدموي (Haemagglutinins) وفحص وجود المستضدات الدموية في الدم (Antigenemia) وقد وجد أن هنالك جدوى أحصائية ذات أهمية وفائدة في التشخيص والمتابعة لمرضى العقم المناعي.

وقد استعملت في هذه الدراسة حيوانات المختبر بقصد التعرف على المناعة التخصصية للنطف Sperm Specific Immunity في كلا من ذكور وإناث الارانب كجزء داعم وساند لدراسة العقم المناعي في الانسان.

وقد تستحث المناطق المتخصصة في احداث الأستجابة المناعية (Epitops) تجاه النطف في الازواج العقم نتيجة لعوامل عدة منها:

١- استجابة مناعية غير معتمدة على خلايا نوع B أو خلايا نوع T.

٢- استجابة مناعية معتمدة على خلايا نوع T .

٣- استجابة مناعية معتمدة على خلايا نوع T مع وجود مولد الجهد المناعي Allergenic Potantial .

٤- جمع أو ربط لكل من الحالات الثلاثة السابقة كما هو واضح من خلال نتائج هذه الدراسة.

هذا وقد شملت الدراسة فحص أختبار السائل المنوي Seminal Fluid Analysis لدى الاشخاص العقيمين والأصحاء وذلك لمعرفة كل من: تركيز النطف Sperm Concentration و التلازن المباشر للنطف Direct Sperm Agglutination و حركة النطف Sperm Motility و حركة الرأس الأهتزازية للنطف .Sperm Shaky Head Movement وكذلك لاسوية شكلياء النطف Abnormal Sperm Morphology ومن خلال هذه المعايير تم تحديد فيما إذا كان المريض مصاب بوهن النطف Asthenozoospermia، قلة النطف Oligozoospermia أو إنعدام النطف (اللانطفية Azoospermia). وقد أظهرت نتائج الدراسة أن الازواج العقم كانوا مصابين أما بالعقم الأولي (1^{o}) أو العقم الثانوي (2^{o}) إذ تمثلت تلك النسب بحوالي٨٩.٦٠ ضد ١٠.٤٠ و ٨٨.٨٩ ضد ١١.١١ عند كل من المصابين بوهن النطف وقلة النطف على التعاقب.

أظهرت هذه الدراسة أن معامل الارتباط Correlation Coefficient على مستوى من الأهمية بين كل من دليل حركة النطف Sperm Motility Index والاضداد المتخصصة بالنطف SSA وكذلك معامل الارتباط بين حركة النطف الاهتزازية واضداد النطف التخصصية. مع عدم ظهور مثل تلك العلاقة بين معامل الارتباط المذكور والتلازن المباشر للنطف Direct Sperm Agglutination.

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List of Abbreviations

Abbreviation	Meaning
ABO	Blood group type
Abs	Antibodies
Ags	Antigens
APC	Antigen presenting cell
ATP	Adenosine triphosphate
ATP_{ase}	Adenosine triphosphatase
(C)	Constant region
C_3	Complement-C_3
C_1	Complement-C_1
CAMP	Cyclic adenosine monophosphate
CD	Cluster of differentiation
CMIS	Common mucosal immune system
CVL	Cervicovaginal lavage
DW	Distilled water
DTH	Delayed-type hypersensitivity
(E)	Estradiol
EAO	Experimental allergic orchitis
EDTA	Ethylene diamine tertra acitic acid
EILS	Enzyme-linked immunosorbent assay
FA-١	Fertilization antigen-١
FS	Formal saline
FSH	Follicle stimulating hormone
GM – CSF	Granulocyte – macrophage colony stimulating factor
GPI	Glycosyl phosphatidyl insoitol
HBSS	Hank's balance salt's solution
HFF	Human follicular fluid

Abbreviation	Meaning
HIV	Human immunodeficiency virus
HLA	Human leucocyte antigen
HLA- DR, DP, DQ	Human leucocyte antigen - DR, DP, DQ allele
IgA	Immunoglobulin A
IgD	Immunoglobulin D
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
$IL_{-(X)no}$	$Interleukin_{-(x)no}$
IFN- γ	Interferon-gamma
KD_a	Kilo-daltion
LDH-$C_ε$	Lactate dehydrogenase-$C_ε$
LIF	Leucocyte migration-inhibition factor
MAC	Membrane attach complex
MALT	Mucosa-associated lymphoid tissues
M-cells	Membraneous cells
MHC	Major histocompatability complex
MIgA	Mucosal immunoglobulin-A
NK-cells	Natural killer cells
P	Progesterone
PAF	Platelet-activating factor
PBS	Phosphate buffer saline
PEG-٦٠٠٠	Polyethylene glycol-٦٠٠٠ media
PG	Prostaglandins
PHAT	Passive haemagglutination test
PIgR	Polymeric immunoglobulin receptor
PMNS	Polymorphonuclear leucocytes

Abbreviation	Meaning
(Pro)	Prolactin
ROS	Reactive oxygen species
RPHAT	Reverse passive haemagglutination test
rpm	round per minute
RSA	Rabbit serum antigen
SAgS	Sperm antigen stock
Sc	Secretory component
SDSt	Semiquantitative-direct slide test
S-IgA	Secretory IgA
S-IgM	Secretory IgM
SMI	Sperm motility index
SN	Normal saline
$SRBC_S$	Sheep red blood corpuscles
SSA	Sperm-specific antibodies
STDs	Seuxal transmitted diseases
T	Testosterone
TB	Tuberculosis
TAT	Tray agglutination test
TCR	T cell receptor
TH Cells	Helper T cells
TGF–β	Transforming growth factor β
TH_1	T-cell helper -١
TH_2	T-cell helper -٢
TNF-β	Tumour necrosis factor β
(V)	Variable region
WBC_s	White blood cells
WHO	World health organization

Abbreviation	Meaning
WSAG	Whole sperm antigen

Chapter One

Introduction

١ – ١ Over view

To understand the immune status of infertile animal and human beings during health and diseases, one should understand the host biology, gamete biology, host – gamete interactions and their products outcome. Thus, in this brief introduction, sperm antigens (Entra and Extra cellular sperm antigen(s)), host (human), sperm antigen – host interaction in the reproductive organ and their outcomes were mentioned and such a mention was mainly on the human immune infertility (Glibert *et al.*, ١٩٨٨; Anil Suri, ٢٠٠٤)

١ – ٢ Reasoning

Infertility is a health issue that usually relates to a particular relationship between two peoples, the male and female partners who deserve equal attention (WHO, ١٩٩٢). Thus, several important assays were done to evaluate the inuse diagnostic tools assessing infertile men and women. Physiological and physiopathological causes of human infertility have throughly been investigated by several workers including Iraqi researchers (Ridha-Albarzanchi *et al*., ١٩٩٢). In comparison, the immunological causes, immune status and immunodiagnosis of infertility of infertile couples have not been investigated (McShane *et al.,* ١٩٨٥; Bronson *et al.*, ١٩٨٦; Eggert-Kruse *et al.,* ١٩٨٩). Thus, the present work has been planned to uncover some immunological aspects of rather infertile couples with no hormonal disturbances, together with their sperm function test in a statistical correlative approaches.

١ –٣ Aims

The aim of the present work is to uncover some aspects of immune infertility among human couples with an emphasis on simulation study of immune infertility in rabbits in both sexes; to verify these goals the following applications were used:

١. Determination of Leucocytes concentration of fertile and infertile patients.

٢. Collection of blood and mucosal secretions from infertile human couples.

٣. Detection of direct sperm agglutinates in seminal fluid analysis.

٤. The use of a battery of immune function test as (semiquantitative agglutination, passive haemagglutination, reverse passive haemagglutination and leucocyte inhibition factors).

٥. Feasibility of immune function test in diagnosis of immune infertility.

٦. Conduction of sperm function tests and classification of infertility.

٧. Correlative statistics for sperm function tests and antisperm antibodies.

٨. Performing sperm specific immunity in male and female rabbits.

Chapter Two

Review of Literatures

Part One: Immunology

٢–١: Immunology

٢–١–١: Immunological Aspects of Reproductive System: Structural Basis of Immune Function

The female reproduction system (Figure-I) includes two ovaries, two oviducts, the uterus and the vagina. In addition to producing oocytes and the mature ova, the ovaries also produce several important female hormones. The male reproductive system (Figure-II) includes the testis, a series of ducts or channels, accessory glands and the penis. The testis also produce the spermatocytes, the sperms in addition to the male sex hormones. The male reproductive mucosal compartment responds as an autoimmune response to sperm antigens (Meshan, ١٩٨٤; Clarke *et al.*, ١٩٨٥; Gilbert, ١٩٨٨; Jones, ١٩٩٤; Patrick *et al.,* ١٩٩٨; Comhair, ١٩٩٩; Wira *et al.*, ٢٠٠٢).

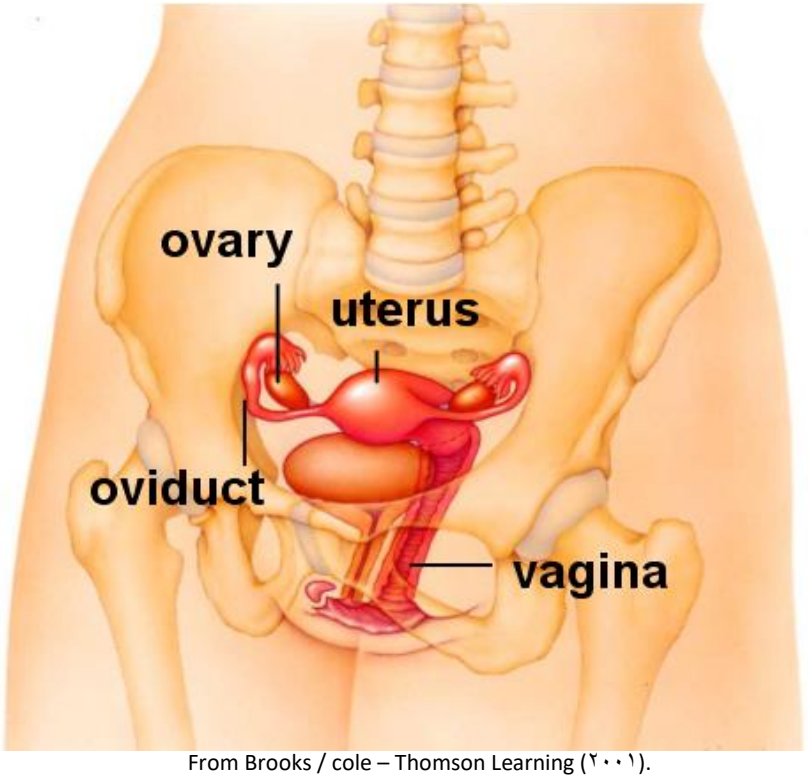
Female mucosal compartment is considerd as lacking mucosal- associated lymphoid aggregates (Yeaman *et al.,* ١٩٩٧; Haefliger *et al.,*٢٠٠٣) as first opinion. The second opinion;

however, advocates that such compartment is partially tolerant to sperm antigens (Fabiani *et al.,* ١٩٩٥; Carlsson, ٢٠٠١).

Finally, the third idea says that the female reproductive mucosa is tolerant to sperm antigens (Mowat and Weiner, ١٩٩٩; Gallucci and Matzinger, ٢٠٠١; Russell and Mestecky, ٢٠٠٢). It seems clear that both male and female genital tracts lack true mucosal inductive immune sites, which are collectively known as mucosa – associated lymphoid tissues (MALT) and typified by intestinal Peyer's patches and similar organized lymphoepithelial structure in lower bowel and upper respiratory tract. Nevertheless, foci of lymphoid and accessory cells, consisting of a core of B cells surrounded by T cells and on outer area of macrophage – like cells have been described in the human vagina, cervix, endometerium, fallopian oviduct and the uterus organs (Haefliger, *et al.*, ٢٠٠٣).

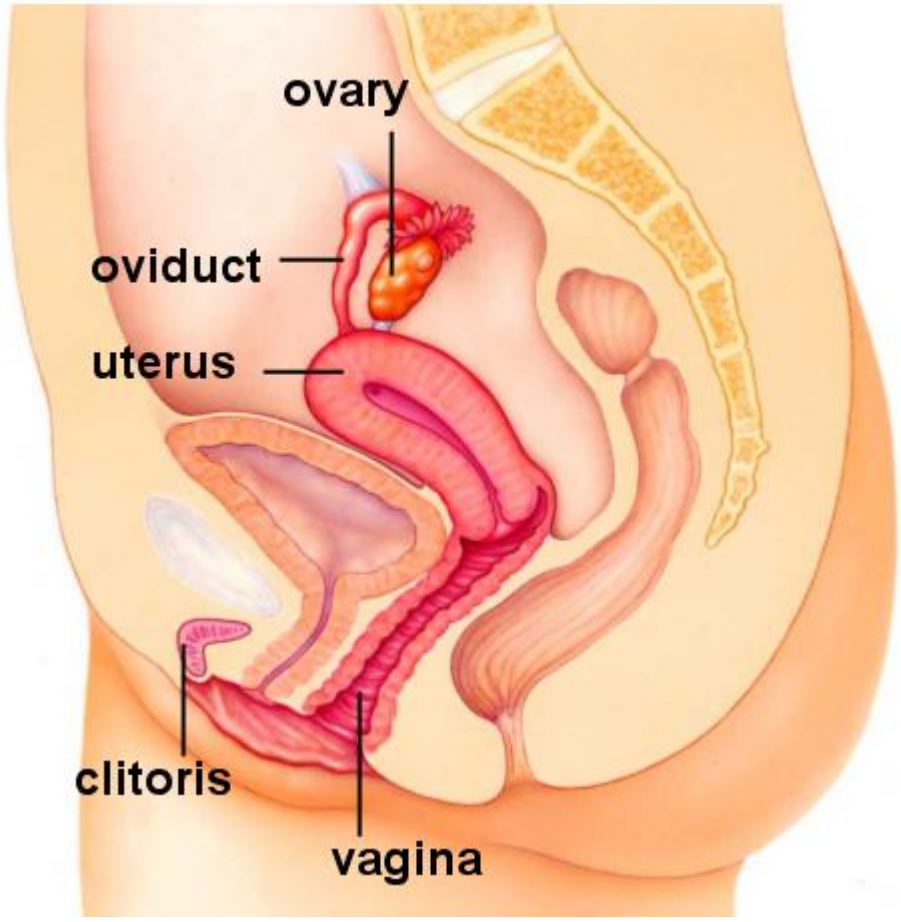
Most studies of the immune responses to sperm focused on humoral immunity. The interaction between sperm and lymphoid cells have only begun to be examined recently. As wells as mucosal immune system of female genital tract has been expanded in recent year (Gilbert *et al.*, ١٩٨٨; Brandtzaeg *et al.,* ١٩٩٣; Parr and Parr, ١٩٩٤).

A- Frontal view



From Brooks / cole – Thomson Learning (٢٠٠١).

B- Lateral view

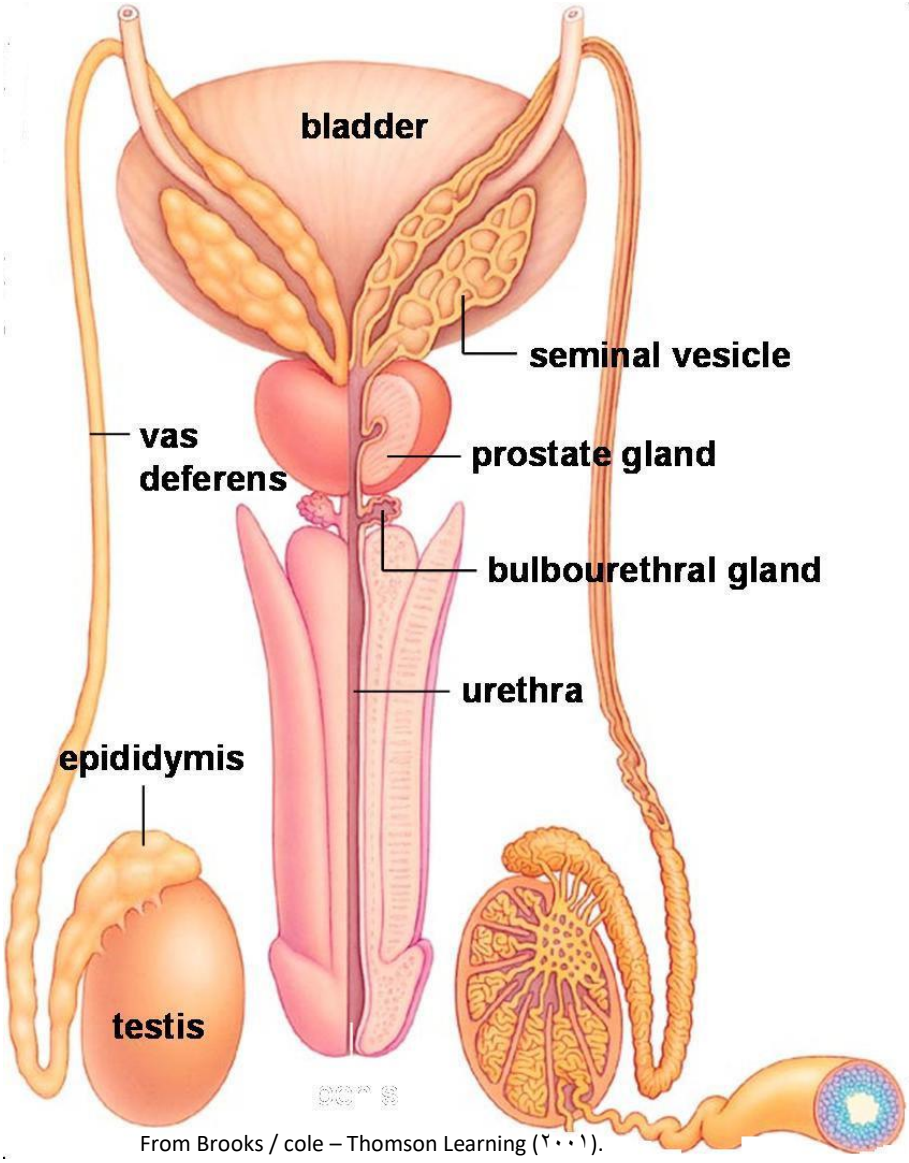


From Brooks / cole – Thomson Learning (٢٠٠١).

Figure: (I)

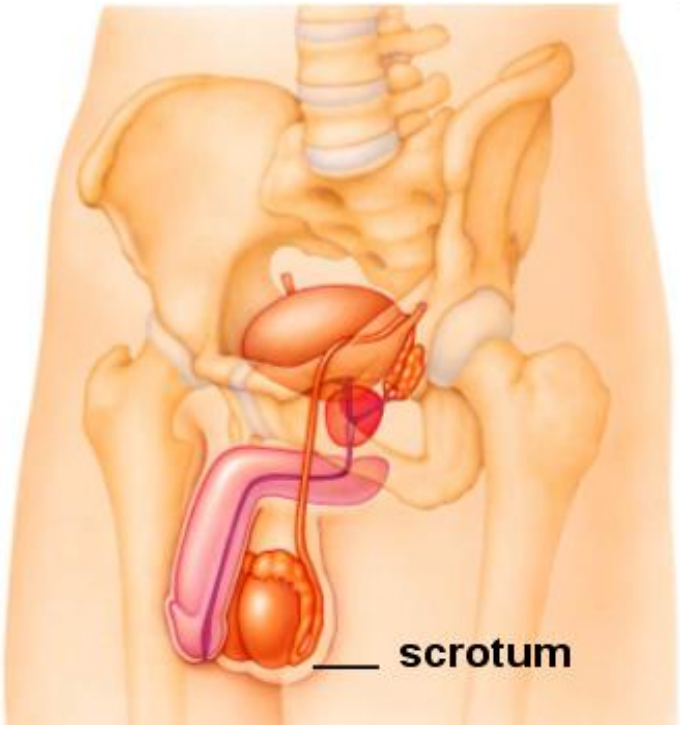
The female reproductive system

A- Frontal view



From Brooks / cole – Thomson Learning (٢٠٠١).

B- Lateral view



From Brooks / cole – Thomson Learning (٢٠٠١).

Figure: (II)

The male reproductive system

٢–١–١–١: Mucosal B Cells

Immune responses in the male and female reproductive tract are induced and disseminated to remote effectors sites (Yeaman *et al.*, ١٩٩٧). Similar to gut mucosa, most of plasma cells in lamina properia of the genital tract are derived from B cells to enhance immunoglobulin production (Brandtzaeg *et al.,* ١٩٩٣; Kutteh and Mestecky*,* ١٩٩٤). These cells are present in subepithelial tissue of female genital tract, particularly in the endocervix and to lesser extent in the fallopian tubes and uterus, has been well documented (Kutteh *et al.*, ١٩٨٨; Crowley *et al.*, ١٩٩٥).

٢–١–١–٢: T Cells

The site where immunocomponent cells are stimulated by antigen (such as sperm antigens) in the reproductive tract is poorly defined (Parr and Parr, ١٩٩٤; Wira *et al.*, ١٩٩٥). Langerhans cells and T cells are found in cervicovaginal epithelium (Lehner *et al.,* ١٩٩٢). Langerhans cells may serve as antigen presenting cells, some T cells clones have cytotoxic functions and others have helper effect .The helper $CD_{٤}$ cells are present in the endocervix suggesting an immunoregulatory role (Wassen *et al.*, ١٩٩٦), whereas a majority of these cells appear to be regulatory cells in that cytokines are produced (Parr and Parr, ١٩٩٤; Lohman *et al.,* ١٩٩٦; Anderson, and Hill, ١٩٩٥). Unlike the intestine, the genital tract is devoid of mediated cells and organized follicles which are analogous to the Pyre's patches (Parr and Parr, ١٩٩٤).

٢–١ –١–٣: Macrophages

Macrophages, primarily involved in innate immune responses, also have a critical role in antigen presentation (sperm antigen(s)) for acquired immunity, as well as, their regulatory secretion of immune soluble factors (Surrey, ١٩٩٧). Macrophages, dendritic cells, endothelial cells, Kupffer cells of the liver, langerhan's cells of the skin and Hofbauer cells of the placenta, and upon activation, they secrete proinflammatory mediators such as interleukin (IL - ١٢) , which is a key cytokine in immune regulation promoting both NK cell and cytotoxic T cell commitment from the T helper O (ThO) to Th١ phenotype characterized by interferon gamma (IFN – γ) secretion. (IFN – γ) secretion by Th١ cells mediates both cellular immunity and organ specific autoimmunity (Surrey, ١٩٩٧).

Macrophages may play a role in mediating infertility by interacting with sperm and destroying gametes, so fertilization in couples with antisperm antibody may be determined not only by the antibody, but also by the presence of genital tract macrophages capable of destroying the antibody – coated sperm (London *et al.*, ١٩٨٥).

As mentioned earlier the presence of MALT in the reproductive system was not established as such other mucosal developing system, but clear aggregate of $CD_{٨}^{+}$ T cells, B cells and

in the male and in cervicovaginal, cervix macrophages – like cells in penile urethral glands of Littre' and uterus of female have been observed (Hong-Yin Wu *et al.,* ٢٠٠٠).

٢–١–٢: Non Specific Cellular Immune Function (Leukocytospermia)

Many attempts had been made to answer the question of whether and to what extent WBCs – in particular polymorphonuclear granulocytes – (PMNs) can affect the quality of semen sample (Comhair *et al.*, ١٩٨٠; Talbert *et al.*, ١٩٨٧; Gonzales *et al.*, ١٩٩٢; Anderson, ١٩٩٥).

Older paper showed a decrease in number and motility of spermatozoa in infertile patients with leucocytospermia (Talbert *et al.*, ١٩٨٧; Eggert *et al.*, ١٩٩٢). Concerning the morphology of spermatozoa a reduction of normal forms was observed along with the decrease of their number and motility (Wolff *et al.*, ١٩٩٠; Gonzales *et al.*, ١٩٩٢).

These older findings have not been confirmed by other studies (Aitken and West, ١٩٩٠; Tomlinson *et al.*, ١٩٩٣; Aitken and West, ١٩٩٤), which involve large numbers of fertile men with leucocytospermia.

On the contrary, most reports seem to agree that leucocytospermia has a negative effect on sperm function tests in a different stages of the sperm – ova interaction processing due to WBCs impartment molecules secretions (Berger *et al.*, ١٩٨٢; Maruyama *et al.*, ١٩٨٥; Chacho *et al.*, ١٩٨٧; Vogelpoel *et al.*, ١٩٩١; Garvella and Lipovac, ١٩٩٣; Chan *et al.*, ١٩٩٤).

When assessing a semen analysis, the number and type of round cells present should be taken into account along with "Conventional" parameters, i.e. number, motility and morphology of spermatozoa (Potitch *et al.*, ١٩٩٣; Yanushpolsky *et al.*, ١٩٩٦).

Round cells in semen can be distinguished into;

A- White blood cells (WBCs) and ***B***- Immature germ cells.

Differentiation between these two cell types is considered of upmost importance for diagnostic and therapeutic purposes. For example, an increased number of WBCs in combination with other criteria may be indicative of genital tract infection (Potitch *et al.*, ١٩٩٣ ; WHO, ١٩٩٩).

On the other hand, an increased number of immature germ cells may be a sign of abnormal spermatogenesis (Nieshlag *et al.*, ١٩٩٧). Two groups of WBCs are present in semen, those with granular cytoplasm and those with agranular cytoplasm. The former includes polymorphonuclear granulocytes, eosinophils and basophils, while the later includes monocytes and lymphocytes. Despite the presence of all above mentioned cells in semen samples, the most abundant are granulocytes – in particular polymorphonuclear (WHO, ١٩٩٩).

The granulocytes were found as the predominant cell type (٥٠ – ٦٠٪), macrophages follows (٢٠ – ٣٠٪), and finally lymphocytes B and T lymphocyte account for very small proportion (٢– ٥٪) (John *et al.*, ١٩٩٩). Other cell types, however, are rare in semen samples (Wolff and Anderson, ١٩٨٨; Eggert *et al.*, ١٩٩٢; Tomlinson *et al.*, ١٩٩٣; Aitken *et al.*, ١٩٩٤).

The presence of leucocytes in seminal fluid can be explained as:

١. Inflammatory response.
٢. Nonspecific cellular immune response.
٣. Cooperation and / or inverse cooperation of natural and adaptive immune responses to foreign antigenic epitopes (WHO, ١٩٩٩; Parslow & Bainton, ٢٠٠١).

٢–١–٣: Sperm Specific Antibodies (SSA)

The antibody is specific immunoglobulin molecule. The immunoglobulin is the gamma globulin part of the serum globulin protein. Globulin protein is a glober glucoprotein (Parslow & Bainton, ٢٠٠١; Roitt *et al.*, ٢٠٠١).

The specific antibody molecule is a four chains unit containing two of heavy and two of light chains. Each of the chains contains variable (V) region and constant region (C). The variable region, however, contains an area of hypervariability nature known as paratope, the site where antigenic epitope of the antigen reacts with their respective antibody molecule. Antibodies are of systemic and mucosal nature. Mucosal antibodies are with secretory pieces and ٢ mercaptoethanol resistance character (Roitt & Rabson, ٢٠٠٠; Parslow *et al.*, ٢٠٠١).

Antibodies are of five classes in accordance with carbohydrate content, amino acid sequence, molecular size and number of fragments. These classes with different heavy chains nature are denoted as:

IgG	monomeric pass through placenta.
IgA	mono & dimeric does not pass through placenta.
IgM	pentameric does not pass through placenta.
IgD	monomeric does not pass through placenta.
IgE	monomeric does not pass through placenta.

IgG, IgA and IgM are free in serum or on mucosal surfaces, while IgD and IgE are of cytophilic nature (Stites *et al.,* ١٩٨٧; Michael *et al.,* ١٩٩٥; Roitt, and Rabson, ٢٠٠٠; Richard *et al.,* ٢٠٠٠; Wandy *et al.,* ٢٠٠٣). B cell surface Ig is responsible for recognition of B cell antigenic epitope. Mast cell and basophil cells contain IgE cross linked by allergen leading to degranulation and vasoactive amine release (immediate hypersensitivity reactions).

Sperm specific antibodies (SSA) are found both in men and women. SSA are systemic (peripheral blood, lymphatic fluid) and mucosal (semen plasma and cervicovaginal secretions) (Clarke *et al.*, ١٩٨٥; ١٩٨٦; Stites *et al.*, ١٩٨٧; Haas, ١٩٩٦).

Systemic:

Five immunoglobulin classes that are mentioned above only; IgG, IgA and IgM have been implicated in infertility. IgM appears early in the immune response followed by IgG and IgA. IgM is extremely efficient at agglutinating complex particulate antigen like sperm (Zina *et al.*, ٢٠٠٥).

Mucosal:

Sperm specific mucosal antibodies are mainly of IgA isotype, however, to lesser extent mucosal IgM or IgG were reported. At mucosal inductive site sperm antigens are taken up by membraneous cells (M) which convey them to antigen presenting cells (APC), such cells present them to helper T cell in the organized lymphoid aggregates (follicles) and in turn T helper cell activate mucosal pro B which migrate through blood and lymph circulation to thoracic lymph duct then return back to lamina properia and intraepithelial lymphocyte aggregates (spread or diffuse aggregates) which constitute the effector site at mucosal compartment of reproductive tract where they homed through adhesion - addressin reaction and produced S-IgM which then switched to IgA type by cytokine influences. The function of M-IgA can be summarized in the following:

١. Reduce fertility.
٢. Reduce pregnancy rate.
٣. Influence gamete development.
٤. Fertilization Processing.
٥. Reproduction failure (Stites *et al.*, ١٩٨٧; Surrey, ١٩٩٧).

Sperm Specific Antibody in Men;

SSA in men are found in blood and mucosal surface. Since they are found in semen, seminal plasma, sera or bound to the outer sperm plasma membrane (Flower and Mariano, ١٩٨٣; Clarke *et al.*, ١٩٨٥; Gilbert *et al.*, ١٩٨٨; Kutteh *et al.*, ١٩٩٦).

Sperm Specific Antibody in Women;

SSA are found in blood, ovarian follicle fluid, vaginal or cervical secretion (Clarke *et al.*, ١٩٨٥; Shulman, ١٩٨٦$_{a,b}$; Mazumdar and Levine, ١٩٩٨).

It is clear that common mucosal immune system (CMIS) is not uniform, and that the human reproductive tract represents components of this system with unique features (Mestecky and Fultz, ١٩٩٩; Russell and Mestecky, ٢٠٠٢).

Locally produced secretory immunoglobulin A (S-IgA) is present in genital secretion, the dominant Ig isotype is immunoglobulin G (IgG), much of which is derived from circulation (Russell *et al.,* ١٩٩٩; Russell, ٢٠٠٢). Neumerous experiments have shown that local instillation of non-replicating antigens into vagina of experimental animals or of human female volunteers can result in the development of specific antibodies in the local secretion (Parr and Parr, ١٩٩٠; Haneberg *et al.*, ١٩٩٤; Wassen *et al.*, ١٩٩٦; Kozlowski *et al.*, ١٩٩٧). The presence of polymeric IgA secretes plasma cells in subepithelial tissue of female genital tract. Polymeric Ig receptor (PIgR), the membrane precursor from of secretory component (SC) have been demonstrated in the overlying epithelium (Kutteh *et al.*, ١٩٨٨). Secretory IgA (S-IgA) is assembled and transported into lumen. The mechanisms whereby IgG from the circulation, or produced by resident IgG secreting plasma cells, are transferred to the lumen remain unclear (Russell, ٢٠٠٢).

Although IgG, IgA and IgM have been reported in both pre- ejaculate and seminal plasma (Tauber *et al.*, ١٩٧٥; Flower and Mariano, ١٩٨٣). Higher levels of IgG than IgA are present in the ejaculate (Flower and Mariano, ١٩٨٣). However, the pre-ejaculate contains more IgA than IgG (Pudney and Anderson, ١٩٩٥). It appears that most IgG which is derived from S-IgA is of local origin (Rumke, ١٩٧٤; Tauber *et al.*, ١٩٧٥; Flower and Mariano, ١٩٨٣).

The secretion of both male and female reproduction systems contain significant levels of S-IgA, but these may be exceeded by IgG levels at least in human and other primates (Belec *et al.,* ١٩٩٥; Belec *et al.,* ١٩٩٥; Crowley *et al.,* ١٩٩٥; Kutteh *et al.,* ١٩٩٦; Brandtzaeg, ١٩٩٧; Hedges *et al.,* ١٩٩٩; Lu *et al.,* ١٩٩٩). Whereas S-IgA is accepted as the distinct product of the CMIS, the source and means of delivery of IgG are less clear; both local production and transudation from the circulation have been implicated (Anderson and Pudney ١٩٩٩ ; Kutteh, ١٩٩٩).

The immune responsiveness of the female genital tract must be seen in relation to its physiological functions in reproduction.

The lower tract (vagina) is colonized with a specialized microbiota and has a partially keratinized pseudostratified epithelium. The upper tract (uterus and fallopian tubes) is normally sterile but must permit the passage of allogenic sperm and support the development of semiallogenic fetus engrafted into the endometrium for prolonged period. Standing guard between these two regions is cervix, which possess a population of subepithelial plasma cells secreting IgG and PIgA and PIgAR- expressing epithelium that can transport S-IgA (Kutteh and Mestecky, ١٩٩٤ ; Kutteh, ١٩٩٩). Although it lacks organized MALT, it is clear that female genital tract can mount immune responses to MHC fetal antigens, sperm acrosomal antigens and certain infectious antigens as well as experimental vaccines (Parr, and Parr, ١٩٩٩).

Physiologically, there is an evidence that immune constituents aggregates are hormone – dependent association (Yeaman *et al.*, ١٩٩٧).

٢–١–٤: Sperm Antigens

Sperm cells consist of head, middle piece and tail. Such cell is rather complex in its structure. It has an array of structures including antigens. Antigens are bioorganic macromolecules that have two basic characters. The immunogenicity and the antigenicity (Parslow *et al.*, ٢٠٠١). The first character is defined as the ability to induce an immune response, while the second, are represent their ability to react with the outcomes of the induced immune response. Such material should be recognized by host immune system as foreign materials. The antigenic materials should be specific and their specificity can be attributed to the surface chemical groups known as epitops. Epitops have set of immunological functions (Stites *et al.*, ١٩٨٧; Parslow *et al.*, ٢٠٠١), such as:

١. Valency.
٢. Specificity.
٣. Immunogenicity.
٤. Antigenicity.

Sperm cells have several antigens with rather complex nature. These were distributed in the acrosome, middle piece and tail as well as the cellular protoplasms (Bronson *et al.*, ١٩٨٤; Pronson *et al.*, ١٩٨٧; Naz, ١٩٩٦).

Of these antigens, some were tissue specific and others, however, were sperm specific. Sperm also has been found to have autoantigens and isoantigens. Sperm, on the other hand, bears ABO and human leucocyte antigen, the membrane associated protein molecule of major histo compatibility complex (MHC). Sperm antigens can be particulate or soluble and / or both . They are mostly of protein in nature (Naz, ١٩٩٦).

To the men, sperm antigens are considered as an autoantigen, while to the female partners are considered as non self isoantigens. So the nature of the immune response in male is rather different than in female (Surrey, ١٩٩٧).

Hence, in men the immune response is of autoimmune nature, while for female partner it may tolerate these antigen or the induction may be through other mucosal parts by than an ordinary immune responses. Sperm antigen(s) in adult human are of sequestrated nature or occulate type. They possibly met immune cells under traumatic conditions (testicular trauma) and surgical intervention (Parslow *et al.*, ٢٠٠١).

In female partner, however, the sex intercourse may expose the mucosa of female reproductive tract to sperm antigens, in rather frequent fasion dependent on sex intercourse frequency. Eight sperm specific antigens are known and are mostly of peptide or protein in nature such as those mentioned below and in the table (٢-١) (Naz, ١٩٩٦).

Some of these sperm specific antigens were strong immunogens, while others were weak immunogens.

١. Lactate dehydrogenase C_4(LDH- C_4):

LDH – C_4 is a sperm specific but species – cross relative enzyme in testis of various animal species (Goldberg, ١٩٨٦; Millan *et al.,* ١٩٨٧; Shelton and Goldberg, ١٩٨٥).

٢. Rabbit Sperm Antigen (RSA):

RSA are three antigens of low molecular weight proteins that seem to function as lectin like molecules and to bind spermatozoa to zona pelluceda (Welch, ١٩٩٠ ; Orand *et al.*, ١٩٩٣).

٣. PHA – ٢٠ Antigen:

This is a protein antigen with a structure of glycosyl phosphatidyl inositol (GPI) – anchored membrane protein with molecular of ٦٤ KD. This antigen is found in the inner acrosomal and plasma membranes (Primakoff *et al.*, ١٩٨٨; Lathrop *et al.*, ١٩٩٠; Lin *et al.*, ١٩٩٣; Hunnicutt *et al.*, ١٩٩٦).

٤. Sp – ١٠ Antigen:

A sperm protein, SP – ١٠ is an intra acrosomal protein in human sperm with molecular weight of ١٨ – ٣٤ KD (Herr *et al.*, ١٩٨٥; Wright *et al.*, ١٩٩٠; Sirinivasan *et al.*, ١٩٩٥).

٥. HAS – ٦٥ Antigen:

It is composed of three soluble glycoprotein of ٥٠, ٤٣ and ٤٢ KD. It is immunogenic in female mice and inhibits fertilization (Liu *et al.*, ١٩٨٩; Liu *et al.*, ١٩٩٢).

٦. Fertilization Antigen – ١ (FA – ١):

It is glycoprotein antigen which is of mono ٢٣ KD and dimeric ٥١ KD forms. It develops in testisduring spermatogenesis and is involved in human infertility both in men and women (Naz *et al.*, ١٩٨٤; Naz, ١٩٨٧; Naz *et al.*, ١٩٩١; Coonrod *et al.*, ١٩٩٤; Naz *et al.*, ١٩٩٥).

٧. Fertilization Antigen – ٢ (FA – ٢):

It is protein antigen with molecular weight of ٩٥ KD isolated from human sperm. It reduces acrosome reaction and the release of acrosine from human sperm cells (Leyton and Saling, ١٩٨٩; Naz *et al.*, ١٩٩٣).

٨. Cleavage Signal Protein (CS – ١):

It is sperm surface antigen. That may provide the first signal for initiation of oocyte cleavage (Naz, ١٩٩٢; Javed and Naz, ١٩٩٢).

Serum Sperm Specific Protein Antigen

Sera of infertile subjects contain a set of ٩٨ sperm autoantigens and iosantigens but not from fertile subjects. At sperm surface, however, there are subset of ٦ auto and isoantigens (Shetty *et al.*, ١٩٩٩).

In another study however, sera from infertile subjects showed several sperm proteins ranging from ٣٤ – ١٠٥ KD, ٦٦ KD, ٨٨ KD and ١٠٥ KD (Pujianto, ٢٠٠٠).

Table (٢-١)

Molecular and Immunological Characteristics of Sperm Specific Antigens (Naz, ١٩٩٦)

No.	Antigen	Molecular Identity		Immunological activity	Cross reaction with human sperm	Involvement in human immuno-infertility
		Weight	DNA			
١	LDH – C_4	١٤٠ KD; ٤ subunits of ٣٥ KD	١١٧١ bp, aa (human) ٣٣١	Active immunization causes a reduction (up to ٥٠٪) infertility various species	Yes	No
٢	RSA	١٣ ± ٢ KD	٢١٨٦ bp, aa (rabbit) ٦٨٠	Active immunization triales not conducted; antibodies inhibit Murine IVF and human SPA	Yes	No
٣	PH – ٢٠	٦٤ KD	١٠١٠ – ٢١٥٦ bp, ٤٦٨ aa (guinea pig); ١٦٨٣bp, ٥٠٩ (٥٩٪ homology between species)	Active immunization causes a complete block in guinea pigs	No	No
٤	SP – ١٠	١٨ – ٣٤ KD; ipH ٤.٩	١١١٧ bp, ٢٥٦ aa (mouse)	Active immunization in baboons causes a partial reduction of infertility	Yes	No
٥	HAS – ٦٣	٣ proteins of ٥٠, ٤٣, and ٢ KD	١٠٦٧ bp, ٢٦١ aa (mouse)	Active immunization did not significantly affect fertility; absinhibit Murine and IVF human SPA	Yes	No
٦	FA – ١	Monomer of ٢٣ KD; dimmer of ٥١ ± ٢ KD	٢ putative human clones presenting being sequenced; decapeptide bioeffective epitope isolated	Active immunization did not significantly affect fertility; Abs completely block Murine IVF, bovine IVF, monkey IVF, human IVF	Yes	Yes
٧	FA – ٢	٩٥ KD	Presenting being cloned and sequenced	Active immunization studies not conducted; Abs completely block human SPA	Yes	Not done
٨	CS – ١	Double band of ١٤ and ١٨ KD	١٨٢٨ bp, ٢٤٩ aa (Human)	Active immunization studies not conducted; Abs inhibit early cleavages of zygotes	Yes	No

٢–١–٥: Sperm Antigen Processing

As the sperm antigens were recognized (Self, Nonself) by mononuclear cells such as macrophages and dendritic cells, sperm antigen were engulfed through phagocytosis and had been gradually broken down or cleaving (antigen processing) by exposure to an acid pH and to cellular proteolytic enzyme (Parslow *et al.*, ٢٠٠١).

Although the bulk of each protein is ultimately destroyed, many short peptides are produced as intermediate in this process and then making those peptides associated with major histocompatability (MHC II) determinants (antigen processing) accessible for recognition by T helper lymphocytes (Stites *et al.*, ١٩٩٨ ; Brodsky, ٢٠٠١).

٢–١–٦: Sperm Antigen Presentation

Macrophages serve as important presentating cells (APCs), that after recognition and opsonization of those affected antigens produce host immune response (Fenton and Vermeulen *et al.*, ١٩٩٦).

Antigen – processing cells present antigen to immune T cells in association with determinants, grouped as either MHC class I (HLA – A, B, C) molecule or MHC class II (HLA, DR, DP, DQ) molecules.

Whereas the MHC class I is synthesized inside the cell and migrate to the surface, they pick up fragments of foreign specific proteins (Ags) (Haregewoin *et al.*, ١٩٩١; Naz, ١٩٩٦; Stites *et al.,* ١٩٩٨).

Antigen presentation by vaginal cells is under hormonal control. Estradiol regulate antigen presentation by vaginal cells and that vaginal cells, in turn influence antigen presentation as well as B and T cell proliferation (Wira *et al.*, ٢٠٠٠).

٢–١–٧: Sperm Antigen Recognition

The sperm specific peptide located on the surface of APCs in conjugated with MHC II molecule was presented to either T helper ١ ($Th_١$) or T helper ٢ ($Th_٢$) or / and both T cell subsets. T cell receptor (TcR), $CD_٣$ and MHC – sperm peptide form trimolecular complex which form the recognition groove, these sending stimulatory signal which leads to Th activation which in turn triggers B cells or other T cell subset to grow up, proliferate and produce antibodies (B cells) or cytokines (T cells) (Parslow *et al.*, ٢٠٠١).

Details on T cells activation can be summaized up in the following: these cells secrete proinflammatory mediators such as interleukin (IL-١٢). IL – ١٢ is key cytokine immune regulation promoting both NK – cell and cytotoxic T lymphocyte activity, also IL – ١٢ modulates IgA synthesis and induce T cell commitment from the T helper O (ThO) to $Th_١$ phenotype characterized by interferon gamma (IFN – γ) secretion (Parslow *et al.*, ٢٠٠١).

IFN – γ secretion by $Th_١$ cells mediates both cellular immunity and organic specific autoimmunity (Surrey, ١٩٩٧).

٢–١–٨: Sperm Primed Memory Lymphocytes

Memory lymphocytes are either memory B cell in association with antibody response or memory T cell in association with T cell mediated immune reaction or T cell mediated delayed hypersensitivity reactions (Playfair & Chain, ٢٠٠١).

As antibody mediated sperm specific immune responses are mounted and vaccination program is in the way in human and animals. Thus, sperm specific memory B cells are found. Like sperm specific memory T cell are also happened (Parslow *et al.*, ٢٠٠١; Playfair & Chain, ٢٠٠١).

Memory T cells are biochemically distinct in that they can be stimulated by lower doses of the antigen. Memory B cells, however, encourage the increases in the maturation of antibody mediated response as whole (Playfair and Chain, ٢٠٠١). Thus memory lymphocytes are formed of effector cells capable of responding rapidly to the reintroduction of the same antigen (Gilbert *et al.*, ١٩٨٨; Parslow *et al.*, ٢٠٠١).

٢–١–٩: Sperm Mediated Cytokine Release

Cytokines secreted peptide of hormone – like nature. They are secreted by immunocompetent cells during cell – cell cooperation in the immune response events. Cytokines are basically of two types; lymphokines secreted by lymphocyte and monokines secreted by monocytes. Cytokines have the function of an intracellular signal transduction which can regulate inflammatory and immune reactions through regulation of growth, motility and differentiation of leucocyte and

non leucocyte cells. The secretion of cytokine together with hormones and neurotransmitters are sorts of chemical signal transduction language that regulate development, repair of tissue and immune responses (Parslow *et al.*, ٢٠٠١; Roitt *et al.*, ٢٠٠١).

There are several common functional features of cytokines as pleotropy, autocrine, paracrine, endocrine and synergism. Cell – cell communication is established through cytokine secretions. The cytokines can be functionally classified into three broad categories:

١. Cytokines that regulate non specific immunity (IFN – γ , IL – ١٢, IL – ١٥, TNF, IL – ٦, IL – ١٠ and chemokine).

٢. Cytokines that regulate specific immunity (IL – ٢, IL – ٤, (Transforming growth factor β (TGF– β), IFN – γ , and lymphatoxins).

٣. Cytokine that stimulates processes of blood cell formation (IL – ٣ and IL – ٧).

Stem cells developed to lymphoid series through IL – ١, IL – ٢ action to NK. IL – ١, IL – ٢ and IL – ٤ evolve T cells. B cells, however are developed through the action of IL – ١, IL – ٢, IL – ٤, IL – ٥ and IL – ٦. (Parslow *et al.*, ٢٠٠١).

Stem cells developed to monocytes and granulocytes through the action of granulocyte-macrophage colony stimulating factor (GM – CSF). (Tabibzadeh and XZ, ١٩٩٢; Simon *et al.,* ١٩٩٣; Hunt *et al.,* ١٩٩٣; Wira *et al.,* ٢٠٠٢).

The Th_1 cytokines release is mostly involved in cellular immunity, whereas Th_2 cytokines activate B cells to grow and proliferate to plasma cell secreting antibodies.

Sperm antigenic epitopes are either Th_1 inducer and / or Th_2 inducers (Surrey, ١٩٩٧; O'Neil *et al.*, ١٩٩٩). Thus, they mediate either Th_1 or Th_2 cytokine release.

It has been found that increased cytokine of Th_1 by the circulating T cells present in women with multiple pregnancy losses and in infertile women (Kwak – Kim *et al.*, ٢٠٠٣).

Semen quality is associated with some proinflammatory cytokines. All leucocytospermic samples have high IL – ٨ concentrations. IL – ٨ is correlated with IL – ٦ concentration in seminal plasma. IL-٨ might be used as sensitive marker for silent male genital tract infection (Eggert – Kruse *et al.*, ٢٠٠١).

٢–١–١٠: Sperm Specific Hypersensitivity

Some of sperm specific T cell epitopes may have the potential to trigger Tdth subset of T lymphocyte on the second exposure to the same epitope.This can be terminated by the

immunologic tissue injury associated with type four delayed type hypersensitivity reactions (Roitt *et al.*, ٢٠٠١).

Immediate type hypersensitivity had reported to human semen plasma in women (Dutch, ١٩٥٨; Halpern *et al.,* ١٩٦٧; Chang, ١٩٦٧; Shah *et al.*, ١٩٨٨; Bernstein *et al.,* ١٩٩٧; Shah, ٢٠٠٠; Shah and Punjabi, ٢٠٠٤).

Male autoimmune response to sperm antigens may be classified as immune complex type three hypersensitivity.Guinean pig orchitis (experimental) can be described as stimulatory type V hypersensitivity (Roitt *et al.*, ٢٠٠١).

٢–١–١١: Evaluation of Immune State of Infertile Couples

٢–١–١١–١: Immune Responses

After sperm antigen presentation to B and T cells, the antigen induced B and T cells ($CD^{+}_{٤}$ and $CD^{+}_{٨}$) are able to migrate via efferent lymphatic vessels and through mesenteric node; they reach the systemic circulation through the thoracic and repopulate not only the lamina properia of the intestine but other distant mucosal sites such as respiratory, urogenital, mammary and salivary gland. The result of this process is that by gut stimulation, distant mucosal sites can be repopulated with IgA producing cells to protect these surfaces. This phenomenon has been termed the Common Mucosal Immune System (CMIS) (Cebra *et al.*, ١٩٩١). However, in spite of the scientific evidence of the ability of CMIS to induce a good local mucosal response, local stimulation is also required (Gabriela *et al.*, ٢٠٠١).

٢–١–١١–٢: Male Genital Tract

Evaluatin of immune parameters in male genital tract consists of immunoglobulins, cytokine level and immune cellular elements in semen (Belec *et al.*, ١٩٩٥; Quayle *et al.*, ١٩٩٧; Anderson *et al.*, ١٩٩٨). Sample must be processed rapidly because immune cells (lymphocytes and monocytes) are present at low number in semen (Anderson *et al.*, ١٩٩٨). Cytokine levels had been quantified in semen and that tumour necrosis factor β (TNF $-\beta$), interleukine $-$ ٨ (IL $-$ ٨) and IL $-$ ٦ can be detected in seminal samples for both HIV positive and negative men (Anderson *et al.*, ١٩٩٨). Interferon gamma (IFN $-\gamma$) can also be detected in semen. Evaluation of cellular elements in semen showed predominance of $CD^{+}_{٨}$ T cells, which showed specific cytotoxic activity (Quayle *et al.*, ١٩٩٨).

٢–١–١١–٣: Female Genital Tract

Whereas the evaluation of female genital tract of quantification of antibodies level, cytokines and cellular immune elements via the cervicovaginal lavage (CVL) as a collection method

(Quesnel *et al.*, ١٩٩٧) and cytobrush for obtained cervical cells for cytokine quantification (Musey *et al.*, ١٩٩٧; Shacklett *et al.*, ٢٠٠٠).

Besides, these techniques for obtaining immune cells subset from female genital tract are neither well-developed nor standardized. Nevertheless, the study demonstrated that IgG Ab is predominat in female genital tract than IgA Ab (Belec *et al.*, ١٩٩٥; Lu *et al.*, ٢٠٠٠) and the endocervical sampling produce highest cellular yields consisting of T cells, B cells and monocytes (Bardeguez *et al.*, ١٩٩٧). $CD^{+}_{٤}$ and $CD^{+}_{٨}$ T cells can both be found and $CD^{+}_{٨}$ cells in preponderance. $CD^{+}_{٤}$ and $CD^{+}_{٨}$ T cells have been detected in uterine, cervical and vaginal tissue biopsies (Givan *et al.*, ١٩٩٧), while, cervical mucosa show slightly more $CD^{+}_{٨}$ than $CD^{+}_{٤}$ cells, but both population have a predominant memory phenotype ($CD_{٤٥}RO^{+}$, $CD_{٤٥}RA^{-}$) and express the chemokine receptor $CCR_{٥}$ (Patterson *et al.*, ١٩٩٨).

Thus, immunodiagnosis of infertility can be summarized as followes: workers on the diagnosis of immune infertility have been putforward several types of immunoassay that can be followed to assess infertility(Gilbert *et al.,* ١٩٨٨). As in:

Assay Types

١. Cellular:

- Cytokine Determination
- Lymphoblast Transformation Test
- Leucocyte Inhibitory Factor

٢. Humoral Antisperm Antibody

- Direct Agglutination
- Standard Tube Agglutination
- Passive Haemagglutination
- Sperm Immobilization Test
- Cytotoxicity Tests
- Immunoflourescence Tests
- Mixed Antiglobulin Test
- Radio Immuno Assay
- ELIZA Test
- Immuno Bead Binding Test (Gilbert *et al.*, ١٩٨٨; Choudhury and Knapp, ٢٠٠٠; Bohring and Kruse, ٢٠٠٣).

٢–١–١٢: Diagnostic Utility of SSA

In a survey on SSA utility in diagnosis of immune infertility, several scores were noted by different investigators as in tables (٢-١١A,B,C) respectively. (Beer and Neaves, ١٩٧٨)

Table (٢–١١ A)

	SSA Positive %		
	Agglutination	**Immunization**	**IFA**
Fertile Non Pregnant Women	٢.٧	–	–
Pregnant	٢١.٤	٠	٢٩.١
Organic Infertility	–	٥.١٥	٣٣.٣
Unexplained Infertility	١٤	٨.٩٥	٢٩.٧

While, Faulk and Fox, (١٩٨٢) had been fixed SSA:

Table (٢-١١B)

Entity	SSA Positive %
Normal Fertile men	٠ – ٢.٨
Normal Infertile men	٣.٢– ٢٠.٦

While, Backrman and Dudley, (٢٠٠١) have been mentioned SSA % of positive as in following table.

Table (٢-١١C)

Entity	SSA Positive %
Fertile Women	١ – ١٢
Infertile women	١٠ – ٢٠

Finally, the studies of Hjort and Mienertz, (١٩٨٦) had mentioned that titer of ١٦ or more may be clinical titer as found in ٤٣ infertile men sera and ٣٠ semen plasma samples of infertile men. Faulk and Fox, (١٩٨٢) had mentioned that those infertile men with SSA titer of ١٠٢٤ can never down to zero and ٥٠ % of that SSA titer of ٣٢ became fertile and succeeded in producing pregnancy in their mates.

Hinting *et al.*, (١٩٨٨) had been referred that ١٦ as a titer of SSA in infertile men sera.

Adeghe *et al.*, (١٩٨٧) found serum SSA among infertile men can never exeed ٦٤ in ٤ out ٢٣
As far as mucosal SSA is concerned, Hjort and Mienertz, (١٩٨٦) said that titer of ١٦ serum samples. or more using TAT was indicative in ٣٠ semen plasma samples of infertile men.

Apparently SSA titer with ١٦ and upward seems to be significant (Hjort and Mienertz, ١٩٨٦; Hinting *et al.*, ١٩٨٨).

٢–١–١٣: Sperm Immunity in Experimental Laboratory Animals

In Guinea pigs, it had been produced experimental allergic orchitis (EAO). Meanwhile, EAO had been reproduced in birds, mice, rabbits and bulls. In addition to, rabbits experimental immune complex orchitis had been induced from following vasectomy. Finally, experimental isoimmunization had been investigated in Guinea pig, mouse, rabbit and bovine (Jones, ١٩٨٨).

sperm specific antibody was determined in vasectomy and vasovasotomy rat, rat sera can detect set of dominant sperm autoantigen (Flickinger *et al.*, ١٩٩٥).

Repair of obstruction and injured vas deference before puberty to forestall development of sperm specific antibody (Flickinger *et al.*, ١٩٩٥).

Immune response, mechanical elements and structural changes in reproductive tract are the multifactors that affect fertility in rat (Flickinger *et al.*, ١٩٩١).

Natural antisperm autoantibodies appeared in Lewis rat after sexual maturation (Flickinger *et al.*, ١٩٩٧), and the epididymal obstruction during development induces antisperm autoantibody at puberty in rats (Flickinger *et al.*, ١٩٩٨).

Part Two: Physiology

٢-٢: Physiology

٢-٢-١: Factors Affecting Sperm Parameters

It had been shown that sperm production is affected by several factors, such as; general health, age (Schwartz *et al.*, ١٩٨٣; Al-Janabi, ١٩٩٢; Centola and Eberly, ١٩٩٩ and Kidd *et al.*, ٢٠٠١); Season of the year, (the peak in autumn and lowest in summer) (Alwachi and Ashir, ١٩٩١); 'smoking, nutrition ,environmental considerations and. .. etc. factors' (Politoff *et al.,* ١٩٨٩; Zovas *et al.*, ١٩٩٩; Steven Sinclair, ٢٠٠٠; Simon, ٢٠٠٥).

Many studies examined the effects of cigarette smoking on fertility, and cumulative evidence suggests that it had a significant negative impact on sperm production, motility and morphology (Thompson, ١٩٩٤); other studies demonstrate that the mutagenic and carcinogenic components of cigarette smoke have adverse effect on rapidly dividing cells, including germ cells in the testis (Stillman *et al.*, ١٩٨٦). Animal investigations had shown that nicotine, cigarette smoke and / or polycyclic aromatic hydrocarbons can cause in some cases testicular atrophy, poor sperm morphology and overall impaired spermatogenesis, as well as the presence of oligospermia and teratospermia. (Stillman *et al.*, ١٩٨٦). Serum levels of prolactine (Pr) and estradiol ($E_٢$) are also elevated in smokers (Thompson, ١٩٩٤).

Klaiber and Broverman, (١٩٨٨) found that the mean serum level of $E_٢$ and production rate of estradiol were significantly greater in smokers than in non smokers that might lead to decrease in sperm concentration. As well as, temperature, nutrition, and stress (Sarrel and Decherny, ١٩٨٥; Giblin *et al.*, ١٩٨٨); diseases as mumps (Andrada *et al.*, ١٩٧٧; Bread *et al*., ١٩٧٧; Shulman *et al.*, ١٩٩٢) and toxic substances affected semen quality (Smith and Asch, ١٩٨٧). Many other factors affected sperm motility *in vitro* such as cyclic Adenosine monophosphate (cAMP) which is an important regulator of sperm motility (Rojas *et al.*, ١٩٩١) depends on Mn^{++} and Mg^{++} as divalent cations and showed optimum pH between ٧ and ٨.٥. The synthesis of cAMP is greatly influenced by Ca^{++} and $HCO_٣^-$ in a concentration manner; these ions are present in seminal plasma and female genital tract. Ca^{++} and $HCO_٣^-$ are physiological modulators of sperm motility and function in human (Rojas *et al.*, ١٩٩١).

Prostaglandins (PG) had been shown to increase sperm motility. They stimulate adenylate cyclase and intracellular levels of cyclic adenosine monophosphate (Tash and Means, ١٩٨٣; Alawchi and Al- shakrchi, ١٩٨٧; Gottieb *et al.*, ١٩٨٨).

Platelet – Activating Factor (PAF) played an important role in mammalian reproduction (Harper, ١٩٨٩). The PAF content in human spermatozoa has a significant and positive relation with sperm motility, concentration indices and implantation rate (Roudebush and Purnell, ٢٠٠٠). Beside their ability to duration of sperm *in vivo* survival time (Kardan and Strzezek, ٢٠٠٢).

React oxygen species (ROS) are produced by spermatozoa leucocytes in both fertile and infertile men as a result of lipid peroxidation. (Rao *et al.*, ١٩٨٩; Aitken *et al.*, ١٩٩٣). Excessive ROS causes oxidative stress, resulting in decreased sperm motility, viability and increased midpiece defects that impair sperm capacitation and acrosome reaction (Aitken, ١٩٩٧; De Lamirande *et al.,* ١٩٩٣; De Lamirande *et al.*, ١٩٩٥).

ROS generated by the WBCs, especially polymorphonuclear leucocytes (PMNS) or granulocytes, can exert a deleterious effect on human spermatozoa as indicated by marked loss of sperm motility and a reduced capacity for oocyte penetration, that contaminate PMNs is a major source of reactive oxygen species activity recorded in human sperm is incontrovertible (Baker *et al.*, ١٩٩٦). ROS effects can partially inhibited in the presence of P – I and human tubal fluid media, human serum albumin, phenol red, glucose, sucrose, polyvinyl alcohol (Alexander *et al.*, ١٩٩٩).

Plante *et al.* (١٩٩٤) concluded that the presence of activated PMNs rather ROS – producing spermatozoa is likely to be a factor responsible for a possible loss of motility in the normal sperm population present in the same sample (Mohamed *et al.,* ١٩٩٩).

Human Follicular Fluid (HFF); Follicular Fliud present to have many different biofunctional fractions that enhanced hyperactivation motility in human sperm, such as estrogen, ١٧B estradiol ($E_٢$), testosterone and progesterone (Frachimont *et al.*, ١٩٨٩). Besides presence of other components like platelet activating factor, glycosamineglycans and albumin in HFF. Therefore HFF were used as efficient stimulatent that improve the sperm motility in IVF test (Minhas and Ripps, ١٩٩٦). Additive HFF was also significantly increased to percentage of sperm motility, grade activity (Al-Hady, ١٩٩٧), and reduce the negative percentage of the penetration assay (Yee and Cumming, ١٩٨٨; Blumenfeld and Nahhas, ١٩٨٩; McClure *et al.*, ١٩٩٠).

Human FF promotes capacitation and the acrosome reaction within a short period. It also stimulates or maintains various sperm motility parameters (Yuan-qing yao *et al.*, ٢٠٠٠). *In vitro* sperm activation by HFF maintained their velocities and hyperactivation motility to be remained

stable for ٣ hours, whereas that of control spermatozoa decreased significantly after ١ hour (Yao *et al.*, ٢٠٠٠).

In accordance with what is mentiond above, sperm motility has been a critical factor in judging semen quality and the grade of this motility influences the fertilization rate. In the lower female reproductive tract, motility is needed to penetrate cervical mucus, while in the upper tract only capacitated sperm with the vigorous beating, of the tail is necessary for penetration of the zona pellucida (Yanagimachi, ١٩٩٦). In addition, successful oocyte fertilization *in vitro* or by artificial insemination requires spermatozoa with adequate motility.

Prostasomes (submicron, membrane – surrounded organelles produced by the epithelial cells of human prostate gland and are present in appreciable amounts in normal semen) (Brody *et al.*, ١٩٨١). Prostasomes, however, were more efficient since they rendered a higher proportion of forwardly motile spermatozoa with a higher amplitude of lateral head displacement at a lower concentration, both parameters being positively correlated the fertilizing potential of spermatozoa (Fabiani *et al.*, ١٩٩٤).

The prostasomes also increased the number of hyperactivated spermatozoa, which is thought to be an important parameter for penetration of zona pellucida and subsequently for fertilization (Coodington *et al.*, ١٩٩١).Hence, prostasomes might be able to protect the spermatozoa against phagocytosis, thus facilitating the survival of the sperm (Kelly *et al.*, ١٩٩١; Lena Carlsson, ٢٠٠١).

٢–٢–٢: Male and Female Infertility Factors

٢–٢–٢–١: Male Infertility Factors

Term infertility is defined as the failure to conceive after one year of frequent unprotected intercourse (Reiss, ١٩٩٨; WHO, ١٩٩٩).

Male Infertility factor may involved of :- Aspermia, Azoospermia, Oligozoospermia, Asthenozoospermia, Necrozoospermia, Teratozoospermia, Leucocytospermia additional to that of Immunological fartors as in the following:

١. **Aspermia:** A case combined with patient can produce completed ejaculation process but without evidence of containing semen. This type of infertility was due to retrograde ejaculation (Guyton, ١٩٩٧).

٢. **Azoospermia:** that means the absence of sperm in semen (Dubin and Amelar, ١٩٧١).Azoospermia can be divided into:

A. Obstructive azoospermia are also called excretory azoospermia that are present with normal spermatogenesis but with obstruction of the vas deference and / or epididymis ductal system, which is present in ٤٠ % of the total azoospermic patient. The cause of this kind of infertility was associated with infection such as gonorrhea, tuberculosis, Chlamydia and bilharzias, or may be associated with congenital absence of the vas deference to reach about ١٠ – ٢٥ % of the azoospermia as well as to that of surgical operation mistake such as hernia.

B. Serious dysfunction of the testicular parenchyma, also called secretory azoospermia which involved maturation arrest (hypospermatogenesis), hypoplasia include all germinal cells (Jequier, *et al.*, ١٩٧٩; Garcia – Diez *et al.*, ١٩٨٣; Yogev *et al.*, ٢٠٠٠). Sertoli cell syndrome due to hypogonadotropic hypogonadism (Rothman *et al.,*١٩٨٢; Garcia – Deiz *et al.*, ١٩٨٣) and finally due to disfunction or the defect of the hypothalamic – pituitary axis (Gross *et al.*, ١٩٨٦).

٣. **Oligozoospermia:** means decrease the concentration of spermatozoa in the seminal fluid (Vermeulen and Comhair, ١٩٧٨). WHO (١٩٩٢), recommended the value of ٢٠ million / ml as the lower limit of normal sperm concentration.

Oligozoospermia may resulte in androgen deficiency caused by pituitary adenoma or prolactinoma with high level of prolactin and low level of serum testosterone (Sueldo *et al.*, ١٩٨٥; Aiman *et al.*, ١٩٨٨; Skakkebaek *et al.*, ١٩٩٤).

There is a significant inverse relationship between seminal prolactin concentration and sperm count, motility in oligozoospermic patient. ٢٠ % of oligozoospermic patients showing elevated FSH and normal LH level, several opinions had been proposed for the high FSH level such as decreased gonadal production of inhibin and decreased gonadal production of sex steroids (Gross *et al.*, ١٩٨٦). FSH level associate directly with spermtogenesis, while there was proved evidence that low sperm count correlated with inactivate of germ cell production with a normal FSH level in many oligozoospermic patients (Matzkin *et al.,* ١٩٩٠).

Varicocele was present as abnormal tortuosity and dilatation of the testicular veins within spermatic cord. Varicocele was found in approximately ٣٠ percent of infertile males (Dubin and Amelar, ١٩٧١; Hendry *et al.*, ١٩٧٥; Rodriguez – Riguan *et al.*, ١٩٧٨; Cockett *et al.*, ١٩٧٩; Aafijes and Vander Vijver, ١٩٨٥; Marks *et al.*, ١٩٨٦; Comhaire, ١٩٩١).Varicocele was found in ٢٤ % of men with abnormal semen especially those oligozoospermic patients.

When mumps orchitis involve pupertal or adult testis, varying degree of 'permanent damage' usually occurs in the seminiferous tubule. Generally orchitis is followed by marked decrease

in sperm quality with sever oligozoospermia or even azoospermia (Shulman *et al.*,١٩٩٢). Testicular damage autoimmunity may be responsible for the oligozoospermia of cases that complained either obstruction, vasectomy of the vas deference, or many other chronic infections (Sharlip, ١٩٨٤; Golomb *et al.*, ١٩٨٦; Zhong *et al.*, ١٩٨٩).

Genetic causes factors including chromosome abnormalities and its variants were almost associated with oligozoosperima and / or azoospermia (Simpson *et al.*, ١٩٩٣; Kun *et al.*, ١٩٩٣; Foresta *et al.*, ١٩٩٨; Gazvani *et al.*, ٢٠٠٠).

٤. **Asthenozoospermia:** sperm motility is one of the most important criteria of semen quality because only motile spermatozoa pass through the female genital tarct to achieve fertilization of oocytes in the ampulla (Amelar *et al.*, ١٩٨٠; Aitken *et al.*, ١٩٨٣).

The ejaculate may be considered abnormal (Asthenozoospermia) if < ٥٠ % of spermatozoa within one hour after ejaculation showed a decrease in sperm motility. So that asthenozoospermia was an indication of a significant reduction in sperm motility and male infertility (Zavos *et al.*, ١٩٨٨; Ryder *et al.*, ١٩٩٠).

The cAMP, calcium, ATPase and energy source played an important role in modulating the function of flagellar axoneme. The ATP was necessary for contraction and generated by metabolic events that took place in the cytoplasm and mitochondria of midpiece (Courtade *et al.*, ١٩٩٨). The concentration of cAMP showed an effect on sperm metabolism, motility and fertilization. $Ca^{+٢}$ has multiple actions on sperm metabolism with appearance of forward progressive motility, and the mechanisms of regulated the free cellular calcium are:

١. Transport and releasing of the accumulated $Ca^{+٢}$ between the mitochondria and the cytoplasmic component of the sperm.

٢. Absorption of $Ca^{+٢}$ through the cell membrane and capturing via the Mg – Ca pump (Fakih *et al.*, ١٩٨٦).

Studies in human suggested that use of sperm stimulate such as cAMP and $Ca^{+٢}$ to the activated sperm media improved the increase of sperm motility of asthenozoospermic patient (Yovich *et al.*, ١٩٩٠).While, other study found that addition of ١٠٠ mM of phosphate critean and ١mM of $Ca^{+٢}$ to the IVF activated media, show significant increases of both velocity and motility of the asthenozoospermia, as the hyperactivity of motile sperm close associated with capacitation that depended on the presence of high cellular levels of $Ca^{+٢}$ and cAMP (Pang *et al.*, ١٩٩٣).

On other hand, the presence of SSA in serum or seminal plasma of infertile men may reduce the motility of spermatozoa and their persistence in the female genital tract (Mathure *et al.*, ١٩٨٤).

Also presence of WBCs in seminal plasma shows a significant correlation with many sperm parmeter, as in sperm motility, which reduce in about ٢٢ % during exhibit of WBCs in semen (Wolff *et al.*, ١٩٩٠).

٥. **Necrozoospermia:** It's another factor of infertility present with ejaculate patients, who had both normal morphological sperm and concentration, but with characterization of complete immotile dead sperm (Dubin and Ameler,١٩٧١). This type of infertility belonged to either microstructural spermatozoal changes (Wilton *et al.,* ١٩٨٨; Ryder *et al.,* ١٩٩٠).Or may be related to epididymal necrospermia which was associated with an obscure pathogenesis of nonmotile dead sperm causes (Kretser *et al.,* ١٩٩٨; Mallidis *et al.,* ٢٠٠٠).

٦. **Teratozoospermia:** Teratozoospermia was regarded as important abnormal infertility factors, which are classified according to WHO as follows:

A- Head shape/size defects, that, are occurance during a series spermatogenesis failure producing of abnormale sperm such as ; macrocephalic, microcephlic, tapering, round, pin & pyriform amorphous head spermatozoa.

B- Neck & midpiece defects, including irregular midpiece, persistent cytoplasmic droplete, bent midpiece or any combination of these (Kruger *et al.*, ١٩٨٦; ١٩٨٨; WHO, ١٩٩٩; Zahalsky *et al.*, ٢٠٠٣).

C- Tail defects, appears with short, multiple, hairpin, broken, irregular width or coiled tails, as well as tail present with terminal droplets, or any combination of these (Kruger *et al.*, ١٩٨٨; WHO, ١٩٩٢; ١٩٩٩; Zahalsky *et al.*, ٢٠٠٣).

٧. **Leucocytospermia**: this factor had been mentioned in (٢-١-٢) dealing with non specific celluler immune function.

٨. **Immunological factors :** this item has been clearly explained in (٢–١–٣) of sperm specific antibody .

٢–٢–٢–٢: Female Infertility Factors

Infertility in women may be associated with one or more of the following factors: -

١. **The cervical factor** : Cervical factor refers to the ability of cervix to nurture and transport sperm into the upper female reproductive tract. In typical female cervix large amounts of clear watery mucus were produced just prior to ovulation .The thin mucus allows sperm easy enter into the fallopian tubes in order to reach egg. After ovulation, the cervical mucus thickness may inhibit the sperm from reaching the fallopian tubes.

Several factors appeared to impair the quality & quantity of cervical mucus. These include:

- Intrauterine exposure to diethylstilbestrol (DES)
- Previous cone biopsy
- Infection
- Use of clomiphene citrate (Clomid)
- Anti-sperm antibodies (Hostility cervix) (Surrey, ١٩٩٧; Stuart Campbell & Less, ٢٠٠٠; Symond and Ian, ٢٠٠٤)

٢. **The Ovulatory factor** : The ovulatory factor refers to the ability of women to normally undergo the process of ovulation .So many problems may interfere with this normal ovulatory process such as LH & FSH organized hormone, that's may be unbalanced when associated with some causes like;

- Polycystic ovarian syndrome (PCOS) also known as hyperandrogenic chronic anovulation is a benign disorder that commonly result in infertility (Franks, ١٩٩٥; Aldolescent Medicine, ٢٠٠٠; Alaf, ٢٠٠٠).
- Hyperprolactinemia–this refers to excessive production of the prolactin.
- Chemotherapy, Infections,or tumors of hypothalamus, pituitary gland which affect the normal release of the hormones.
- Early Premature ovarian failure (Menopause) in this case the ovaries are unable to produce a developing egg .

٣. **The Uterine Factor :** refers to any abnormalities of the uterus or the uterine lining that may interfere with implantation of the embryo and /or maintenance of the pregnancy. Hence, the uterine contributing factors are:

- Fibroids.
- Abnormally shape uterus (as "T shape uterus").
- Poor endometrial lining associated with a dvanced age.
- Repeated clomiphene citrate treatment .
- Scarring and inflammation from endometeriosis .
- Luteal phase defect (Symonds and Ian, ٢٠٠٤).

٤. **The Pelvic Factor :**Pelvic factor primarily refers to problems affecting the fallopian tubes and other structures with the pelvic cavity. Patent fallopian tubes and a normal relationship between the pelvic organs are essential to enable sperm to reach the egg and travel to the

uterus after fertilization. Factor interfering with this process can impede pregnancy. There are various conditions which can contribute to infertility due to the pelvic factor:

- Pelvic Inflammatory Disease (PID): PID can result from any number of infections that occured within the pelvic area. Such as Seuxal transmitted diseases (STDs), *Gonorrhea* and *Chlamydia.* To a lesser extent, other microorganisms like *E. coli*, Group B *Streptococci*, *Peptostreptococci* and genital *mycoplasma*. (Fauci*,* ١٩٩٨).Frequent infections can cause scarring and damage to the fallopian tubes, which are significantly impact fertility.
- Endometriosis: Endomterial implants can cause scarring and adhesions inside the fallopian tubes and the pelvic cavity, thus impacting fertility.
- Prior pelvic surgery: Prior surgery in the pelvic area can cause bands of scar tissue to form in such a way that they interfere with the normal relationship between the pelvic organs. (*Hardman,* ١٩٩٦; Fauci*,* ١٩٩٨; D'Hooghe *et al.,* ١٩٩٩). As well as to many other factors such as; drug abuse, sever inllness, systemic disease, stress, fatigue, heavy/strenuous exercise and diet (Clarke & Baker, ١٩٩٣; Surrey, ١٩٩٧; Adolescent Medicine, ٢٠٠٠; Symonds and Ian, ٢٠٠٤).

Chapter Three

Materials and Methods

٣–١: Solutions

٣–١–١: Physiological Normal Saline (NS)

This solution was prepared at a concentration of ٠.٨٥٪ by dissolving ٠.٨٥ gm of sodium chloride (NaCl), (BDH Company), in small amount of distilled water (D.W.), then complete to final volume of ١٠٠ ml with distilled water and autoclaved for ١٥ min. at ١٢١°C, ١٥pound/inch^{٢}. Normal saline was used in titration of antibodies in systemic and mucosal preparation of sonicate normal human sperm and preparation of the other needed solutions (Cruickshank *et al.*, ١٩٧٥).

٣–١–٢: Formal Saline (FS)

This solution was prepared by adding ٠.٥ ml of ٤٠٪ formaldehyde (H–CHO), (BDH Company), to ٩٩.٥ ml sterile normal saline to final concentration of formalin in this solution ٠.٥٪. this solution was used as a solvent for immunoglobulin from seminal plasma, cervical mucous secretion, serum of fertile and infertile men and women (Kwapiniski, ١٩٧٢; Cruickshank *et al.,* ١٩٧٥).

٣–١–٣: Phosphate Buffer Saline PBS (pH = ٧.٢)

This buffer was prepared by dissolving one buffered disc in ١٠٠ ml distilled water and sterilized by autoclaving (١٢١°C, ١٥ pound / inch^{٢} for ١٥ minutes) in accordance with the instructions of the manufacture (BDH Company).

٣–١–٤: Alsever's Solution (pH = ٦.١)

To prepare this solution, the following ingredients were dissolved in ١٠٠ ml of distilled water:

Glucose (BDH) ٢.٠٥ grams.

Na–citrate (BDH) ٠.٨ grams.

NaCl (BDH) ٠. ٤٢ grams.

pH was adjusted to ٦.١ by ١٥٪ of citric acid.

The solution was sterilized by autoclaving (١٢١°C, ١٥ pound / inch^{٢}, for ١٠ minutes). This solution was used as anticoagulant, preservative and transport medium for sheep red blood corpuscles (Garvey *et al.*, ١٩٧٧).

٣–١–٥: Polyethylene Glycol (PEG–٦٠٠٠) Solution

This solution was prepared at a concentration of ٦٪, by dissolving ٦ gm of PEG–٦٠٠٠ (OH $(C_{٢}H_{٤}O)_{n}$ H), (BDH Company), molecular weight (٦٠٠٠–٧٥٠٠) in small amount of Tris buffer solution as in (٣–١–٦). Then the volume was completed to ١٠٠ ml in volumetric flask. It was used to separate immunoglobulin of serum and urogenital (male, female) secretory tract and gastrointestinal (male, female) secretory tract (Johnstone and Thorpe, ١٩٨٢; Al-Sa'adi, ١٩٩٨; Shnawa and Thewaini, ٢٠٠٢).

٣–١–٦: Tris Buffer Solution

This buffer solution was prepared by dissolving ١٢ gm of Tris powder ($NH_{٢}C$ $(CH_{٢}OH)_{٣}$), (TAAB Company), molecular weight (١٢١.١٤) in small amount of D.W., then the volume was completed to ١ litter. The pH of the Tris buffer were neutralized to become (٧.٠) by using ٠.١ molary HCl. The solution was used for preparing of polyethylene glycol solution (Johnstone and Thorpe, ١٩٨٢).

٣–١–٧: Hank's Balance Salts Solution (HBSS)

This solution was used for cell suspension, preservative and diluant cellular media. It was prepared by dissolving ١ gm of Hank's balance salts powder in ١٠٠ ml of distilled water to be mixed gradually.

Toxic chemical compound of sodium azide $NaN_{٣}$ molecular weight (٦٥.٠١, BDH Company) were added of (١/١٠٠ ٠٠٠) mg to the mixture. In this work the Hank's solution were used as stock solution, diluant solution and concentrated sperm constant suspended solution (Frei *et al.*, ١٩٩٥).

٣–١–٨: Tanic Acid Solution

This solution was prepared at a concentration of ٠.٥٪ by dissolving ٠.٥ gm powder of Tanic acid ($C_{٧٦}H_{٥٢}O_{٤٦}$), molecular weight (١٧٠١.٢٢, BDH Company), in small amount of D.W., then was completed the final volume of ١٠٠ ml. this solution was used to remove the surface antigens of sheep red blood corpuscles (Garvey *et al.*, ١٩٧٧).

٣–١–٩: Suspension of Sheep Red Blood Corpuscles (SRBCs)

This suspension blood was prepared as follows: fresh blood was taken from the neck jugular vein of sheep and bottled into sterilized bottle containing Alsever's solution in a proportion volume blood: volume Alsever's solution and mix gently and preserved at ٤°C (refrigerator). The blood–Alsever's solution mixture was centrifuged at ٢٥٠٠ rmp for ٥ minutes, and sediment (red corpuscles) was washed three times with PBS. Finally the red corpuscles were re-suspended in Alsever's solution to a final concentration of red corpuscles ١٠٪ (Garvey *et al.*, ١٩٧٧).

٣–١–١٠: Liquid Paraffin–Alsever's Preservative Solution

This solution was prepared by mixed of equal proportion of liquid paraffin ($C_nH_{٢}N+_{٢}$) and the previous prepare of (٣–١–٤)Alsever's solution. The pH solution will be neutralized to become ٧–٧.٢ and then sterilized by autoclaved at ١٢١°C, ١٥ pound / inch^{٢} for ١٥ minute. The solution will be ready to be mixed with cervico–vaginal mucous secretion. As well as it was help for preservation and freezing the sample until use, it was avoiding the immunoglobulin from cryopreservation damaged.

٣–٢: Media

٣–٢–١: Leucocyte–Migration Medium

This medium was prepared by dissolving ١.٥٪–٢٪ agar–agar (Master) in one litter of distilled water, sterilized by autoclaving (١٢١°C, ١٥ pound / inch^{٢} for ١٥ minute) and then poured into sterilized Petri-dishes (١٠ cm in diameter). The medium was used as a migration medium for leucocytes when measuring leucocytes inhibitory factor (LIF) in blood samples (Rose and Biggazzi, ١٩٨٢).

٣–٢–٢: Basal Medium Eagle (BME)

This medium was prepared following Sigma company instructions as dissolving ١ gm of Eagle medium in ١٠٠ ml distilled water and sterilized by membrane filtration (٠.٤٢ µm Whatman). This BEM was used as cell nutritive solution helpful for measuring migration inhibitory factor assay (Soborg, ١٩٦٩).

٣–٣: Disposable Products

٣–٣–١: Tuberculin Syringe

One milliliter graduated sterilized disposable plastic syringe size $G_{٢٧}$ (Medical ject /Pakistan) was used for injecting the sensitizor sonicate human sperm (as antigens) into the skin (epidermis) of the experimental model rabbits.

٣–٣–٢: Blood Collecting Syringes

Sterilized disposable plastic syringe size G٢٢ (Medical ject / Pakistan) was used for both collecting the blood from patients and for injecting sensitizor antigens to a multiple site injection of experimental rabbits for preparation of the antisera sonicate donor sperms.

٣-٤: Equipments and Tools

Instrument	Company	Country
Autoclave	Webeco GMbH	Germany
Hot air oven	Gallenkamp	England
Incubator	Gallenkamp	England
Refrigerator	Frigidaire	French
Compound light microscope	Olympus	Japan
Microscope optical reader	Medic	Italy
Haematocrit	Clay Adams	England
Plain tube	FAMA–Dispo	Japan
Centrifuge	Damon–IEC Division	USA
Sensitive balance	Sartorius	Germany
Anticoagulant tube	FAMA–Dispo	Japan
Water path	Memmert	Germany
Distillar	Memmert	Germany
Sonicater prep-١٥٠	Memmert	Germany
Cooled centrifuge chilpsin -٢	Memmert	Germany
Glass slides and cover slips	Meheco	Germany
Micro pipettes ١.٠, ١٠٠.٠ µm	Volac, Slamed	England, Germany
Petri-dishes	Sterilin	England
Disposal syringe ١, ١٠ ml	Medical ject	Pakistan
Epidrofe tube ٠.٥, ٢.٥ ml	Meheco	Germany
Tips micropipette ٣٠, ٥٠, ١٠٠ µm Milipore filters ٠,٤٢µm	Meheco Satoris	Germany Germany
Gas pak system(incubator)	Gallenkamp	England
Heparinized capillary tube	Meheco	Germany
Anatomy and surgical tools	Gallenkamp	England
Porcelain mortar	Meheco	Germany
Pastur's pipettes	Volac	U.K.
Clay plasticine	Coloclay	China
Liquid paraffin	Riedel- dehaen - Hanowver	England
Sodium azide	Sigma	USA
Hank's balanced salts	Sigma	USA
Ph-meter	Meheco	Germany

٣–٥: Patients

The study consists of one hundred and twenty five (٧٩ males and ٤٦ females) who were involved in this study during their attendance of Babylon maternity and child care teaching hospital of Babylon university. The clinical information data of those infertile patients are shown in table (٣–٥B).

Those patients were subgrouped according to infertility type which includes; (٤٨) of asthenozoospermic, (١٨) of oligozoospermic and (١٣) of azoospermic patient males and their wives were studied of; ٢٥, ١٥ and ٦ couple infertile females respectively.

All those patients were evaluated by consultant of Urologist and Gynecologist surgeons assessing for the presence or absence of varicocele, hydrocele, cryptochidism, inguinal hernia operation, present or absent of the congenital abnormalities, complete history regarding sexual habits, venereal diseases, chronic diseases such as diabetes, febrile disease at least throughtly during last one year were undertaken. Whereas infertile females were investigated for abnormal urogenital tract, endometritis, chronic diseases, cystic ovary, fallopian tubes obstructions, number of recurrent abortions. Each patient enrolled in this study was interviewed by spermatology and case sheet was filled as in table (٣–٥A).

In addition to the questioner for: age, alcohol consumption, smoking habits, ABO blood group, exposure for ionizing radiation and the type of infertility. The immunologic and physiologic investigation were done as presented in the study menu(Figure-III).

Table (٣–٥A) Infertile Patients Data Sheet

١	P. Name:
٢	Address:
٣	Age:
٤	Period of Marriage:
٥	Relation with Wife:
٦	Number of Wives:
٧	Number of prior or recurrent abortion:
٨	ABO Blood Group Male: Female:
٩	Occupation:
١٠	Smoking:
١١	Alcohol Consumption:
١٢	Addicted Drugs:
١٣	Exposure to Ionizing Radiation:
١٤	Chronic and other Diseases (such as; Diabetes, Malaria, Infectious, HIV, Asthma, Allergy, Gonorrhoea & other venereal diseases):
١٥	Varicocele testis:
١٦	Hydrocele testis:
١٧	Surgical testicular biopsy or treatments:
١٨	Cryptochidism (unilateral or bilateral):
١٩	Orchitis:
٢٠	Hernia (Inguinal hernia):
٢١	Mumps (pre-pubertal or pubertal):
٢٢	Testicular trauma:
٢٣	Female; (Cystic ovary, Endometritis, Vaginitis, Urogenital tract abnormalities and obstruction of fallopian oviducts, other uterine and pelvic diseases):
٢٤	Others:

Table (٣–٥B) Clinical Information Data

(Some Possible and Notable Predisposing Factor for Infertility)

Factors	Rate of Asthenospermic Patients	Rate of Oligospermic Patients	Rate of Azospermic Patients
- History of Infection(s):			
Mumps	١٨ / ٤٨ (٣٧.٥٪)	٩ / ١٨ (٥٠٪)	٧ / ١٣ (٤٢.٨٥٪)
Gonorrhoea	٢١ / ٤٨ (٤.١٦٪)	١ / ١٨ (٥.٥٥٪)	١ / ١٣ (٧.٦٩٪)

- Occupation:			
Ionizing radiation	٣ / ٤٨ (٦.٢٥٪)	١ / ١٨ (٥.٥٥٪)	١ / ١٣ (٧.٦٩٪)
Chronic metal exposure	١ / ٤٨ (٢.٠٨٪)	-	-
Gases inhalation	٢ / ٤٨ (٤.١٦٪)	-	-
Paint(s)	١ / ٤٨ (٢.٠٨٪)	-	-
Smoking	٩ / ٤٨ (١٨.٧٥٪)	٤ / ١٨ (٢٢.٢٢٪)	٤ / ١٣ (٣٠.٧٦٪)
- Surgical Intervention:			
Varicocele	٦ / ٤٨ (١٢.٥٪)	٦ / ١٨ (٣٣.٣٣٪)	١ / ١٣ (٧.٦٩٪)
Inguinal hernia	٣ / ٤٨ (٦.٢٥٪)	٢ / ١٨ (١١.١١٪)	-
Testicular biopsy	-	١ / ١٨ (٥.٥٥٪)	٣ / ١٣ (٢٣.٠٧٪)
Ectopi testis	-	-	٢ / ١٣ (١٥.٣٨٪)
Fibrosis testis	١ / ٤٨ (٢.٠٨٪)	-	١ / ١٣ (٧.٦٩٪)
- Testicular trauma	٢ / ٤٨ (٤.١٦٪)	-	-
- Gonadal Abnormalities:			
Testis			
Atrophied testis	٢ / ٤٨ (٤.١٦٪)	١ / ١٨ (٥.٥٥٪)	٣ / ١٣ (٢٣.٠٧٪)
Single testis	-	١ / ١٨ (٥.٥٥٪)	١ / ١٣ (٧.٦٩٪)

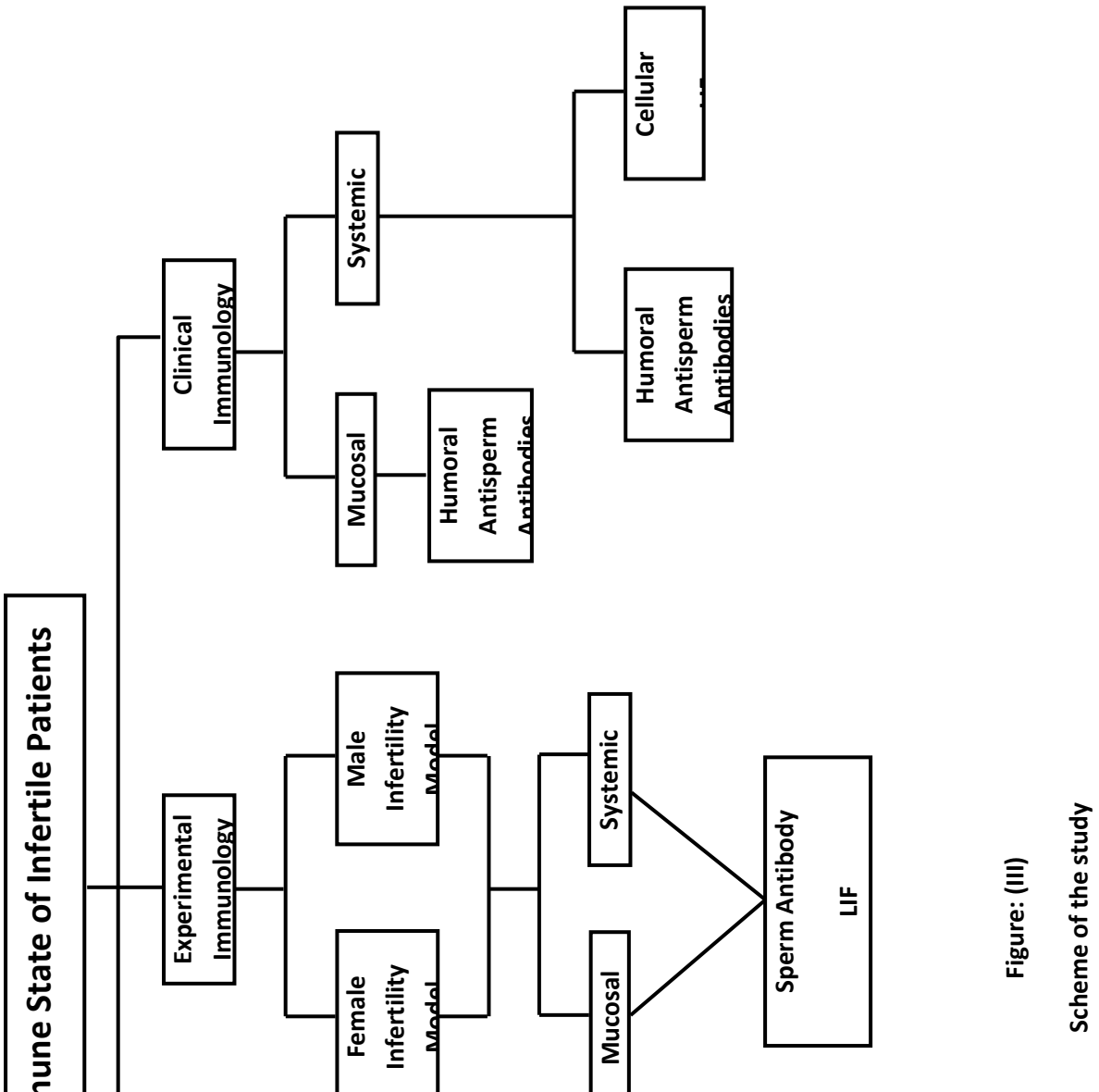


Figure: (III)

Scheme of the study

٣–٦: Controls (Study Normal Subjects)

Twenty healthy fertile subjects (١٠ male and ١٠ female) were also studied as a couple controls. Physical and clinical examinations were showed by urologist and gynecologist clinician specialists.

Blood (serum), seminal fluid (seminal plasma) and cervical mucous secretion (during the mid menstrual cycle) were taken from those twenty control subjects to asses the previous mentioned immunological tests.

٣–٧: Semen Collection

Semens or the samples of seminal fluid was collected (after ٣–٤ day of abstinence) directly into a clean, dry and sterile disposable Petri–dish by masturbation in room near the laboratory.

Some infertile patients would like to collect their semen at home, which are present to be (٢) of single male out of the (٧٩) examining semens samples. These patients were given special instructions in order to protect the semen sample from the possible effects of temperature (extremes not < ٢٠°C and not > ٤٠°C) during transport to the laboratory.

The container was labeled with the following information: name, age, abstinence period and time of sample collection.

The specimens were incubated at ٣٧°C for ٣٠ minutes to allow liquefaction. The specimen was examined in details by macroscopic and microscopic examinations.

The standard form of WHO, (١٩٩٩) was used to record the results of seminal fluid analysis (Table ٣–٧).

Table (٣–٧)

Formula used to Record the Results of Seminal Fluid Analysis (SFA)

Patient's name:		Lab. Reference No.:	
Patient's age:		Examination date:	
Abstinence day:		Time examined:	

- Macroscopical Examination:	**Normal Value**
Volume:	٢–٦ ml
Color:	Whitish–gray
Liquefaction time:	≤ ٣٠ minutes
Viscosity:	< ٣ cm
pH:	٧.٤–٨.٢
- Microscopical Examination:	**Normal Value**
Leucocytes concentration:	< ١ X ١٠^{٦} / ml
Phagocyte concentration:	< ٠.٥ X ١٠^{٦} / ml
Sperm agglutination:	٠ %
Sperm concentration:	>٢٠ X ١٠^{٦} / ml
Sperm motility (%):	> ٥٠٪
Sperm grade activity:	≥ ٣
Sperm Shaky head movement (%):	٠ %
Sperm viability (%):	> ٦٠٪
Sperm hypo-osmotic swelling test (%):	> ٦٠٪
Abnormal sperm morphology (%):	< ٤٠٪

٣–٨: Blood Collection

Five ml of blood was collected from an antecubital vein by disposable syringe. First, the skin was cleaned and made sterile by ٧٠٪ ethyl alcohol and allowed to dry before being punctured. The blood was slowly withdrawn by the piston of the syringe without an attempt to withdraw blood faster than the vein was filling (Lewis *et al.*, ٢٠٠١).

After that, the blood was delivered carefully from the syringe and divided into two equal volume, first with anticoagulant tube (AFMA–Dispo–EDTA tube), that was mostly processed for LIF (Soborg, ١٩٦٩).

At critical rare occasion, it was preserved in refrigerator for overnight, then processed for measuring systemic leucocytes migration inhibitory factor …, and the second half blood volume without anticoagulant were left in room temperature for ١٠ minutes and then centrifuged for ٥ minutes at ٣٠٠٠ rpm. The serum was collected by Pasture pipette and decomplementized and preserved at freeze in tube at–١٨°C, laboratory processing which was carried out without delay (Lewis *et al.*, ٢٠٠١).

٣–٩: Preparation of Whole Sperm antigen(s)

١. Collect semen sample via masturbation from proved healthy fertile donor.

٢. Incubate the semen sample at ٣٧°C for at least ٣٠ minutes to allow complete liquefaction happened.

٣. Dilute the specimens with equal volume of physiological normal saline and mixing gently for homogenized to diluents liquidated sample.

٤. Centrifuge the sample at ٥٠٠٠ rmp for ٥ minutes in a cooling ultracentrifuge to avoid protein denaturation .

٥. Separate the supernatant that represent the seminal plasma, than the bottom disc like pellet that represent the cellular components of the ejaculate sample.

٦. Wash the pellet containing cellular components sperms, spermatides, spermatocytes, with ١٠ ml of physiological normal saline and then centrifugation for about three frequent repeated times at ٥٠٠٠ rpm for ٥ min., the supernatant will be discarded and the bottom disc like pellet will be preserved for suspension.

٧. Re-suspend the pellet in (point ٦) in ٥ ml of normal saline to make the suspension solution ready for sonicated processing.

٨. As mentioned before, the last washed and the cellular pellets were sonicated in a cooling circumstances via coated two glass special containers : the outer of containing a piece of ice and the inner contain the suspension sonicate sample (Figure -IV).

٩. Multiple sonication trials were done for getting and fixing the optimal amplitude sonication waves and suitable sonication time that appeared to present as (١٦–٢٢ amplitude waves), in average of (١٩ amplitude sonicate waves) for ١٥ minutes, (Table ٣–٩).

١٠. Sonicate sample was centrifuged for ٥٠٠٠ rpm for ١٥ minutes in cooling centrifuge, after that pellet was discarded and the obtained supernatant preserved, that represent spermatozoal human sperm sap antigens, which now be ready to be used for covering or mixing with tanned red blood sheep corpuscles to investigate and assess the immunological tests, (Garvey, ١٩٧٧) (Table ٣–٩).

Table (٣–٩)

Sonication Procedure

Entity Features	Amplitude	Sonication

	Sonication Waves	time
١. disruption the pellet (sperm and other cellular components) to small isolated parts as; tails, midpieces and intact heads especially for sperms.	٨–١٢ (١٠)	٥ minutes
٢. Same structural features of (١) were appeared in this amplitude, time sonication waves, but, with lower of intact head and mid piece.	١٤–١٦ (١٥)	١٠ minutes
٣. Total number of disruptive sperm parts which have to be seen very clear in the head compartments with a very low appearance of mid piece among these pieces besides to the disappear of the intact heads, during the first ٥ min. of sonication with appearance of sperm sap motions and the Brownian waves.	١٦–٢٢ (١٩)	١٥ minutes *N.B

* N.B.; (The elected conditions)

A.

B.

Figure: (IV)

Photograph soniprep – ١٥٠

A. soniprep with it's compartments **B. soniperp during the work**

٣–١٠: Preparation of Leucocytes Sensitizers

Leucocytes sensitizer preparation takes the same serial steps procedure of (٣–٩ preparation of antigens).

٣–١١: Immunology of Infertile Patients

٣–١١–١ Test for Cellular Immune Responses

٣–١١–١–١ Leucocyte Migration–Inhibitory Factor (LIF)

LIF is defined as protein substances nature factor(s), that is released due to sensitization of lymphocytes and might be other possible cells, which act to inhibit the migration of leucocytes, particularly polymorphnuclear (PMNs) cells, away from their site of release. Assessment for these factors was established to be used as indicator for the cellular immune test (Medical Dictionary online, ٢٠٠٤).

LIF test was performed in accordance with (Soborg, ١٩٦٨):

I. Using heparinized capillary tube, blood was drawn in duplicate for each patient (male and female) and filling one end with plasticine.

II. The blood heparinized capillary tubes were centrifuged in haematocrit for ٥ minutes.

III. The capillaries were broken little above the buffy coat.

IV. These cutted capillaries were applied in ١٥ mm agar–agar well in Petri–plates.

V. ٥٠ µl eagle medium and ٥٠ µl sensitizer antigen (sonicate human donor sperm) were added to test wells and ٥٠ µl eagle and ٥٠ µl physiological normal saline were added to a control well.

VI. In a humid jar, the plates were incubated in upright position overnight at ٣٧°C.

VII. The optical reader lens were used to read the migration of leucocytes with and without the sensitizer.

VIII. LIF percent was calculated, as in following equation:

$$\text{LIF Percent} = \frac{\text{Distance with sensitizer}}{\text{Distance without sensitizer}} \times 100$$

Inhibition more than ٣٠٪ was significant.

٣–١١–٢ Test for Mucosal and Systemic Immunity

٣–١١–٢–١ Test for Mucosal and Systemic Humoral Immunity

٣–١١–٢–١–١ Tray Agglutination Test (TAT or PHA)

٣–١١–٢–١–١–A Mucosal

First, filling the whole tray agglutination wells with ٥٠ µl of normal saline (١٢ wells), then add only to the first well ٥٠ µl of prepared immunoglobulin suspension of male and female mucosal tested patients (such as: seminal plasma suspended immunoglobulin or cervico–vaginal mucus secretion [C.M.S] suspended immunoglobulin.

Now, mixing the contents of the first well carefully with micropipette and then ٥٠ µl of the above mixed well is transferred to the second well to be mixed carefully and ٥٠ µl of this mixture will be transferred to the third well and so on till the eleventh well to obtain serial dilutions of ; ١:٢, ١:٤, ١:٨, ١:١٦, ١:٣٢, ١:٦٤, ١:١٢٨, ١:٢٥٦, ١:٥١٢, ١:١٠٢٤ and ١:٢٠٤٨, that conceded with elimination of ٥٠ µl before diluting the well number ١٢ (control).

Again, the whole wells of the tray were filled with ٥٠ µl of antigenic coated sheep red blood corpuscles (SRBCs) and moving the tray as forward and backward rocky movement and then incubated at ٣٧°C for ٢٤ hr., then the results were scored as taken reciprocally of the highest dilution that gives clear positive results (titer).

٣–١١–٢–١–١–B Systemic

The serum immunoglobulin of male or female infertile patients, was diluted in a serial decimal–double dilution manner, in which ٠.١ ml of serum was added to ٠.٩ ml of normal saline. ٥٠ µl of the above dilute serum mixture was used to obtain the serial dilutions of: ١:٢٠, ١:٤٠, ١:٨٠, ١:١٦٠, ١:٣٢٠, ١:٦٤٠, ١:١٢٨٠, ١:٢٥٦٠, ١:٥١٢٠, ١:١٠٢٤٠ and ١:٢٠٤٨٠.

For each of well serum patient dilution in the set, ٥٠ µl of antigenic coated red blood sheep suspension was added to the reacted mixture which was gently shaken, then incubate the plate specimens at ٣٧°C for ٢٤ hr. and reading the reciprocal highest dilution that shows obvious positive titration results.

٣–١١–٢–١–١–C: Separation of Immunoglobulin from Seminal Plasma and Cervical Mucus Secretion

٣–١١–٢–١–١–C_I: Seminal Plasma Immunoglobulin Separation Assay

The test semen samples were diluted and shaken well with the equal amount of normal saline.

Semen sample was centrifuged for ٥٠٠٠ rpm for ٥ minutes, discarded the bottom and took about ٤ ml of the supernatant to be added to ٤ ml of PEG–٦٠٠٠ and these mixed samples were kept

into ٤°C refrigerator for ١ hr., helping for precipitate seminal plasma immunoglobulin, then centrifuged the sample for ٥٠٠٠ rpm for ٢٠ minutes. The resulted pellet will be re-suspended again with ١ ml of formal saline and discarded the supernatant, now the re-suspended seminal plasma immunoglobulin were ready for the immunological tests (Johnstone and Thorpe, ١٩٨٢; Shnawa and Thewaini, ٢٠٠٢).

٣–١١–٢–١–١–C_{II}: Cervical Mucus Immunoglobulin Separation Assay

I. Amount of ٠.٥ cervical mucus secretion (C.M.S.) was collected during the day of the mid menstrual cycle by a special experienced Gynecology via the retoctor vaginal speculum use and particular catheter collector design.

II. C.M.S. (٠.٥ ml) was immediately mixed with ٠.٥ ml previously prepared of liquid paraffin Alsever's preservative solution, labled sample were be freezing until the time needed use.

III. Centrifuge the sample in (II) for ٥٠٠٠ rpm for ٥ minutes.

IV. Remove the supernatant sample carefully by cleaning and sterilizing Pasture pipette.

V. Physiological normal saline was added to the C.M.S. sample to become about ٢ ml volume and mixed with Pasture pipette at least for ٥ minutes.

VI. Re-centrifuge the sample at ٥٠٠٠ rpm for ٥ minutes, then take the supernatant and discarded the bottom residue.

VII. Mix the supernatant with an equal amount of PEG–٦٠٠٠ and put sample in ٤°C for ١ hr. to help for precipitate the cervical mucus immunoglobulin.

VIII. Centrifuge the sample again at ٥٠٠٠ rpm for ٢٠ minutes.

IX. The pellets were taken and re-suspended with shaking in ١ ml of formal normal saline.

X . The C.M.S. immunoglobulin was being ready for immunological or titration assays (Shnawa and Thewaini, ٢٠٠٢)

٣–١١–٢–١–٢: Modified Confirmative Direct Slide Agglutination Test (Semiquantitative Direct Slide Test)

To ensure the results of this test, the following tests were done:

١. Direct Slide Agglutination:

To ٢٥ µl of patient serum on the surface of clean sterile glass slide, ٢٥ µl of whole sperm antigens (WSAg) were added and rocked forward and then backward, with ١–٢ minutes, a cloud of fine mesh like agglutination was developed.

٢. Standard Sperm Tube Agglutination Test:

I. Serial decimal double dilution technique of patient sera was made to final ٠.٥ ml for each dilution; ١:١٠, ١:٢٠, ١:٤٠, ١:٨٠, ١:١٦٠, ١:٣٢٠ and so on.

II. To each of these dilutions, ٠.٥ amounts of (WSAg) suspension of ٣-١١-٢-١-٢-١ was added.

III. Serum–Antigen mixtures in those tubes were incubated for ١/٢, ١ and ١١/٢ hrs. at ٣٧°C incubator.

IV. The reciprocity of highest dilution that gives clear positive agglutination results was considered to be the titer.

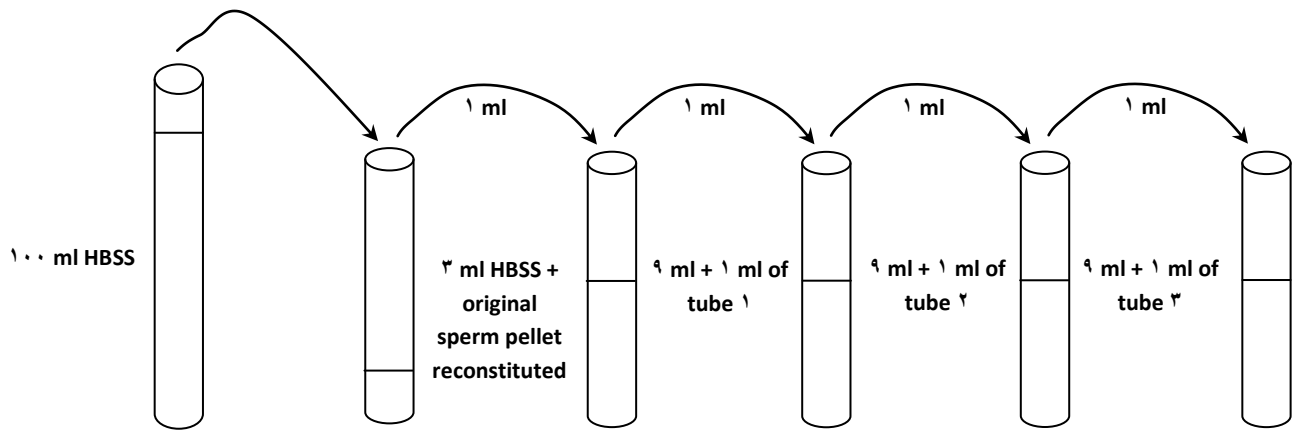
V. Clear cut opaque granular matte like film in the tube bottoms. When flickered it becomes granular in clear matrix of reaction mellue.

٣. Direct Semiquantitative Sperm Slide Agglutination Test: On black glass slab of ٦ squares ٨٠, ٤٠, ٢٠, ١٠, ٥ µl of patient serum was layed on each of these squares. To each of these drops of sera in the squares, ٢٥ µl of (WSAg) was added and applicator stick mixed. Fine mesh like cloud was developed with ١–٢ minutes at room temperature. The smaller serum drop that gives clear agglutination results was the titer.

Direct	Semiquantitative
+	١٦٠

٣–١١–٢–١–٢–١: Sperm Antigen for Standard Agglutination Test

A normospermic of ٣ ml donor semen sample was count of ٦٠ × ١٠^٦ sperm / ml was attempted. It was centrifuged at ٢٠٠٠ rpm for ٥ minutes. Then saline washed for three times at ٢٠٠٠ rmp for ٥ minutes and reconstituted to ٣ mls with HBSS, therefore, recounted again and found to be ٤٠ × ١٠^٦ sperm / ml. This was considered as a sperm antigen stock (SAgS). From this SAgS, sodium azide preserved HBSS were prepared in ٥ tubes each of ١٠ mls and preserved at ٤°C till use as in Figure (V).



٣–١١–٢–١–٣: Reverse Passive Haemagglutination (RPHA) Immune Test

٣–١١- ٢–١–٣–١: A Mucosal, and

٣–١١ ١–١–٣–١: B Systemic Antigenemia Test

An application of the steps procedures in passive haemagglutination test of ٣–١١- ٢–١–٣–١A Mucosal and ٣–١١- ٢–١–٣–١B Systemic humoral immune test were repeated with that of reverse passive haemagglutination (Antigenemia) test. But with one exception of red blood sheep corpuscles (RBSCs), they altered their coating with the rabbit antibodies (antisera) instead of the antigenic sonicate sperms suspension.

٣–١٢: Laboratory Animals

Experimental animals of this work consist of twenty four indigenous *Oryctolagus cuniculus* rabbits (Hime and Donaghure, ١٩٧٩), ٤–٦ months old, ٢.٥–٣ kg body weight.

They were maintained in microisolator cages for ١ month adaptation before the experiment starting (Schneider *et al.*, ١٩٩٠), as well as to the housed in semiopened shade.

Animals, all of the experimental time, were provided with concentrated diet and green forage along with water *ad libitum* and they were divided into two categories each of two replicates (Beek *et al.*, ١٩٩٣), as in Figure (VI)

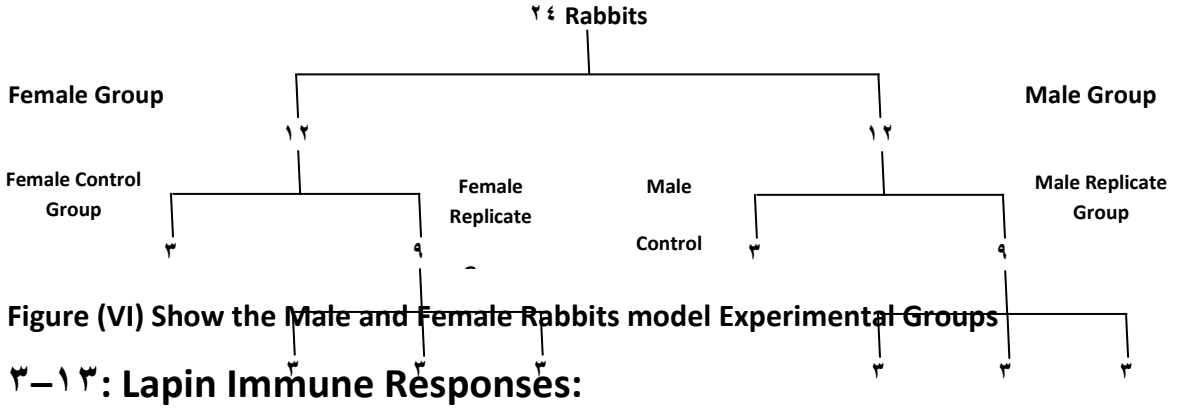


Figure (VI) Show the Male and Female Rabbits model Experimental Groups

٣–١٣: Lapin Immune Responses:

٣–١٣–١: Semen Sperm Antigen and Preparation of immunogen

Sperm sonicate antigen(s) was prepared as mentioned in section (٣–٩). Same antigen(s) were helpful as skin allergen and for antigen antibody reactions for passive haemagglutination.

٣–١٣–٢: Serology

Micro-haemagglutination for passive and reverse passive haemagglutination was done as in (Garvey *et al.*, ١٩٧٧), for both serum and mucosal immunoglobulin that include:

١. Male, intestinal appendix secretory immunoglobulin.

٢. Female, intestinal appendix secretory immunoglobulin.

٣. Male, right and left testis genital tract secretory immunoglobulin.

٤. Female, cervicovaginal genital tract secretory immunoglobulin.

As mentioned before the mucosal immunoglobulin was separated from seminal plasma of man, cervico–vaginal human and animal samples had been done. Intestinal appendix and the testicular stroma of animal rabbits according to (Shnawa and Thewaini, ٢٠٠٢). As in section (٣–١١–٢–١–١–C), with one exception of that scraped and crush the mucosa and submucosa of those cervico–vaginal and intestinal appendix were treated in a sterile mortar to prepare and sediment their immunoglobulins as in (٣–١١–٢–١–١–$C_{I\ and\ II}$).

٣–١٣–٣: Immunization Program

٣–١٣–٣–١: Immunization Program of Male Rabbit

Dose	Route of Immunogen Administration	Time of Injection
In the right side of male rabbit, ١ ml of immunogen (Sensitizer sperm antigens) were divided as follow doses:		٥ day interval
٠.٣ ml	I.M. thigh injection	
٠.٣ ml	S.C. adjacent to the prescapular lymph node	
٠.٢ ml	S.C. Skin subcutaneous injection	
٠.٢ ml	Intratesticular injection (right testis only)	
The injection regime was repeated on the alternative left side of the male rabbit. Then on day (٢٠) of time intervals, male rabbits were left for (٧) days after the fourth last dose immunization. Whereas, these male were introduced for DTH test, that persist for (٧٢) hr. before animal were being bleed.		

٣–١٣–٣–٢: Immunization Program of Female Rabbit

Dose	Rout of Immunogen Administration	Time of Injection
In the right side of female rabbit, ١ ml of immunogen (Sensitizer sperm antigens) were divided as follow doses:		٥ day interval
٠.٣ ml	I.M. thigh injection	
٠.٣ ml	S.C. adjacent to the prescapular lymph node	
٠.٢ ml	S.C. Skin subcutaneous injection	
٠.٢ ml	Intra cervico–vaginal region (Hong-Yin *et al.*, ٢٠٠٠)	
The injection regime was repeated on the alternative left side of the female rabbit.		
Then on day (٢٠) of time intervals, female rabbits were left for (٧) days after the fourth last dose immunization.		
Whereas, these female were introduced for DTH test, that persist for (٧٢) hr. before animal were being bleed.		

٣–١٣–٤: Blood Collection

Blood of treated rabbit was collected after the DTH had been done.

That means collected blood had been done after ٧ days of the fourth immunization trial plus ٧٢ hour (٣ days) of DTH assessment.

Blood collection was done via the heart puncture to obtain anticoaggulated blood sample for LIF test and coagulated blood sample to obtain serum for passive haemagglutination (PHA) and reverse passive haemagglutination (RPHA) test, for detecting the presence of antibody, antigenemia titer respectively.

٣–١٣–٥: Leucocyte Inhibition Factor (LIF)

This test was done as mentioned earlier with the cellular immune response of the infertile human patients at (٣–١١–١–١).

٣–١٣–٦: Skin Delayed Type Hypersensitivity (DTH) Test

٠.١ ml of the skin sensitizer allergen was intradermally injected in deeply shaved skin in one side of rabbit abdomen. Results were watched during ٣, ٦, ١٢, ١٨, ٢٤, ٤٨, and ٧٢ hrs. post injection (Garvey *et al.*, ١٩٧٧; Burrell, ١٩٧٩; Statnes Serum Institute, ٢٠٠٢).

٣–١٤: The Classification Criteria for Infertility:

In brief, definition of infertility as mentioned earlier means the absence or unable to produce a successful pregnancy after ١ year of continuous and unprotected intercourse, and when that if were happened leads to initiate infertility, that's manifested either to be primary or secondary infertility type.

In addition to the presence of many participation causes that's being interact to reduce the normal criteria of standard spermatology (WHO, ١٩٩٩), there is a restricted class of infertility. While, the most of relative dependent infertility criteria, were those whom sperm function parameter associations are:

١. Sperm concentration.
٢. Sperm motility percent (Progressive active forward movement sperm).
٣. Grade sperm activity.
٤. Abnormal sperm morphology percent.
٥. Sperm viability percent.

Finally the approved criteria for classification of infertility were;

١. Azoospermia.
٢. Oligozoospermia.
٣. Asthenozoospermia.
٤. Oligoasthenozoospermia.

٣–١٥: Seminal Fluid Analysis:

٣-١٥-١: General

The sampling method, sampling precusions, the evaluation of appearance, volume, odor, liquefaction time, viscosity and pH were made according to WHO, ١٩٩٩.

٣–١٥–٢: Leucocytes and Phagocyte Concentration

Semen contained cellular elements other than spermatozoa, such as epithelial cells, prostate cells, spermatogenic cells and leucocytes. Such cells are collectively known as “Round Cells” (Tomlinson *et al.*, ١٩٩٣).

The presence of leucocytes in semen is termed as leucocytospermia; excessive number of leucocytes may be associated with infection and poor sperm quality (Wolff *et al.*, ١٩٩٠). Leucocytospermia was graduate as table (٣–١٥–٢)

Table (٣–١٥–٢) Graduation of Leucocytospermia

Grade	Entity
Less than one million leucocytes	Normal semen
١–٢ million leucocytes	Mild leucocytospermia
٣–٥ million leucocytes	Moderate leucocytospermia with marked infection
More than ٥ million leucocytes	Very marked infection with clusters of leucocytes engulfing sperm (phagocytosis)

Leucocytospermia was recorded using ٥ µl drop on clean glass side with cover slip and counted at ٤٠٠X magnification.

٣-١٥-٣: Dircet Sperm Agglutination

The grading system of sperm agglutination was recorded in the following table (٣–١٥–٣):

Table (٣–١٥–٣) Sperm Grade Agglutination

Grade Agglutination	Percentage
– (No sperm agglutination)	٠٪
+ (+ve; Normal sperm agglutination)	≤ ١٠٪
++ (Mild sperm agglutination)	١١–٢٠٪
+++ (Marked sperm agglutination)	٢١–٤٠٪
++++ (Very marked sperm agglutination)	> ٤١٪

The agglutination sperm percentage was estimated as in the following formula (WHO, ١٩٩٩):

$$\text{Sperm Agglutination (\%)} = \frac{\text{No. of agglutinated spermatozoa}}{\text{Total No. of spermatozoa}}$$

٣-١٥-٤: Sperms Concentration

The sperms concentration were counted from the mean number of spermatozoa in ten random microscopic fields multiplied by the factor of one million. Total sperm count was calculated as sperms concentration per (ml) multiplied by semen volume of the ejaculate (Hinting, ١٩٨٩; WHO, ١٩٩٩).

٣-١٥-٥: Sperm Motility

Sperm motility and grade activity were established as the mean number of those spermatozoa that showed progressive motility in straight line motion out of ٢٠٠ spermatozoa (WHO, ١٩٩٩).

Grade activity, however, was determined as in the following table (٣–١٥–٥).

Table (٣–١٥–٥) Sperm Grade Activity

Grade	Qualification
٠	Immotile sperm.
١	Local circular slow motility.
٢	Slow forward progression.
٣	Good forward progression; straight line movement.
٤	Very good forward progression; straight line movement.
٥	Excellent forward progression; straight line movement.

The infertility entities were:

No spermatozoa = Azoospermia.

Less than twenty million = Oligozoospermia.

Less than ten million = Sever Oligozoospermia.

Less than ٥٠٪ of motile spermatozoa classified as Asthenozoospermia.

The sperm motility index (SMI) was calculated as sperm motility % multiplied by grade activity of spermatozoa (Al-Hady, ١٩٩٧; Shaban, ١٩٩٩).

٣–١٥–٦: Sperm Morphology

Preparation of semen slide was made from each patients and from controls. Sperm head, sperm tail and medpiece abnormalities were recorded out of ٢٠٠ sperm (WHO, ١٩٩٩), as in the following formulas:

$$\text{Normal Sperm Morphology } \% = \frac{\text{No. of Normal Sperm}}{\text{Total Sperm Count}} \times 100$$

$$\text{Abnormal Sperm Morphology } \% = \frac{\text{No. of Abnormal Sperm}}{\text{Total Sperm Count}} \times 100$$

More than ٤٠٪ abnormal sperm morphology was classified as teratospermia and considered to be associated with subfertility. Such abnormalities were classified as follows :

١. Primary abnormalities: Tapered head, macrocephalic double head, microcephalic head and amorphous head.

٢. Secondary abnormalities: Short tail, double tail, coiled tail, bent tail or broken tail (Acosta *et al.*, ١٩٨٦).

٣–١٦: Statistical Analysis

Statistical test was used depending on the nature of the data. In addition to the standard statistical methods to determine the mean, standard error (SE), atypical type of student's t–test so called paired t–test was used to compare between the plasma, mucosal antibodies titers for azoospermic, oligozoospermic and asthenozoospermic parameters of SSA results of male and female infertile patients (Duncan *et al.*, ١٩٨٣; Ott, ١٩٨٨).

Multi regression and correlation coffiecient was done during the study of antisperm antibody titers and direct sperm agglutination, sperm motility index and shakey head motion. In addition to, multi factorial analysis of variance (MAVOVA) to report the level of statistical significance between the means of infertile studied groups (Daniel, ١٩٩٩).

Chapter Four

Results

٤–١ Basic Cellular Immunology

٤–١–١: Leucocytospermia

Semen of infertile men has shown maximum leucocytes concentration among Oligospermic then asthenozoospermic and azoospermic patients. Leucocytospermia was ranged among infertile groups from $٢.٣٨٤\times١٠^{٦} \pm ٠.٩٢$ leucocytes /ml to $٦.١٦٦\times١٠^{٦} \pm ١.١٠٦$ leucocytes /ml.

The Inter-groups ranges of leucocyte concentrations Were; ٢×١٠^{٦} – ٢٥×١٠^{٦}, ٤×١٠^{٦}–٢٠×١٠^{٦} and ٢×١٠^{٦}–١٤×١٠^{٦} leucocytes /ml; for asthenozoospermic, Oligozoospermic and azoospermic patients, respectively (table ٤–١ A)

Table (٤–١A)

Leukocytes Concentration Among Infertile Groups

Infertility Patient Group	Leukocytes Mean ± SE	Rate of Leukocytes positive: total patient	Leukocytes Positive range
Asthenozoospermic patient	٥.٦٣٨٣×١٠^٦ ± ٠.٥٦٢٨	٤٥:٤٨ (٩٣.٧٥٪)	٢×١٠^٦ _٢٥×١٠^٦
Oliyozoospermic Patient	٦.١٦٦٧×١٠^٦ ± ١.١٠٦٣	١٨:١٨ (١٠٠٪)	٤×١٠^٦ _٢٠×١٠^٦
Azoospermic Patient	٢.٣٨٤٦×١٠^٦ ± ٠.٩٢٣٦	٨:١٣ (٦١.٥٣٪)	٢×١٠^٦ _١٤×١٠^٦

٤ –١–٢: Leucocyte–Migrating Inhibitory Factors (LIF)

The results of leucocyte inhibitory factors using sperm sonicate as sensitizer and peripheral blood in a capillary technique have shown that significant leucocyte inhibition was noted among asthenozoospermic and Oligozoospemic males and their female couples. While they were non significant leucocyte migration inhibition among azoospemic patients and their female couples (table ٤ –١ B).

Table(٤ –١ B)

Leucocyte inhibitory factor using sperm sonicate sensitizer and peripheral blood leucocyte capillary technique (Soberg, ١٩٦٨) among infertile subjects.

Infertility type	LIF		
	Mean	Median	Range
Male			
Asthenozoospermia	٠.٥٣	٠.٤٨	٠.٢٧–٠.٧٠
Oligozoospermia	٠.٤٩	٠.٤٩	٠.٣٣–٠.٦٦

Azoospermia	٠.٩٠	٠.٨٧	٠.٧٥–١.٠
Female couple of			
Asthenozoospermia	٠.٥٠	٠.٤٩	٠.٣٣–٠.٦٦
Oligozoospermia	٠.٥١	٠.٥٤	٠.٤٢–٠.٦٦
Azoospermia	٠.٩٠	٠.٩١	٠.٨٣–١.٠
Male control group	٠.٩٠	٠.٩١	٠.٨٣–١.٠
Female control group	٠.٩٦	٠.٩٣	٠.٨٦–١.٠

٤–٢ Basic Humoral Immunology

٤–٢–١: Direct Sperm Agglutination In Seminal Fluid

The direct sperm agglutinin percentage was found most frequent at ٢٥–٤٩ % among asthenozoospermic patient in ٢٧ patients. Meanwhile they range from ٥–٢٤ % in highist frequent as ١١ out of ١٨ Oligozoospermic patient and they were nill at azoospermic patients.

Table (٤–٢)

Direct sperm agglutination as head to head, tail to tail or mixed in term at % of total sperm count in seminal fluid analysis.

Infertility type	Direct sperms agglutination
Asthenozoospermic patients	٠–٤٪ – ٥–٢٥٪ ١٥ ٢٥–٤٩٪ ٢٧ ٥٠–٧٤٪ ٦ ٧٥–١٠٠٪ –
Oligozoospermic patients	٠–٤٪ ٤

	٥–٢٤٪ ١١ ٢٥–٤٩٪ ٣ ٥٠–٧٤٪ – ٧٥–١٠٠٪ –
Azoosapermic patients	Nill
Fertile subject (Control group)	٠–٤ % ١ (٢ %) ٥–٢٥٪ ٥ (٥–١٠٪) ٢٥–٤٩٪ – ٥٠–٧٤ % – ٧٥–١٠٠ % –

٤–٢–٢: Sperm Specific Mucosal and Systemic Agglutinins

The means of semiquantitative tests of systemic sperm specific agglutinins were higher than those of mucosal agglutinins. The diagnostic sensitivity was ٦٦.٦٦٪ and diagnostic specificity was ٨٢.٣٥٪, for asthenozoospermic patients. The male couple titers were higher than those of female couple titers.

Whereas, the Oligozoospermic patients appear to show means of systemic sperm specific agglutinins higher than those of mucosal agglutinins, with diagnostic sensitivity and specificity were of ٧٢.٠٪, ٨٨.٨٨٪ respectively and male couple titers higher than those female couple titers.

While azoospermic patients showed systemic and mucosal sperm specific agglutinins mean titer levels appeared within a same direction to those of asthenozoospermic, oligozoospermic studied patients, with difference in diagnostic sensitivity and diagnostic specificity respectively (٦٠٪, ٩٣.٣٣٪). Male couple antibody titers were higher than female couple titers (table ٤-٣).

Table (٤–٣)

Sperm Specific Autoantibody Using Washed Sperm Cell (٤٠×١٠^{٦}/ml) Antigen and Semiquatitative Slide Agglutination Among Infertile Subjects.

Infertility type		Seropositive rate	Titer		
			Mean	Median	Range
Asthenozoospermia observations	Male couple mucosal	٢٦:٤٨	٢١٥.٣٨	١٨٠.٠	٤٠—٣٢٠
	systemic	٢٦:٤٨	٢١٤٠.٦١	١٧٠٠	٢٠٠—٣٢٠٠
	Female couple mucosal	١٩:٢٥	١٦٣.١٥	١٧٠.٠	٢٠—٣٢٠
	systemic	١٩:٢٥	١٧٦٠.٨٤	١٧٠٠.٠	٢٠٠—٣٢٠٠
Statistical features: (quality control) **Index of sensitivity** ٥٠.٠٪ **Index of specificity** ٧٨.٨٥٪ **Diagnostic of sensitivity** ٦٦.٦٦٪ **Diagnostic of specificity** ٨٢.٣٥٪					
Infertility type		**Seropositive rate**	**Titer**		
			Mean	**Median**	**Range**
Oligozoospermia observations	Male couple mucosal	١١:١٨	١٩٦.٣٦	٢٠٠.٠	٨٠—٣٢٠
	systemic	١١:١٨	١٧٨٠.١٨	١٨٠٠.٠	٤٠٠—٣٢٠٠
	Female couple mucosal	٧:١٥	١٤٢.٨٥	١٨٠.٠	٤٠—٣٢٠
	systemic	٧:١٥	٦٥٠.٧١	٩٠٠.٠	٢٠٠—١٦٠٠
Statistical features: (quality control) **Index of sensitivity** ٦١.١١٪ **Index of specificity** ٨٧.٥٠٪ **Diagnostic of sensitivity** ٧٢.٠٪ **Diagnostic of specificity** ٨٨.٨٨٪					
Infertility type		**Seropositive rate**	**Titer**		
			Mean	**Median**	**Range**
Azoopermia observations	Male couple mucosal	٢:١٣	٢٠٠.٠	٢٠٠.٠	٨٠—٣٢٠
	systemic	٢:١٣	١٨٠٠.٠	١٨٠٠.٠	٤٠٠—٣٢٠٠
	Female couple mucosal	١:٦	٢٠.٠	٢٠.٠	٢٠ *
	systemic	١:٦	٢٠٠.٠	٢٠٠.٠	٢٠٠ *
Statistical features: (quality control) **Index of sensitivity** ٣٣.٣٣٪ **Index of specificity** ٨٥.٧١٪ **Diagnostic of sensitivity** ٦٠.٠٪ **Diagnostic of specificity** ٩٣.٣٣٪ * **It is one single case .**					

٤–٢–٣ :Sperm Specific Mucosal and Systemic Haemagglutinins (HA)

The means of systemic sperm specific haemagglutinins were higher than of mucosal haemagglutinins. The diagnostic sensitivity was ٦٦.٦٦٪ and diagnostic specificity was ٨٢.٣٥٪, for asthenozoospermic patients. The male couple titers were higher than those of female couple titers.

While the means of systemic sperm specific haemagglutinins in Oligozoospemic patients were found to be higher than those of mucosal haemagglutinins. And, the diagnostic sensitivity and the diagnostic specificity were ٧٢٪, ٨٨.٨٨٪ respectively ; with the male couple titers were higher than those female couple titers. Matching picture were present with azoospermic criteria, showing a diagnostic sensitivity was ٦٠٪ and a diagnostic specificity was ١٠٠٪ for those infertile patients, with signs of male couple titers were higher than those female couple titers (table ٤-٤).

Table (٤–٤)

Sperm Specific Autoantibody Using Sperm Cell Sonicate Antigen Coated Ovine Erythrocyte Microhaemagglutination Among Infertile Subjects.

Infertility type		Seropositive rate	Titer		
			Mean	Median	Range
Asthenozoospermia observations	Male couple mucosal	٢٦:٤٨	٣٣٩.٦٩	١٠٤٠.٠	٣٢-٢٠٤٨
	systemic	٢٦:٤٨	٢٠٠٤.٩٢	٥٢٨٠.٠	٣٢٠—١٠٢٤٠
	Female couple mucosal	١٩:٢٥	١٢٨.٨٨	٢٧٢.٠	٣٢—٥١٢
	systemic	١٨:٢٥	١٢١٧.٠٥	٢٥٧٠.٠	٢٠—٥١٢٠
Statistical features: (quality control)					
Index of sensitivity	٥٠.٠٪				
Index of specificity	٧٨.٥٧٪				
Diagnostic of sensitivity	٦٦.٦٦٪				
Diagnostic of specificity	٨٢.٣٥٪				

Infertility type		Seropositive rate	Titer		
			Mean	Median	Range
Oligozoospermia observations	Male couple mucosal	١١:١٨	١٩٤.٩٠	٢٧٢.٠	٣٢—٥١٢
	Male couple systemic	١١:١٨	١١٣٤.٥٤	٢٧٢٠.٠	٣٢٠—٥١٢٠
	Female couple mucosal	٧:١٥	٩٣.٧١	١٣٦.٠	١٦—٢٥٦
	systemic	٧:١٥	٣٠٨.٥٧	٣٦٠.٠	٨٠—٦٤٠
Statistical features: (quality control)					
Index of sensitivity	٦١.١١٪				
Index of specificity	٨٧.٥٠٪				
Diagnostic of sensitivity	٧٢.٠٪				
Diagnostic of specificity	٨٨.٨٨٪				

Infertility type		Seropositive rate	Titer		
			Mean	Median	Range
Azoopermia observations	Male couple mucosal	٢:١٣	١٦٠.٠	١٦٠.٠	٦٤—٢٥٦
	systemic	٢:١٣	١٤٤٠.٠	١٤٤٠.٠	٣٢٠—٢٥٦٠
	Female couple mucosal	١:٦	١٦.٠	١٦.٠	١٦*
	systemic	١:٦	١٦٠.٠	١٦٠.٠	١٦*
Statistical features: (quality control)					
Index of sensitivity	٣٣.٣٣٪				
Index of specificity	٨٥.٧١٪				
Diagnostic of sensitivity	٦٠٪				
Diagnostic of specificity	١٠٠٪				

* **It is one single case .**

٤–٢–٤: Sperm specific mucosal and systemic antigenemia

The reverse passive haemagglutination test for detection of antigenemia appeared to show the means of systemic sperm specific antigenemia higher than those mucosal antigenemia. The diagnostic sensitivity and diagnostic specificity were ١٠٠٪, ٨٠٪ for the asthenozoospermic patients. With appearently male couple titers were higher than those of female couple titers.

While Oligozoospermic patients were found to show means of systemic sperm specific antigenemia higher than those of mucosal antigenemia, and their diagnostic sensitivity, diagnostic specificity were ١٠٠٪, ٨٠٪ in respect, with clear male couple titers compared with absent female couple titers.

Whereas the antigenemia levels of azoospermic patients have shown no observable means for both mucosal and systemic sperm specific antigenemia (table ٤-٥).

Table (٤–٥)

Mucosal and Systemic Sperm Antigenemia Using Antisperm Sonicate Coated Ovine Erythrocyte and Microhaemagglutination Among Infertile Subjects.

Infertility type		Seropositive rate	Titer		
			Mean	Median	Range
Asthenozoospermia observations	Male couple mucosal	٩:٢٢	٦٤.٠	٨٠.٠	٣٢–١٢٨
	systemic	٩:٢٢	٣٧٣.٣٣	٤٠٠.٠	١٦٠–٦٤٠
	Female couple mucosal	٣:٦	٥٣.٣٣	٦٠.٠	٤٠–٨٠
	systemic	٣:٦	٣٣.٣٣	٣٠.٠	٢٠–٤٠
Statistical features: (quality control)					
Index of sensitivity ١٠٠٪					
Index of specificity ٧٥٪					
Diagnostic of sensitivity ١٠٠٪					
Diagnostic of specificity ٨٠٪					

Infertility type		Seropositive rate	Titer		
			Mean	Median	Range
Oligozoospermia observations	Male couple mucosal	٣:٧	١٦.٠	٢٠.٠	٨–٣٢
	systemic	٣:٧	١٧٣.٣٣	١٨٠.٠	٤٠–٣٢٠
	Female couple mucosal	٠:٨	–	–	–
	systemic	٠:٨	–	–	–
Statistical features: (quality control)					
Index of sensitivity ١٠٠٪					
Index of specificity ٧٥٪					
Diagnostic of sensitivity ١٠٠٪					
Diagnostic of specificity ٨٠٪					

٤–٣ Seroprofiles Of Infertile Couples

٤–٣–١: Seroprofile Of Infertile Males

Three B cell function tests were attempted to plot the seropfile of asthenozoospermic, Oligozoospermic and azoospermic males as well as test for mucosal and systemic antigenmia tables (٤–٦, ٤–٧ and ٤–٨).

Direct sperm agglutination may and may not be associated with rise up of sperm specific antibodies in sera or mucosal surfaces.

Rise up of specific antibodies were associated with negative sperm antigenemia and vice versa.

Serum antibodies titers were higher than those of mucosal antibodies. The sperm specific agglutinin titre levels were parallel to those titre levels of haemagglutinins. This situation is quite wright for both mucosal and systemic humoral responses.

There were some patients that showed negative results for agglutinins, hameagglutinins and antigenemia.

The ratio of systemic antibody to mucosal antibodies were ١:١, ٢:١, ٥:١, ١٠:١, ٢٠:١ and ٤٠:١.

A thirteen out of ٢٢ asthenozoospermic patients were of negative antigenemia, four out of ٧ oligozoospermic patients were of negative antigenemia .While seven out of ٩ were of negative antigenemia and negative for sperm specific antibodies among azoospermic patients.

Table(٤–٦)

The Seroprofile of Asthenozoospermic Male

sequence	Antigenemia		Passive Heamagglutination (PHA)		Semiquantitave Slid Agglutination	
	Mucosal	Serum	Mucosal	Serum	Mucosal	Serum
١.	–	–	١٢٨	٦٤٠	١٦٠	١٦٠٠
٢.	–	–	٥١٢	٢٥٦٠	٣٢٠	٣٢٠٠
٣.	–	–	٢٥٦	٤٦٠	٣٢٠	١٦٠٠
٤.	–	–	٣٢	٣٢٠	٤٠	٤٠٠
٥.	–	–	١٢٨	١٢٨٠	١٦٠	٣٢٠٠
٦.	–	–	١٢٨	١٦٠	١٦٠	٢٠٠
٧.	–	–	٢٥٦	٢٥٦٠	٣٢٠	٣٢٠٠
٨.	–	–	١٢٨	١٢٨٠	٣٢٠	٣٢٠٠
٩.	–	–	٢٥٦	٥١٢٠	٣٢٠	٣٢٠٠
١٠.	–	–	١٢٨	٥١٢٠	١٦٠	٣٢٠٠
١١.	–	–	١٠٢٤	٢٠٤٨	٣٢٠	٣٢٠٠
١٢.	–	–	١٢٨	١٠٢٤	٣٢٠	٣٢٠٠
١٣.	–	–	٦٤	٦٤٠	٨٠	٨٠٠
١٤.	–	–	١٠٢٤	٢٥٦٠	٣٢٠	٣٢٠٠
١٥.	–	–	٥١٢	١٢٨٠	٣٢٠	٣٢٠٠
١٦.	–	–	١٢٨	١٢٨٠	١٦٠	٣٢٠٠
١٧.	–	–	٦٤	٢٥٦٠	١٦٠	٣٢٠٠
١٨.	–	–	٣٢	١٢٨٠	٤٠	١٦٠٠
١٩.	–	–	١٢٨	٣٢٠	١٦٠	٤٠٠
٢٠.	٦٤	٦٤٠	-ve	-ve	–	–
٢١.	٦٤	٣٢٠	-ve	-ve	–	–
٢٢.	-ve	-ve	-ve	-ve	–	–
٢٣.	-ve	-ve	-ve	-ve	–	–
٢٤.	-ve	-ve	-ve	-ve	–	–
٢٥.	٣٢	١٦٠	-ve	-ve	–	–

Table (٤ –٦)

The Seroprofile of Asthenozoospermic Male (Continue)

sequence	Antigenemia		Passive Heamagglutination (PHA)		Semiquantitave Slid Agglutination	
	Mucosal	Serum	Mucosal	Serum	Mucosal	Serum
٢٦.	–	–	٦٤	٦٤٠	٨٠	٨٠٠
٢٧.	–	–	٢٥٦	٢٥٦٠	٣٢٠	٣٢٠٠
٢٨.	–	–	٢٠٤٨	٥١٢٠	٣٢٠	٣٢٠٠
٢٩.	–	–	٦٤	٦٤٠	١٦٠	٨٠٠
٣٠.	–	–	١٢٨	٣٢٠	١٦٠	٤٠٠
٣١.	–	–	١٠٢٤	٦٤٠	٣٢٠	١٦٠٠
٣٢.	–	–	٦٤	٣٢٠	٨٠	٨٠٠
٣٣.	٣٢	٣٢٠	-ve	-ve	–	–
٣٤.	-ve	-ve	-ve	-ve	–	–
٣٥.	-ve	-ve	-ve	-ve	–	–
٣٦.	-ve	-ve	-ve	-ve	–	–
٣٧.	-ve	-ve	-ve	-ve	–	–
٣٨.	-ve	-ve	-ve	-ve	–	–
٣٩.	٦٤	٦٤	-ve	-ve	–	–
٤٠.	٣٢	٣٢٠	-ve	-ve	–	–
٤١.	١٢٨	٣٢٠	-ve	-ve	–	–
٤٢.	١٢٨	٣٢٠	-ve	-ve	–	–
٤٣.	٣٢	٣٢٠	-ve	-ve	–	–
٤٤.	-ve	-ve	-ve	-ve	–	–
٤٥.	-ve	-ve	-ve	-ve	–	–
٤٦.	-ve	-ve	-ve	-ve	–	–
٤٧.	-ve	-ve	-ve	-ve	–	–
٤٨.	-ve	-ve	-ve	-ve	–	–

Table (٤–٧)

The Seroprofile of Oligozoospermic Male

sequence	Antigenemia		Passive Heamagglutination (PHA)		Semiquantitave Slid Agglutination	
	Mucosal	Serum	Mucosal	Serum	Mucosal	Serum
١.	–	–	٢٥٦	١٢٨٠	٣٢٠	٣٢٠٠
٢.	–	–	–	٦٤٠	٣٢٠	١٦٠٠
٣.	–	–	–	٦٤٠	٣٢٠	١٦٠٠
٤.	–	–	–	١٢٨٠	١٦٠	٣٢٠٠
٥.	–	–	٢٥٦	٣٢٠	٣٢٠	٨٠٠
٦.	–	–	٦٤	٥١٢٠	٨٠	٣٢٠٠
٧.	–	–	١٢٨	١٢٨٠	١٦٠	١٦٠٠
٨.	–	–	٦٤	٣٢٠	٨٠	٤٠٠
٩.	-ve	-ve	-ve	-ve	–	–
١٠.	-ve	-ve	-ve	-ve	–	–
١١.	٣٢	٣٢٠	-ve	-ve	–	–
١٢.	-ve	-ve	-ve	-ve	–	–
١٣.	٨	١٦٠	-ve	-ve	–	–
١٤.	٨	٤٠	-ve	-ve	–	–
١٥.	–	–	١٢٨	٦٤٠	١٦٠	١٦٠٠
١٦.	–	–	٦٤	٣٢٠	١٦٠	٨٠٠
١٧.	–	–	٣٢	٦٤٠	٨٠	١٦٠٠
١٨.	-ve	-ve	-ve	-ve	–	–

Table (٤–٨)

The Seropofile of Azoospermic Male:

sequence	Antigenemia		Passive Heamagglutination (PHA)		Semiquantitave Slid Agglutination	
	Mucosal	Serum	Mucosal	Serum	Mucosal	Serum
١.	–	–	٦٤	٣٢٠	٨٠	٤٠٠
٢.	–	–	٢٥٦	٢٥٦٠	٣٢٠	٣٢٠٠
٣.	-ve	-ve	-ve	-ve	–	–
٤.	-ve	-ve	-ve	-ve	–	–
٥.	-ve	-ve	-ve	-ve	–	–
٦.	-ve	-ve	-ve	-ve	–	–
٧.	-ve	-ve	-ve	-ve	–	–
٨.	-ve	-ve	-ve	-ve	–	–
٩.	-ve	-ve	-ve	-ve	–	–
١٠.	-ve	-ve	-ve	-ve	–	–
١١.	-ve	-ve	-ve	-ve	–	–
١٢.	-ve	-ve	-ve	-ve	–	–
١٣.	-ve	-ve	-ve	-ve	–	–

٤–٣–٢: Seroprofiles of Infertile Females

Two B cell function tests and one reverse passive haemagglutination test using sperm specific sonicate antibodies coat sheep erythrocyte, and patient sera as well as mucosal proteins solutions separated by Polyethylene Glygol (PEG, ٦٠٠٠) to test for mucosal as well as systemic antigenemia. These tests were used to plot seroprofile of infertile female couples tables (٤ –٩, ٤ –١٠, and ٤–١١). Rise up of sperm specific antibodies associated with negative antigenemia, positive antigenemia, however, was associated with nill sperm specific antibody. Systemic antibodies were higher than those of mucosal. Haemagglutinins were parallel to that of agglutinins.

Table (٤ –٩):

The Seroprofile of Female of Asthenozoospermic Patients

sequence	Antigenemia		Passive Heamagglutination (PHA)		Semiquantitave Slid Agglutination	
	Cervical mucus secretion Mucosal	Serum	(C.M.S) Mucosal	Serum	(C.M.S) Mucosal	Serum
١.	—	—	٣٢	٣٢٠	٨٠	٤٠٠
٢.	٨	٤٠	-ve	-ve	—	—
٣.	—	—	١٢٨	٦٤٠	١٦٠	٨٠٠
٤.	—	—	٢	٢٠	٢٠	٢٠٠
٥.	—	—	١٢٨	١٠٢٤	١٦٠	٣٢٠٠
٦.	—	—	٣٢	١٦٠	٨٠	٤٠٠
٧.	—	—	١٢٨	٦٤٠	١٦٠	١٦٠٠
٨.	٨	٤٠	-ve	-ve	—	—
٩.	٨	٤٠	-ve	-ve	—	—
١٠	—	—	٣٢	٣٢٠	٤٠	٨٠٠
١١	—	—	١٢٨	٥١٢٠	٣٢٠	٣٢٠٠
١٢	—	—	٥١٢	٢٥٦٠	٣٢٠	٣٢٠٠
١٣	—	—	٣٢	١٦٠	٤٠	٢٠٠
١٤	—	—	١٢٨	٦٤٠	١٦٠	١٦٠٠
١٥	—	—	٢٥٦	١٢٨٠	٣٢٠	٣٢٠٠
١٦	—	—	١٢٨	٦٤٠	١٦٠	١٦٠٠
١٧	—	—	٦٤	٣٢٠	١٦٠	١٦٠٠
١٨	—	—	١٢٨	٢٥٦٠	١٦٠	٣٢٠٠
١٩	—	—	٦٤	١٢٨٠	٨٠	١٦٠٠
٢٠	—	—	١٦	٣٢٠	٤٠	٤٠٠
٢١	—	—	١٢٨	٢٥٦٠	٣٢٠	٣٢٠٠
٢٢	-ve	-ve	-ve	-ve	—	—
٢٣	-ve	-ve	-ve	-ve	—	—
٢٤	-ve	-ve	-ve	-ve	—	—
٢٥	—	—	٢٥٦	٢٥٦٠	٣٢٠	٣٢٠٠

Table (٤–١٠)

The Seroprofile Of Female Of Oligozoospermic Patients

sequence	Antigenemia		Passive Heamagglutination (PHA)		Semiquantitave Slid Agglutination	
	Cervical mucus secretion Mucosal	Serum	(C.M.S) Mucosal	Serum	(C.M.S) Mucosal	Serum
١.	-ve	-ve	-ve	-ve	–	–
٢.	-ve	-ve	-ve	-ve	–	–
٣.	–	–	١٦	٣٢٠	٨٠	٤٠٠
٤.	–	–	١٢٨	٦٤٠	١٦٠	١٦٠٠
٥.	–	–	١٢٨	٣٢٠	١٦٠	٤٠٠
٦.	–	–	٦٤	١٦٠	١٦٠	٢٠٠
٧.	-ve	-ve	-ve	-ve	–	–
٨.	–	–	٢٥٦	٣٢٠	٣٢٠	٨٠٠
٩.	-ve	-ve	-ve	-ve	–	–
١٠.	-ve	-ve	-ve	-ve	–	–
١١.	-	-	٣٢	٣٢٠	٤٠	٤٠٠
١٢.	-ve	-ve	-ve	-ve	–	–
١٣.	-ve	-ve	-ve	-ve	–	–
١٤.	-ve	-ve	-ve	-ve	–	–
١٥.	–	–	٣٢	٨٠	٨٠	٨٠٠

Table(٤–١١)

The Seroprofile of Female Of Azoospermic Patients

sequence	Antigenemia		Passive Heamagglutination (PHA)		Semiquantitave Slid Agglutination	
	C.M.S Mucosal	Serum	C.M.S Mucosal	Serum	C.M.S Mucosal	Serum
١.	-ve	-ve	-ve	-ve	–	–
٢.	–	–	١٦	١٦٠	٢	٢٠٠
٣.	-ve	-ve	-ve	-ve	–	–
٤.	-ve	-ve	-ve	-ve	–	–
٥.	-ve	-ve	-ve	-ve	–	–
٦.	-ve	-ve	-ve	-ve	–	–

٤–٤ Immune status of Infertile Couples

Various leucocytospermia levels were noted among males with asthenozoospermia, oligozoospermia as well as azoospermia. Tables (٤–١٢, ٤–١٣, ٤–١٤, and ٤–١٥).

Likewise, various degrees of direct sperm agglutination in seminal fluid samples were noted. Low levels of sperm antigenemia were noted among female couple of asthenozoospermic patients as in table (٤–٩). In comparison high levels of sperm antigenemia were noted among male couple asthenozoospermic patient (١٦٠–٦٤٠; ٣٢–٦٤). Leucocyte inhibitory factor in infertile couples may appear as either both significant or both nonsignificant as will as one of them nonsigificant .Circulating sperm specific agglutinins,

haemagglutinins were higher than mucosal agglutinins and haemagglutinins male and/or female couples at times may be of seronegative results for sperm specific antibodies both at mucosal and systemic compartments.

Thus, asthenozoospermic patients may be of humoral and/or cellular immune response specific for sperm immunogens.

Oligozoospermic patients showed various degrees of leucocytspermia (٢–١٦ × ١٠^{٦}), as well as various grades of direct sperm agglutination in seminal fluid analysis (١٠–٣٠٪). LIF results were either both couple significant or both are nonsignificant or one of them nonsignificant. Circulating agglutinin and haemagglutinins were higher than those of mucosal. Females may be seronegative for sperm specific antibodies.

Azoospermic patients and their female couples showed nonsignificant LIF, negative antigenemia. Four of them were also negative for sperm specific antibodies.

٤–٤–١: Immune Infertility and ABO Erthocyte Surface Antigens

The immune infertility is mostly associated with the erythrocyte surface antigen type – A in all of diseases categories mentioned before, since it approximates (٤٠٪) association. As shown in table (٤–١٦).

Table (٤–١٦)

Human Erythrocyte Membrane Associated ABO Blood Group Antigens and–their Possible Correlation To Immune Infertile Patients (couple)

Infertile type		ABO antigens rate for male and female			
		A	B	AB	O
Asthenozoospemic couple	Male	١٠:٢٥(٤٠٪)	٣:٢٥(١٢٪)	٥:٢٥(٢٠٪)	٧:٢٥(٢٨٪)
	Female	٧:٢٥(٢٨٪)	٨:٢٥(٣٢٪)	٣:٢٥(١٢٪)	٧:٢٥(٢٨٪)
Oligozoospermic couple	Male	٦:١٥(٤٠٪)	٣:١٥(٢٠٪)	٤:١٥(٢٦٪)	٢:١٥(١٢٪)
	Female	٣:١٥(٢٠٪)	٥:١٥(٣٣٪)	١:١٥(٦٪)	٤:١٥(٢٦٪)
Azoospemic couple	Male	٢:٦(٣٣٪)	١:٦(١٦٪)	١:٦(١٦٪)	٢:٦(٣٣٪)
	Female	٢:٦(٣٣٪)	٢:٦(٣٣٪)	٠:٦(٠)	٢:٦(٣٣٪)
Normospermic couple (control)	Male	٣:١٠(٣٠٪)	٣:١٠(٣٠٪)	٢:١٠(٢٠٪)	٢:١٠(٢٠٪)
	Female	٣:١٠(٣٠٪)	٢:١٠(٢٠٪)	٢:١٠(٢٠٪)	٣:١٠(٣٠٪)

٤ –٥ The Immunologic Causes of Infertility

There were remarkable sperm specific autoantibodies more frequent in the infertile men ٣٩:٧٩(٤٩.٣٦٪) (asthenozoospermia, Oligozoospermia and azoospermia) than those of ٢٧:٤٦(٥٨.٦٩٪) infertile women studied cases, while in ٤٦ couples the positive sperm specific autoantibodies were found in ٢٧ cases of infertile couples (asthenozoospermic, Oligozoospermic and

azoospermic couples) comparing with those of men and women alone. On the other hand, the case of sperm specific antigenemia infertility patients was represented by ١٢:٤٠(٣٠٪) of infertile men, (asthenozoospermia, Oligozoospermia and azoospermia) comparsion with sperm specific antigenemia in women that's represented by ٣:١٢ (٢٥٪) of infertile women. The table shows DTH expressed association by T-Cell mediated hypersensitivity in rate of; ٤٢:٧٦ (٥٥.٢٦٪) men and ١٥:٤٦ (٣٢.٦٠٪) women, which is closely relative associated with the presence of sperm specific autoantibodies in men and women to demonstrate both LIF and autoantibodies in rate of ; ٢٣:٧٦ (٣٠.٢٦٪) men and ١٥:٤٦ (٣٢.٢٦٪) women of the studied cases table (٤ –١٧).

Table (٤-١٧)

The Immunologic Causes Of Infertility

Cause	Rate
Sperm specific autoantibody in men	٣٩:٧٩ (٤٩.٣٦٪)
Sperm specific autoantibody in couple	٢٧:٤٦ (٥٨.٦٩٪)
Sperm specific autoantibody in women	٢٧:٤٦ (٥٨.٦٩٪)
Sperm specific antigenemia in men	١٢:٤٠ (٣٠٪)
Sperm specific antigenemia in women	٣:١٩ (١٥.٧٨٪)
T-Cell mediated hypersensitivity to sperm antigen	٤٢:٧٦ (٥٥.٢٦٪)
Sperm specific antigen (s)	١٥:٤٦ (٣٢.٢٦٪)
Mixed autoantibody and T-Cell mediated	٢٣:٧٦ (٣٠.٢٦٪)
Hypersensitivity	١٥:٤٦ (٣٢.٢٦٪)

٤-٦ Lapin Model for Immunity to Sperm Antigens

Both male and female rabbit immune systems were attempted. The immunogen was sperm sonicate antigens(s) and the immunization protocol

was multisite injection protocol (IM, SC and intratesticular injection) in males, while the multisite injection protocol for female includes IM, SC and cervicovaginal injection routes.

The immunopriming intervals were the same for both sexs. Sperm specific haemagglutinin levels in sera were higher than those of mucosal immunoglobulin in both sexes. Peripheral blood LIF tests were significant inhibition % in female model and one of male model and nonsignificant with other-two male. DTH skin test (Figure-VII) results were strong positive in female. While they were strong in that one of significant LIF only and weak in other male rabbits table (٤–١٨ , ٤–١٩).

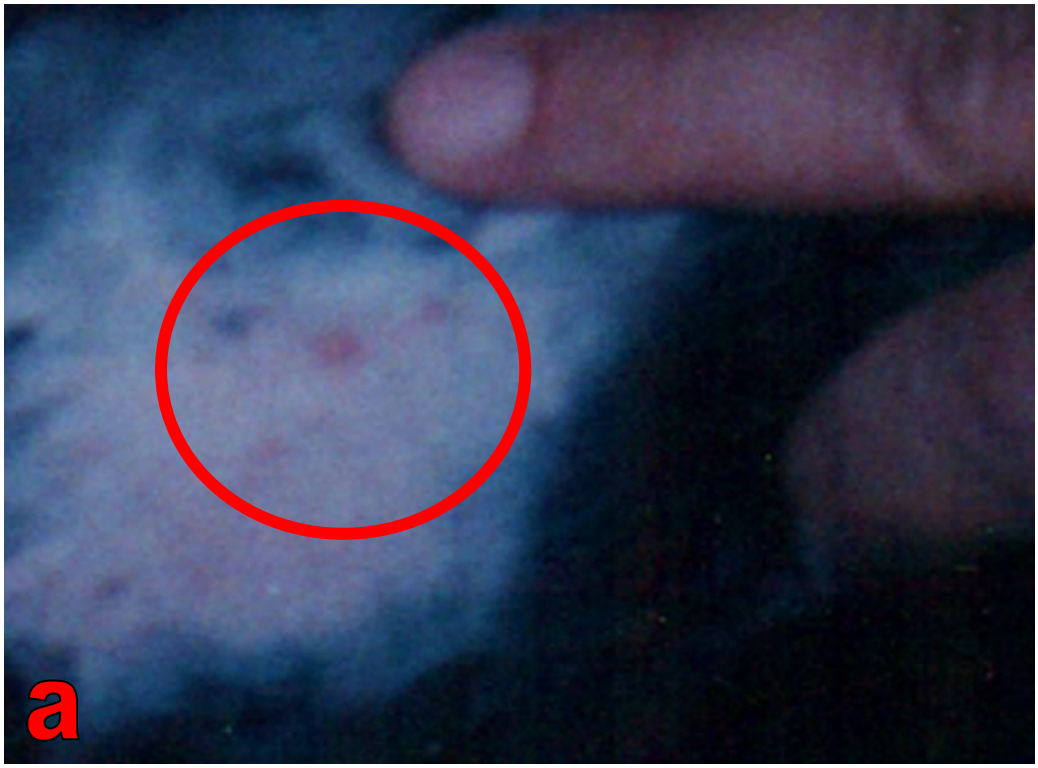


Figure: (VII)

Photomicrograph of sperm specific skin delayed type hypersensitivity (Tuberculin type) in rabbit male (a) rabbit female (b) whom pre-primed with sperm sonicate protein(s)

Table (٤-١٨)

The Humoral and Cellular Immune Function of Female Rabbits To Normal Human Sperm Protoplasmic Antigen(s).

Investigation	**Response at the below mentioned days post to the multisite injection of the antigen(s) to female rabbits**		
	٣٤ days	٣٧ days	٤٠ days
I. Celluar	EIN	EIN	EIN
١- Skin DTH*	١٦ mm*	١٦ mm*	١٧ mm*
٢- LIF Peripheral blood	٠.٥٠* (s)	٠.٥٦١* (s)	٠.٦٣٦* (s)
II. Humoral HA test			
١- Serum	٤٠٩٦	٥١٢	٥١٢
٢- Appendix	٢٠٤٨	١٠٢٤	١٠٢٤

٣- Genital tract (mucosa, submucosa of vagina, cervix and uterus)	٢٥٦	١٠٢٤	١٠٢٤

* Mean of the readings

(s) Significant

EIN Erythema, Induration and Necrosis

Table (٤-١٩)

The Humoral and Cellular Immune Function of Male Rabbits To Normal Human Sperm Protoplasmic Antigen(s).

Investigation	Response at the below mentioned days post to the multisite injection of the antigen(s) to male rabbits		
	٣٤ days	٣٧ days	٤٠ days
I. Celluar	EIN	EIN	EIN
١- Skin DTH*	١٦ mm*	١٢ mm*	١١ mm*
٢- LIF Peripheral blood	٠.٥٤٥* (s)	٠.٨٥* (non s)	٠.٨١٨* (non s)
II. Humoral HA test			
١- Serum	١٠٢٤	٢٠٤٨	٢٥٦
٢- Appendix	٥١٢	٢٥٦	٥١٢
٣ Genital tract			
A- Injected testis	٢٠٤٨	٥١٢	٥١٢
B- Non injected testis	٥١٢	١٢٨	٢٥٦

* Mean of the readings

(s) Significant

EIN Erythema, Induration and Necrosis

٤–٧ Clinical Physiology

٤–٧–١: Infertility Physiology and Immunophysiology; The infertility Factor and sperm function tests;

The primary (1°) infertility type recorded high percentage than secondary (2°) infertility. In both asthenozoospermic (٨٩.٦٠ vs ١٠.٤٠ respectively) and oligozoospermic (٨٨.٨٩ vs ١١.١١ respectively) as referring patients in table (٤–٢٠–A).

Table (٤-٢٠-B) showed the means of sperm function test (sperm concentration, sperm motility percent and abnormal sperm morphology percent) of oligozoospermic and asthenozoospermic infertile patients complaining for immunological factors.

٤–٧–٢: Effect of SSA on Sperm Function test ; A Statistical Approach

The result revealed a positive significant correlation coefficient between SSA and shaky head motion of spermatozoa (Figure-VIII). While there was inverse positive significant correlation coefficient between SSA and sperm motility index of spermatozoa of immune infertile patients (Figure-IX).

Table (٤–٢٠)

The Infertility Type and Sperm Function Tests.

A-Infertility type

Infertility type	Asthenozoospermia	Oligozoospermia
$^{o}_{1}$ infertility single	٢٠:٢٣ (٨٦.٩%)	٣:٣ (١٠٠%)
$^{o}_{1}$ infertility couple	٢٣:٢٥ (٩٢%)	١٣:١٥ (٨٦.٦%)
$^{o}_{1}$ infertility total	٤٣:٤٨ (٨٩.٦٠%)	١٦:١٨ (٨٨.٨٩%)
$^{o}_{2}$ infertility single	٣:٢٣ (١٣%)	٠:٣ (٠%)
$^{o}_{2}$ infertility couple	٢:٢٥ (٨%)	٢:١٥ (١٣.٣%)
$^{o}_{2}$ infertility total	٥:٤٨ (١٠.٤٠%)	٢:١٨ (١١.١١٪)

B- Sperm function tests

Sperm parameter	Asthenozoospermic patients (٤٨)	Oligozoospermic patients (١٨)
Sperm concentration	٦٦.٥٦ × ١٠^{٦} ± ٢.٨٠ *	٨.٣٣ × ١٠^{٦} ± ١.٣٣
Sperm motility percent	٣٠.٢٥ × ١٠^{٦} ± ١.٨٠*	٢٥.٥٥ × ١٠^{٦} ± ٥.١١
Sperm abnormal morphology	٣٠.٢٧ × ١٠^{٦} ± ٢.٧٣*	٥٦.٥٥ × ١٠^{٦} ± ٤.٧٧
Grade activity	١.٨٠ ± ٠.٠٨٨*	١.١٩ ± ٠.٧٩

* Mean ± SE

( ) Percentage

٤–٨ Correlation of Mucosal to Systemic Immunity (Mucosal Versus Systemic Immune Status of Infertility); Statistical Approach

١. Higher correlation coefficient was noted between serum antibody and mucosal antibodies specific for sperms (Table ٤–٢١)

٢. Direct sperm agglutination % was of non significant correlation with the sperm specific antibodies. Both by correlation coefficient and regression analysis studies (Figure-X)

Table (٤ –٢١)

Correlation Of Mucosal and Systemic Immune Status Of Infertility Patients

Local serum	**Mean** ٢٩٦.٦٤٨٦ ٢٢٤٤.٣٢٤٨	**SD** ٤٠٠.٧٩٢ ٣٧٠٢.٤٤١	
		Local	Serum
Local	Pearson corr.	١.٠٠٠	٠.٣٧١*
	Sig.(٢–tailed)	–	٠.٠٢٤
	N^+	٣٧	٣٧
Serum	Pearson corr.	٠.٣٧١*	١.٠٠٠
	Sig.(٢–tailed)	٠.٠٢٤	–
	N^+	٣٧	٣٧

* High significant

+ Number of patients

٤-٩ Feasibility of passive Haemagglutination, Reverse passive Haemagglutination and Leucocyte Inhibitory factors in diagnosis of infertility in man

Z statistic was persuaded to evaluate (assess feasibility) the differences between the percentage of positive results of any two applied immunologic tests on the immune infertility patients. Four statistical observation were reported percentages as tabulated in table (٤–٢٢ A and B). The first percentage designated as $p_{١}$, the second as $p_{٢}$. The summation of the two, $p_{١}$ and $p_{٢}$ was as signed as $\hat{p}$ and $\hat{q}$ is equal to the value of ١٠٠ minus the summation of $p_{١} + p_{٢}$. The calculated (Z) value is measured as in the following formula $Z_c = \frac{\hat{p}_1 - \hat{p}_2}{\sqrt{\hat{p}\hat{q}(1/n_1)+(1/n_2)}}$.

The standardized Z value matched from the area under the curve, standard table values, when it occurred between (-١ and +١). So far it approaches -١ or +١ it will be highly significant.

As previously mentioned the feasibility was matched as the differences between any of two percentages. Thus, the differences between passive haemagglutination and reverse passive haemagglutination; the difference between semiquantitative agglutination and reverse passive haemagglutination; as well as between leucocyte migrating inhibitory factor and the reverse passive haemagglutination test, were found highly significant.

Table (٤–٢٢)

A. Feasibility of The Immunologic Tests in Diagnosis of Infertility

Observations test	Positive total %
١. Sperm sonicate coat ovine erythrocyte	٤٩.٣٦٪
٢. Semiquantitative slide sperm agglutination	٤٩.٣٦٪
٣. Antisperm sonicate coat ovine erythrocyte microhaemagglutination (antigenemia)	٣٠.٠٪
٤. Leucocyte inhibition factors	٥٣.١٦٪

B. Statistical Features

Statistical features	(PHA) standard Agg. – Antigenemia	Agglutination – Antigenemia	LIF– Antigenemia
$P_١$	٤٩.٣٦	٤٩.٦٣	٥٣.١٦
$P_٢$	٣٠	٣٠	٣٠
$P^{\wedge}$	٧٩.٣٦	٧٩.٣٦	٨٣.١٦
$q^{\wedge}$	٢٠.٦٤	٢٠.٦٤	١٦.٨٤
Zc	٢.٥٣١٦	٢.٥٣١٦	٢.٣٩١
P ٠.٠٥	٠.٩٩٤٦ **	٠.٩٩٤٦ **	٠.٩٩ **

** Highly significant.

Chapter Five

Discussion

٥–١: Basic cellular immunology

٥–١–١: Leukocytospermia

The ejaculation invariably contains other than spermatozoa. These include polygonal epithelial cells from the urethral tract, spermatogenic cells and leucocytes, which have been collectively referred to as (round cells). Leukocytes are present in most human ejaculations (Wolff and Anderson, ١٩٨٨ a,b; Aitken and West, ١٩٩٠; Barratt *et al.,* ١٩٩٠; Aitken and West, ١٩٩٤; Aitken and Fisher, ١٩٩٤); the predominant cell type being the neutorphil.

Accurate assessment of the number of leucocytes is important because the excessive presence of these cells may indicate the existence of reproductive tract infection. A threshold concentration of leucocytes beyond which fertility will be impaired is difficult to define. The impact of these cells depends upon the site at which the leucocyte enters the semen. Furthermore, leucocytospermia may be associated with defects in the semen profile including reductions in volume of ejaculate, Sperm concentration, and sperm motility, as wells as the loss of sperm function as a result of oxidation stress (Aitken *et al.,* ١٩٨٩; Aiteken and west, ١٩٩٠) and / or the secretion of cytokines (Hill *et al.,* ١٩٨٧).

As a general guide, a normal ejaculate should not contain more than ١×١٠ ٦ leucocyte /ml (WHO, ١٩٩٩). Such concentration (١ ×١٠٦/ml) was closely different from those results of leucocytospermia of asthenozoospermic, oligzoospermic and azoospermic patients, which are apparently with high concentration of leucocytes; ٥.٦٣٨ ×١٠٦ ±٠.٥٦٢ (٩٣.٧٥٪), ٦.١٦٦× ١٠ ٦± ١.١٠٦ (١٠٠٪), and ٢.٣٨٤ ×١٠٦ ±٠.٩٢٣ (٦١.٥٣ %) respectively as shown in table (٤–١). Infections may include the testicular tissue, vas deference or the sexual

accessory gland (e.g. prostate, seminal vesicles and bulbo-urethral gland). Besides, the relationship between the presence of genital tract infection is controversial (Comhair *et al.,*١٩٨٠; WHO, ١٩٩٢).

Our results of the leucocytospermic patients agreed with those of Talbert *et al.,* (١٩٨٧); Eggert *et al.,* (١٩٩٢) which had been referred to decrease in number and motility of spermatozoa, Leucocytospermia of the asthenozoospermic, oligozoospermic and azoospermic patients study also agreed with those study of Wolff *et al.,* ١٩٩٠; Gonzales *et al.,*١٩٩٢; Potitch *et al.,* ١٩٩٣; Yanushpolsky *et al.*, ١٩٩٦). Whom concerning the morphology of spermatozoa and the reduction of normal forms, as well as the decrease of concentration motility spermatozoa which are relatively evident. In view of susceptibility of human spermatozoa to oxidative stress, the presence of neutrophils is likely to be damaged, particularly if the infiltration occurs at the level of the rete testes or epididymis.

Conversely, the entry of leucocytes, at the moment of ejaculation, via the prostate or seminal vesicles is probably less harmful because of the powerful antioxidant effects of seminal plasma (Jones *et al.,* ١٩٧٩) . Hence this may be attributed to the fluctuating effect of leucocytospermia. Thus, such leucocytospermia may be beard in mind as:

I. Indication of genital infection .

II. Indication of urogenital infection .

III. Influxes of tissue harbouring leucocytes.

IV. Cellular adjunct to infertility

V. A part of normal nonspecific cellular immune surviellence expressed by human immune system.

٥–١–٢: Leucocyte migration- inhibitory factor (LIF)

LIF test may be considered as another confirmative clinical diagnostic test for patients showed positive immunological SSA responses of infertile couples.

Among the most popular cytokines playing a role in cell mediated immunity to the Allergen (antigen) are leucocyte migration- inhibitory factor (LIF) and other cytokines (IL–٤ and IFN–γ) that participate in this function (Bernhagen *et al.,*١٩٩٨) . Therefore a good feature was being studied of *in vitro* method for detection of cell mediated immunity in clinics issue should:

١. Be specific for cell-mediated immune reaction

٢. Correlate with *in vitro* manifestations of the reaction

٣. Be quick and use a minimum of venous blood as the source for both Immune reactive and indicator cells (Al-Sa'adi, ٢٠٠٤).

The inhibition of leucocytes migration from capillary tubes is one of the most popular *in vitro* methods for detection of cell-mediated immune reactions (Silobrcic *et al,*١٩٧٥; Bacher *et al*., ١٩٩٦; Benigni *et al.,* ٢٠٠٠) in clinics. The action of sperms antigen (s) *in vitro* upon immunocopetent results in an inhibition of cell migration if the cell originate from the immunological infertile subjects (male and /or female), which are in a state of cellular hypersensitivity to the same antigen (Soborg, ١٩٦٨).

Immunological infertile couple patients table (٤–١ B) showed significant leucocyte migration inhibition indices in asthenozoospermic and oligozoospermic couples and non significant LIF in each of the azoospermic, and control couple groups.

These results of LIF depend generally on two main factors:

١. The sensitivity of cells to the specific sensitizer . and

٢. the antigen concentration (Soborg, ١٩٦٨)

The last factor (Sensitizer concentration) was being similar in all the four groups (asthenozoospmia, oligozoospermic, azoospermia, and controlled coupled groups). Therefore the variation in the results of LIF could be attributed to the first factor, sensitivity of cells. Juttner and Bernhagen, (١٩٩٨) stated that LIF plays an important role in delayed-type hypersensitivity (DTH). Shah and Punjabi, (٢٠٠٤) showed that the male autoimmune reactions to sperm specific antigen(s) may be associated with immune complex type three hypersensitivity.

While Dutch, (١٩٥٨) a gynecologist, found that human seminal plasma allergy in women is an exceptionally rare phenomenon and it is usually caused by sensitization to proteins present in the seminal fluid, leading to immediate hypersensitivity manifestations during or soon after coitus (Chang, ١٩٦٧; Halpern *et al.,* ١٩٦٧; Best *et al.,* ١٩٨٨; Shah *et al.,* ١٩٨٨; Bernstein *et al.*, ١٩٩٧; Shah, ٢٠٠٠).

Roitt *et al.,* (٢٠٠١), mentioned that experimental animals such as gunia pig ocrhitis had been classified as stimulatory type V hypersensitivity.

Other investigators found that there was a significant correlation demonstrated between the intensity of LIF and extent of experimental pulmonary TB and correlates well with the tuberculin skin reaction of DTH,as it was present in the applied injection of sonicate sperm sensitizer in the male and female rabbits experiments (tables ٤–١٨ and ٤–١٩) (Trnka and Skvor, ١٩٧٩). Peritonial macrophages taken from animals exhibiting DTH are

markedly inhibited from spreading *in vitro* in presence of specific antigen (Dekaris *et al.,* ١٩٧١)

Likewise, a sharp decrease in mean LIF index was detected following the sensitization of rats by tuberculin (Veselic *et al.,* ١٩٧٢). The ability of host cells to be sensitized to specific antigens determined to a large extent the difference in LIF values from patient to patient. In addition to the sensitivity of cells, other factors may effect the results of LIF including those concerning with the producer cells, LIF is produced by antigen sensitized lymphocytes, pituitary glands, cells of brain, kidney, lung, prostate, testis and macrophages (Das, ٢٠٠٠).

Any abnormalities in the production of this factor may cause a variation in the inhibition of leucocyte migration. (Dai *et al.,* ١٩٩٨) reported that macrophage function including (MIF) was not impaired in protein malnutrition.

Hence, the LIF results, of this study agreed with most of the previous mentioned investigators, whom elicitor the significancy effects of the LIF in human and experimental mammalian animals, as it was present in LIF of infertile couples (table ٤–١ B; mean, median and range) of the asthenozoospermic, oligozoospermic, azoospermic and control groups which present with increased LIF significant effect as result of exposure of the immune system to the same sperm specific antigen(s), that are specifically associated with cells production, usually of T- cells DTH (Terr, ٢٠٠١).

While the non-significant values of LIF for azoospermic and control groups were in agreement with that reported by (Veselic *et al.,* ١٩٧٢). These non-significant values may be attributed to either the lack of antigenic sensitization or to the absence of long- term antigenic sensitization, which is necessary for the expression of inhibitory function.

٥–٢: Basic Humoral Immunology

٥–٢–١: Direct Sperm Agglutination

Agglutination of spermatozoa means that motile spermatozoa stick to each other – head to head, mid piece to mid piece, tail to tail or in a mixed way, e.g mid piece to tail. The adherence either of immotile spermatozoa to each other or of motile spermatozoa, or debris is considered to be non specific aggregation (WHO, ١٩٩٩). So the agglutination of the sperm cells either specific or non- specific causes clumping which prevent the motility and activity of sperm (WHO, ١٩٩٩).

The seminal fluid infection (SFI) may indicate that sperm agglutination is not specifically immune reaction. But it may be due to the cytotoxic materials which secret from the inflammatory cells which cause sperm clumping and agglutination (Saeed, ١٩٩٩)

Rohde *et al.,* (١٩٩٩) reported the association of nonspecific sperm agglutination with adenovirus infection. The reported range of direct sperm agglutination (table ٤ -٢) was ٢٥-٤٩ % among infertile patients. Such percentage ranges are in agreement with (Lynch *et al.,*١٩٨٦; Naessen *et al.,* ١٩٨٦; Montag *et al*., ١٩٩٨a; Ridha Al-Barazanchi *et al.,* ١٩٩٢; Waheda, ١٩٩٣; Allaw, ١٩٩٩; and Rohde *et al.,*١٩٩٩) .

However, zero % agglutination were also noted among few oligozoospermic patients, but with serum and/ or mucosal SSA. Such findings may be interpretated on the basis of an intermittent expression of direct sperm agglutination in various ejaculations of semen (WHO,١٩٩٩).

٥–٢–٢: Sperm Specific Mucosal & Systemic Agglutinins

The Clinical diagnosis for each of the previous asthenozoospermic, oligozoospermia & azoospermic male and female couples infertility. Attempts were made to use another modified semiquentitative test dependent on the whole sperm surface antigens for defection the titers of autoimmune and isoimmune of SSA in each of the mucosal & systemic former examined sample of the infertile couple to promot the pervious PHAT test and keep quite accuracy matched reading as in many of the investigated studies of SSA detection titers such as De-Almeida *et al.* (١٩٨٦), Hjort and Meinertz (١٩٨٦) whom, compared two different ways of testing for SSA; the mixed antiglobulin reaction (MAR) test demonstration of antibodies of IgG and IgA classes bound *in vivo* to sperm membrane antigens and the gelatin agglutination test for detection of nonbound SSA in serum and seminal plasma. While Andreou *et al.,* (١٩٩٥) used the most reliable and clinically relevant method through evaluated results of the direct and indirect SSA detection tests in serum and seminal plasma using the spermMAR and immunobead (IB) techniques for the detection of immunoglobulin (Ig) classes G, A and M, and compared the results with those of tray agglutination test and the adenosine triphosphate release cytotoxicity test. The results obtained indicate that the spermMAR tests for IgG and IgA are more accurate and biologically relevant, as well as easier to perform than the respective IB tests, and the former test must be considered the method of first choice.

To our case, however, it seems feasible to use sperm specific agglutinins adjunctely in diagnosis of immune infertility.

Hence, our results of semiquantitative modified test were clearly matched reading the positive and negative presence of SSA in each of the mucosal & systemic tested samples for the same infertile couples of PHAT examinations, that may give definitive clinical diagnosis of the existence of SSA

which are in share of impairment infertility problems. Sperm agglutinins were found of comparable quality control parameter values for index of sensitivity, index specificity and so on..

٥–٢–٣: Sperm Specific Mucosal & Systemic Haemagglutinins

Since there is no goal standard for diagnosis of immunological infertility, a comparison between different methods and of their results with defining the most reliable and clinically relevant method is at most recommended (Andreou *et al.*, ١٩٩٥). We have evaluated the serum, seminal plasma and cervicovaginal mucus secretion of both male & female infertile patients at status of the mucosal and systemic modified Haemagglutinins estimated titers.

Studies performed on animals & humans demonstrated that the mucosal and systemic compartments of the immune system display a significant degree of mutual independence (Alley & Mestecky, ١٩٩٨). Immunoglobulin (Ig) present in external secretions or systemic fluids are represented by molecules of different physicochemical and biological properties. In external secretions of humans and many mammalian species, the dominant Ig is secretory IgA (S-IgA), consisting of polymeric IgA (PIgA) with J chain, and secretory component (Sc) derived from epithelial cells (Mestecky & McGhee, ١٩٨٧). However, the secretion of female and male genital tracts differs from other external secretions (e.g.; saliva or milk) as well as from plasma in the proportion of Ig isotypes and forms.

The cells engaged in the production of antibodies and to a lesser degree, various population of T cells destined for systemic and mucosal compartments display different tissue distribution, origin of precursors, and maturation patterns (McDermott and Bienenstock, ١٩٧٩; Phillips-Quagliata and Lamm, ١٩٨٨). Approximately ٧٠٪ of all Ig-producing cells in the human body are

found in mucosal tissues; the remaining ٣٠٪ are found mainly in the bon marrow (the most important source of plasma IgG and Ig A) and in the spleen and lymph nodes (Brandtzaeg, ١٩٨٥; Conley and Delacroix,١٩٨٧). In human cervicovaginal secretions or cervical mucus, there are show higher levels of IgG than IgA; this contrasts with other typical external secretions, such as saliva, milk and intestinal fluids, in which S-IgA is the dominant isotype (Mestecky and Russell, ١٩٩٩).

There is an evidence that Igs produced are locally and transported in the vaginal canal (Jalanti and Islaker, ١٩٧٧). The subepithelial connective tissue of the human vagina contains dispersed IgA- & J chain-positive plasma cells, the multilayered epithelial cells do not stain for SC (Kutteh *et al.*,١٩٨٨; Mestecky *et al.,* ١٩٩٦). Nevertheless, both IgA and IgG positive epithelial cells are frequently found on luminal surface and dispersed among the multilayered epithelium. These data suggest that certain population of vaginal epithelial cells can acquire, with some degree of selectivity, locally produced and plasma derived proteins. However, mechanisms of Ig uptake are unknown, and the functional significant of intraepithelial Ig for defense of vaginal mucosa remains to be determined (Mestecky & Russell, ١٩٩٩). While IgG, IgA and IgM have been reported in both pre-ejaculate and seminal plasma (Rumke, ١٩٧٤; Tauber *et al.,* ١٩٧٥; Flower and Mariano, ١٩٨٣), their relative levels have varied, perhaps due to differences in collection procedures, methods and standard used in Ig measurements, and the presence of proteolytic enzymes that are essential in liquefaction of semen but also degrade especially IgM (Tjkronegoro and Sirisinha,١٩٧٤). Higher levels of IgG than IgA are present in the ejaculate (Flower and Mariano, ١٩٨٣). However, the pre-ejaculate contains more IgA than IgG (Pudney and Anderson, ١٩٩٥). It appears that most IgG is derived from the circulation, while IgA, which is mainly represented by S-IgA is

of local origin (Rumke, ١٩٧٤; Tauber *et al.*,١٩٧٥). Anderson & Pudney (١٩٩٢) reported prominent SC expression in normal epididymis, seminal vesicles, prostate and the epithelial cells in Littre's glands in the penil urethra (Perra *et al.,* ١٩٩٤; Pudney and Anderson, ١٩٩٥). Therefore, immmochemical and immunohistochemical data suggest that both plasma-derived and locally produced Ig are present in seminal fluid.

These types of Igs may be stimulant, synthetic & released resulted with an immune response (IR) to self antigens producing autoimmune response (AIR) as present with SSA reaction in the genital tract of infertile male or non-self antigens as isoimmune response (IIR) as with SSA reaction in the genital tract of infertile women . Thus, seminal plasma (SP) and cervicovaginal exterinal secretion that shares common features with both typical secretions and plasma specifically, SP and cervicovaginal contains naturally occurring S-IgA Abs to environmental Ags of microbial origin & to an orally administered bacterial vaccine & plasma derived IgG Abs to systemically injected vaccine, Therefore, both mucosal and systemic immunization with various types of Ags can induce humoral immune responses and this finding should be considered in immunization strategies to induce humoral responses against autoimmune, isoimmune & sexually transmitted diseases (STD_s), (Zina *et al.*, ٢٠٠٥).

Thus, circulating sperm specific haemagglutinins were mostly higher than mucosal titres. Clinical titres appeared to range from (١٦٠-١٠٢٤٠). These titre ranges were in agreement with other workers (Royle *et al.,*١٩٨١; Cimino *et al.*, ١٩٨٧; Cimino *et al.,* ١٩٨٧; Kubota, ١٩٨٧; Meinertz, ١٩٨٧) and disagrees with other group of workers (De-Almeida *et al.,* ١٩٨٦; Micic *et al.,*١٩٩٠; Collins *et al.,* ١٩٩٣). The results of quality control parameters were parallel with that of others working on SSA and infertility (Adeghe*,* ١٩٨٧; Hjort and Meinertz, ١٩٨٦; Shulman, ١٩٨٦ a,b; Hinting *et al.,* ١٩٨٨; Gilbert *et al.,* ١٩٨٨; Parr and

Parr, ١٩٩٠; Haneberg *et al.,* ١٩٩٤; Wassen *et al.,* ١٩٩٦; Kozlowski *et al.,* ١٩٩٧; Mestecky and Fultz, ١٩٩٩; Choudhury and Knapp, ٢٠٠٠; Russel and Mestecky, ٢٠٠٢; Bohring & Eggert - Kruse, ٢٠٠٣; Zina *et al.,* ٢٠٠٥).

٥–٢–٤: Sperm Specific Mucosal and Systemic Antigenemia

In any immune disorders in human being, whatever its nature, the nature of immune tissue injury that parallel it can be mediated by either antibody in blood or in tissue fluids or antigens in same fasion. Meantime, antigens may be present in higher concentration in blood and tissue mellu from insitue denovo released or eventually introduced, in case, where it may regulate the immune responses. In the present issue, however, stander sperm specific sonicate antibodies were coated to tunned ovine erythrocyte to detect level of sperm antigenemia in immune infertile sperm specific antibody negative patients. In these patients, sperm antigenemia were evident to various degrees (٣٢-١٢٨) and (١٦٠-٦٤٠) for mucosal & systemic respectively (table٤-٥). These results were consistent with findings of other workers using infertile immune sera and found around ٩٨ protein sperm specific antigens. Thus, antigenemia can be a cause of infertility in men (Linnet, ١٩٨٣; Meshan, ١٩٨٤; Haas, ١٩٨٧; Shai and Noat, ١٩٩٢; Snow and Ball, ١٩٩٢; Benoff *et al.*, ١٩٩٣; Jones, ١٩٩٤; Surrey, ١٩٩٧; Shetty, ١٩٩٩; Pujianto, ٢٠٠٠; Bohring *et al.*, ٢٠٠١) and women which have not been tackled previously for antigenemia. Hence on summing up, one may state the following:

١- Mucosal and systemic antigenemia were evident both in male and female infertile couples.

٢- It is reported in women for first instance.

٣- It can be as infertility causal.

٤- It may be useable as infertility probe.

٥–٣: Evaluation of Immune Function Parameters

The sperm antigen(s) in male is of sequestrated type (Occluate) (Gillbert *et al.*, ١٩٨٨; Setchell *et al.*, ١٩٩٠; Flickinger *et al.*, ١٩٩٥; Bronson, ١٩٩٩a). Its release, however, needs proking of the testicle-blood barrier (Tung, ١٩٨٠; Gillbert *et al.*, ١٩٨٨; Campbell's, ١٩٩٢; Naz, ١٩٩٦) through trauma. The male immune system can recognize these antigen as an autoantigen and consequently the response of autoimmune type.

Sperm specific peptide antigen is linked to MHC II molecule on surface of APC which present it to T helper ٢ which in turne triggers B lymphocyte to grow up, proliferate and expand, then, do secret sperm specific antibodies and/or cytokine ١٠ & ١٢ in peripheral lymph glands as well as bone marrow (Tabibzadeh and Xz, ١٩٩٢; Simon *et al.*, ١٩٩٣; Hunt, ١٩٩٣; Chegini *et al.*, ١٩٩٤; Eggert-Kruse *et al.*, ٢٠٠١; Kwak-Kim *et al.*, ٢٠٠٣; Mao *et al.*, ٢٠٠٥).

Meanwhile APC bearing sperm peptide in conjugation with MHC II presents these epitopes to mucosal T helper cells, such T helper cells triggers pro B cells and primed them. Hence, sperm epitope primed pro B cells do migrate through lymph and blood circulation to thoracic lymph duct to effecter sites where it is homed there and to secret antibodies. In female, however, the mucosal compartments tolerate sperm antigens naturally. So sex-intercourse, in sites other than intravaginal or intravaginal during menstruation, late in menstruation with rater frequent influxes of sperm antigens may follow similar immunobiologic events for production of serum and mucosal antibodies. By these immunobiologic mechanisms, sperm specific antibodies were produced in infertile couples both at mucosa and peripheral blood (Tauber *et al.*, ١٩٧٥; Flower & Mariano, ١٩٨٣; Stites *et al.*, ١٩٨٧; Parr & Parr, ١٩٩٠; Haneberg *et al.*, ١٩٩٤; Michael *et al.*, ١٩٩٥; Wassen *et al.*, ١٩٩٦; Kozlowski *et al.*, ١٩٩٧; Quayl

et al., ١٩٩٧; Anderson *et al.*, ١٩٩٨; Lu *et al.*, ٢٠٠٠; Richard *et al.*, ٢٠٠٠; Roitt & Rabson, ٢٠٠٠; Russell, ٢٠٠٢).

Sperm specific peptide epitope in conjugation with MHC II on APC surface may be presented to T helper ١ which triggers T cytotoxic or T dth to produce cytokines at both mucosal and systemic compartment, thus, sperm specific antibody and cell mediated immunity were established for male and female infertile patients.

The immunologic investigations that have been done for those infertile couples (Tables (٤–١ A&B; ٤–٢; ٤–٣; ٤–٤; ٤–٥)), have revealed the following immunologic features:

I- Cellular immune features
   - i- Variable degrees of leucocytospermia not correlated with SSA levels.
   - ii- Significant leucocyte inhibitory factors at most cases.

II- Humoral immune features
   - iii- Variable degrees of sperm agglutination in semen.
   - iv- Sperm specific agglutinin and haemagglutinins.
   - v- They are circulating and mucosal.
   - vi- Sperm specific mucosal and **/**or systemic antigenemia.
   - vii- Direct sperm agglutination in semen wouldn't correlated with levels of mucosal and systemic sperm specific antibodies.

The sperm specific antibody levels and LIF can be used as diagnostic probe for immune infertility in couples as in the following table:

Table (٥-٢٣)

Utility of LIF Test as Diagnostic Probe for Immune Infertility Couples

Cellular	Mucosal	Systemic
LIF		
Nonspecific	٧١-٩٨	٧١-٩٨
Border line	٦٩-٧٠	٦٩-٧٠
Significant	< ٦٩	< ٦٩
Humoral		
Base-line	٢-٤	١٠-٢٠
Suspect	٨-١٦	٤٠-٨٠
Clinical infertility	٣٢-٢٠٤٨ or more	١٦٠-١٢٨٠

٥-٤: Seroprofiles

The infertile couples patients can be classified into seronegative and seropositive for sperm specific antibodies or for sperm specific antigens. Meantime in each couple they are either both of seronegative or both of seropositive (Agglutinins & Haemagglutinins) (tables ٤-٦ ,٤-٧ and ٤-٨), or it may be with one of the couples is seronegative and the other seropositive. Such seronegative results may be real absence of sperm specific immune responses and/or the agglutinin and haemagglutinin assays are with specificity and sensitivity limits below that need for detection of the mounted immune responses.

Seronegative couples can be attributed to non antibody or non antigen mediated infertility, such as obstruction, surgery, infections (Cockett *et al.*, ١٩٧٩; Sharlip, ١٩٨٤; Aafijes and Vander Vijver, ١٩٨٥; Golomb *et al.*, ١٩٨٦; Marks *et al.* ١٩٨٦; Shahmansh *et al.*, ١٩٨٦; Zhong *et al.*, ١٩٨٩; Wolff *et al.*, ١٩٩٠; Comhaire, ١٩٩١; Campbell's *et al.*, ١٩٩٢; Gonzales *et al.*, ١٩٩٢; Parslow, ٢٠٠١).

From the aforementioned seroprofile studies on these infertile couples (tables ٤–١٠, ٤–١١ and ٤–١٢), one may suggest a serologic classification of infertility couples as in the followings:

Class I: Seropositive, antigenemia negative

a. Agglutinins
   i. Mucosal ii . Systemic or iii. Both
b. Haemagglutinins
   i. Mucosal ii . Systemic or iii. Both

Class II: Seronegative, antiganemia positive

i. Mucosal ii . Systemic or iii. Both

Class III: Seronegative, antigenemia negative

٥–٥: The Immune Statue

The infertile couples (tables ٤–١٢, ٤–١٣, ٤–١٤ and ٤–١٥) have shown to be of immune mediated and non immune mediated types (Cebra *et al.*, ١٩٩١; Qualye *et al.*, ١٩٩٨; Comhair, ١٩٩٩; Gabriela *et al.*, ٢٠٠١; Eggret-Kruse *et al.*, ٢٠٠١). Meanwhile the immune mediated could be humoral, cellular and/or both. The cellular can be significant and non significant leucocyte inhibitory factor, with various degrees of leucocytospermia (Hill *et al.*, ١٩٨٧; Lanenfeld *et al.*, ١٩٨٩; Cumming *et al.*, ١٩٩٠; Papadimas *et al.*, ١٩٩٠; YanuhpolsKy *et al.*, ١٩٩٦; Dai *et al.*, ١٩٩٨; Bernhagen *et al.*, ١٩٩٨; and Das, ٢٠٠٠).

Meanwhile one of the couples may be immune mediated and the other can be non-immune mediated. The non-immune mediated is due to obstruction, surgery, infection or trumal causes. Therefore, on summing up the results and discussion of the immune status of infertility patients, one may suggest an immunologic classification for infertility as in the followings:

Class I: Immune mediated, both couple affected

a- Humoral (Mucosal, Systemic).immune responses

or

b- Cellular. immune responses

or

c- Humoral & cellular.immune responses

Class II: Immune mediated, one of the couple affected

a- Humoral (Mucosal, Systemic).

or

b- Cellular.

or

c- Humoral & cellular.

Class III: Non immune mediated, both couple.

٥-٦: Immune Infertility and ABO Erythrocyte Surface Antigens

Good knowledge about (ABO) blood group has expanded to include a diverse and numerous array of antigenic determinates on erythrocytes. Approximately ٦٠٠ eryrthrocyte antigens are known, each antigen is controlled by one gene. The antigenic determinants of blood group are produced either directly (for proteins) or indirectly (for carbohydrates) by alleles at a single gene locus or at other gene loci so closely linked that crossing over is extremely rare.

There is an evidence of many other systems which may directly interact with ABO and H systems: e.g. Lewis and Secretor secretion of ABH substances in body fluids (saliva, sweat, milk, seminal plasma, tears etc). On erythrocyte and endothelial surfaces, most of antigens are bound to glycosphingolipids (Parslow *et al.*, ٢٠٠١).

The results of ABO erythrocyte surface antigens and their distribution among infertile couples show that type A blood group is present to be more susceptible than the other types of ABO blood group; this may be due to genetic predisposition with ABO blood group system. This also may indicate that there is an association between ABO antigen for those of infertile subject (couples), and that may be explained as subject genetics predilection for specific immunological infertility as in table (٤-١٦).

٥–٧: The Immunologic Causes of Infertility

Circulating and/or mucosal sperm specific antibodies in husband, wife and/or in both of them can be the most evident causes of immune infertility among our patients ٢٧:٤٦ (٥٨.٦٩٪). To lesser extent, however, the sperm specific antigeremia and sperm DTH ١٥: ٤٦ (٣٢.٩٦٪) per each.

Mixed humoral and cellular causes can match ٢٣:٧٦(٣٠.٢٦٪) (tables ٤-١٧). Sperm specific ab as causative for infertility was thoroughly mentioned by other works (Meinertz, ١٩٨٧; Adeghe, ١٩٨٧; Bronson and Cooper, ١٩٨٧; Kutteh *et al.*, ١٩٩٥; Shetty *et al.*, ١٩٩٩; Herr *et al.*, ١٩٩٩; Kurpisz and Domagale, ٢٠٠٤).

The noted antigenemia among infertile couple were recently mentioned by several workers (Gilbert, ١٩٨٨; Shetty *et al.*, ١٩٩٩; Pujianto, ٢٠٠٠; Choudhury and Knapp, ٢٠٠٠; Rao *et al .,* ٢٠٠٣).

Sperm DTH, however was sought in laboratory animal studies (Filickinger *et al.*, ١٩٩٥; ١٩٩٦; ١٩٩٧; ١٩٩٨; ٢٠٠٠) and as semen sensitivity "hypersensitivity" in women (Dutch, ١٩٥٨; Chang, ١٩٦٧; Halpern *et al.,* ١٩٦٧; Shah *et al.,* ١٩٨٨; Bernstein *et al.,* ١٩٩٧; Shah, ٢٠٠٠; Shah and Panjabi, ٢٠٠٤).

٥–٨: Lapin Model for Immunity to Sperm Antigens

During this study, utilization of male and femal replicate rabbits were used as in (Figure-VI). These scientifically reliable & dependent animals attempted to simulate of human sperm specific immunity in lab. animal and that can be extrapolated in human being via the investigations of cellular (Skin DTH and LIF peripheral blood) and humoral systemic (serum), and mucosal (appendix and male genital, female genital compartments).

Prepared human sonicate sperm was examined for sperm specific haemagglutinin, which was present in higher levels serum than those of mucosal in both sexs, and that will be agreed with that of human estimations; at the same time it appears higher in female more than in male for both of the humoral serum, appendix and genital tract, this may be related firstly to the highest foreginess of the sperm sonicate in female, does not posses the specialist tissue for producing this type of cell (Sperms), than of male, whose already produce these types of cells and/or to some time same sperm specific isoantigens, or due to cross –reaction with human sperm (O'Rand and Porter, ١٩٨٢; Shelton and Goldberg, ١٩٨٥; Goldberg, ١٩٨٦; Leyton and Saling, ١٩٨٩; Welch *et al.*, ١٩٩٠; Wright *et al.*, ١٩٩٠; O'Rand *et al.*, ١٩٩٣; Lin *et al.*, ١٩٩٣; Naz *et al.*, ١٩٩٣; Coonrod *et al.,* ١٩٩٤; Sirinivasan *et al.*, ١٩٩٥; Naz *et al.*, ١٩٩٥). And secondly may because, the multiple site and multi-repeated injection that differ in female than of male using immunization of mucosal surfaces such of vaginocervical route, that may probably high sensitive site for

induce immune competents response against many of strong antigens (Rebello *et al.*, ١٩٧٥; Wassen *et al.*, ١٩٩٦; Kutteh and Mestecky, ١٩٩٦; Russell, ١٩٩٩; Hong-Yin *et al.*, ٢٠٠٠; Ogra *et al.*, ٢٠٠١; Russell, ٢٠٠٢).

Whereas such reflexes are present in the LIF test, DTH skin test showed a significance respond, which being higher in female than of male, and that may also be explained as mentioned earlier.

All these lapin model immunity results will be precipitating each of B-cell dependent and T cell dependent to established the humoral, cellular & memory cells immune responses.

Thus, rabbit immune system responds well to human sperm protein immunogen(s). The immune response was of humoral mucosal and systemic specific to human sperm antigen(s). the systemic cellular were as significant LIF results and positive skin delayed hypersensitivity reactions.

٥–٩: Clinical Physiology

٥–٩–١: Infertility Physiology and Immunophysiology; the infertility factor and sperm function tests:

Previously, we mentioned the definition of infertility as the lack of conception after one year of unprotected intercourse (WHO, ١٩٩٩). Accordance to the above definition, about ١٥٪ of couples have infertility problems (Glover *et al.*, ١٩٩٩). Approximately ٢٤٪ of those infertile couple have an exclusive male factor, ٢٤٪ have factor in both male and female, and ٤٠٪ have predominant female factor (WHO, ١٩٩١; Glover *et al.*, ١٩٩٩).

While the ١٢٪ remaining couples do not show any demonstrable infertility cause in either partners (WHO, ١٩٩١). Therefore, the couples which have never achieved pregnancy at any time the infertility is described as $^{o}1$

Infertility, whereas if infertility follows a pregnancy, whatever the outcome, it is called $^{o}_{2}$ infertility as referee in(٤-٧-١) (Illions *et al.*, ١٩٩٨).

Dependence on that, the results of table (٤-٢٠-A) shows high number of infertile patients with $^{o}_{1}$ infertility to be eight time more than number of infertile patients with $^{o}_{2}$ infertility couple for both asthenozoospermic and oligozoospermic patients. It is well know that $^{o}_{1}$ infertility is due to male or female infertility factors or both, while the $^{o}_{2}$ infertility was mostly of female factor and to a lsser extent of male factor, which are present after birth of one or more than one child.

Also the high percentage of $^{o}_{1}$ infertility may be attributed to Iraq people circumstance such as; malnutrition, female weight loss, growth retardation and alteration in psychological status of the individual, which are collectively associated with reduction in body resistance to diseases which may affect reproductive organs. As it was clearly observable with the past time of Iraqi conditions (stress of wars), those stressful factors may increase the percentage of $^{o}_{1}$ and $^{o}_{2}$ infertility, but the effect on the infertile patients with $^{o}_{1}$ infertility is more powerful than the infertile patients with $^{o}_{2}$ infertility. As well as to the continuous exposures to environmental pollutants and toxic materials will increase the damage of reproduction system of male & female (Strauss *et al.*, ١٩٨٥; Vermeulen, ١٩٩٣; Boivin and Scanlan, ١٩٩٨; Sharara *et al.*, ١٩٩٨; Thonneau *et al.*, ١٩٩٨; Fargerli *et al.* ١٩٩٩). Pollutants, toxic materials may result in decrease in body defense mechanism and exposed the reproductive organs to the microbial infection as well as the damaging effects, that well present in Iraqi people particularly during the last two decades.

Many researchers revealed that high concentration of ejaculation leucocytes percent and elevation titers of sperm specific auto and/or isolocalized antibodies cause a significant decrease of normal sperm parameters. These auto-iso-antibodies in external secretion of infertile couple has a disruptive action on sperm motility, fertilization, embryo development and pregnancy successfulness (Sharlip, ١٩٨٤; Shulman, ١٩٨٦ a,b; Zhong *et al.*, ١٩٨٩; Wolff *et al.,* ١٩٩٠; Gonzales *et al.*; ١٩٩٢; Garvella and Lipovac, ١٩٩٣; and Zina *et al.*, ٢٠٠٥). Therefore, results of this study table (٢٠–٤–B), showed a wide range retardation of sperm parameter assy. Such as; sperm concentration, motility, grade activity, and normal sperm morphology, that have been evolved both of the asthnozoospermic and oligozoospermic patients to be agreed with those of researchers mentioned results.

٥-٩-٢: Effect of SSA on sperm function test: a statistical approach

For controlling fertility, both gametes (sperm and oocyte) have proteins on the surface that are unique, cell specific, immunogenic and accessible to antibodies.

Immunological interaction with such molecules can cause block of sperm cervical penetration and thus fertilization (Naz *et al.*, ١٩٩٥).

It has been demonstrated that sperm cells has both autoantigens / isoantigens (Rao *et al.*, ٢٠٠٣), potential to generate an immunological response in both men and women, which is capable of causing infertility (Anil Suri, ٢٠٠٤). From the past, our results elicite with a significant positive correlation coefficient ($r = ٠.٢٢٤$) were observed between shaky head motion and sperm specific antibodies (SSA) as showed in (Figure-VIII).

Impact studies, referred that, agglutination and shaky head motion may related to the presence of sperm specific antibody and or seminal fluid

infection (SFI). The shaky head motion, agglutination were causes sperm clumping and than prevent motility and reduce the grade activity of the sperm. Best semen parameter were observed after removal of those SSA and SFI, so this results in a good agreement with data reported by (Adeghe, ١٩٨٧; Bielsa *et al.*, ١٩٩٤; Al-Hady, ١٩٩٧) whom treatment of that of SFI to be resulted in marked reduction in sperm shaky head motion, agglutination and significant improvement in sperm motility, sperm grade activity and sperm motility index as in (Figure-IX).

Hence, (Figure-IX) shows positive inverse significant correlation coefficient (r = ٠.١٣٤) between sperm motility index and sperm specific antibodies.

These results also agreed with ZarmaKoupis-Zaros *et al.* (٢٠٠٠) reported that, sperm motility index considered as useful in predicting fertilization rate, and with that of Ridha-Albarazanchi *et al.* (١٩٩٢) who found positive correlation between sperm agglutination and SSA titer in patients with immunological infertility. Sperm specific antibodies significantly reduce sperm motility and grade activity due to their immobilizing, agglutinating & sperm blocking fertilization interfere or interacting activities (Alexander *et al.*, ١٩٨٦; Lynch *et al.*, ١٩٨٦; Primakoff and Hyatt, ١٩٨٦; Livi *et al.*, ١٩٩٣; Eggert-Kruse *et al.*, ١٩٩٣; De-Almeida, ١٩٩٣; Montag *et al.*, ١٩٩٨_b; Rao *et al.*, ٢٠٠٣).

Results in (Figure-IX) shows well agreement with those investigators who found that sperm specific bound-Igs washig may appear to be significantly improvement motility, grade activity and then after significantly of sperm motility index (Ridha Al-barzanchi *et al.*, ١٩٩٢; and Al-Hady, ١٩٩٧).

٥–١٠: The Correlation between Mucosal and Systemic Sperm Specific Immunity

At times, some workers advocated that antibiodies at mucosal surface are transudated from peripheral blood to mucosal surfaces and local body fluids (Rumke, ١٩٧٤; Tauber *et al.*, ١٩٧٥; Belec *et al.*, ١٩٩٥; Yeaman *et al.*, ١٩٩٧). Then, a group of workers had been putforward a concept of well organized mucosal immune system with several compartments. They, advocate that it is entirely distinct (Holmgren *et al.*, ١٩٩٢; Haneberg *et al.*, ١٩٩٤; Kutteh & Mestecky, ١٩٩٤; Kutteh & Mestecky, ١٩٩٦; Mestecky & Fultz, ١٩٩٩; Ogra *et al.*, ٢٠٠١; Russell & Mestecky, ٢٠٠٢). There have been other third opinion, in which their advocators state that both systemic and mucosal are linked together in some way or other. Using bacterins of C. fetus SS fetus in a lapin model Shnawa and Thewiani, (٢٠٠٢) putforward a prove that these immune systems are linked together. Upon investigating the immune status of infertile couples statistics provided us an evident correlation between the mucosal and systemic sperm specific antibodies (Tables ٤-٢١). As pearson correlation coefficient indicated, the study correlation coefficient between serum and mucosal SSA titers. This situation could be as a results of frequent stimulation through several exposure of the immune competent cells to sperm specific antigen during usual or probably unusual sex intercourses (Bronson *et al.,* ١٩٨٣; Wolff & Schill*,* ١٩٨٥; Wira and Sande, ١٩٨٧; Mestecky and Russell, ١٩٩٩; McGhee *et al.*, ١٩٩٩). While, there were non significant statistics between direct sperm agglutination and SSA titres (Rhida-Albarzanchi *et al.*, ١٩٩٧; and ١٩٩٨; Comhair, ١٩٩٩; Rohde *et al*., ١٩٩٩; WHO, ١٩٩٩). These finding will appear to be in close relation with that of the direct sperm agglutination as it was persent (Figure-X) assessment .

٥-١١: Feasibility of Immune Function Tests for Diagnosis of Infertility ;statistial approach

Leucocytospermia is one of the marks noted among semen of infertile subjects, but it is not clear-cut evidence for it. Likewise, direct sperm agglutination can't be correlated with SSA and clinical state of the infertile subjects. Thus it can't be used alone as criteria for immune infertility. Meantime, there is a notion among clinical serologist held far ago, that one single serologic test can't be dependable for serodiagnosis of a disease or syndrome or disease condition. So, a battery of test should be experimentally evaluated for their feasibility in use for diagnosis of certain condition(s).

Thus, table (٤-٢١A & B) has shown the Z statics for the difference between two percentages of positive by different tests on the same patient. Whereby, the battery of passive haemagglutination, agglutination, reverse passive haemagglutination as well as proved to be collectively evident for immunodiagnosis of infertility. While the best of which can be the LIF followed agglutination and Haemagglutination test. (Gilbert *et al.*, ١٩٨٨; Choudhury & Knapp, ٢٠٠٠; Parslow *et al.*, ٢٠٠١; Bohring & Eggert-Kruse, ٢٠٠٣).

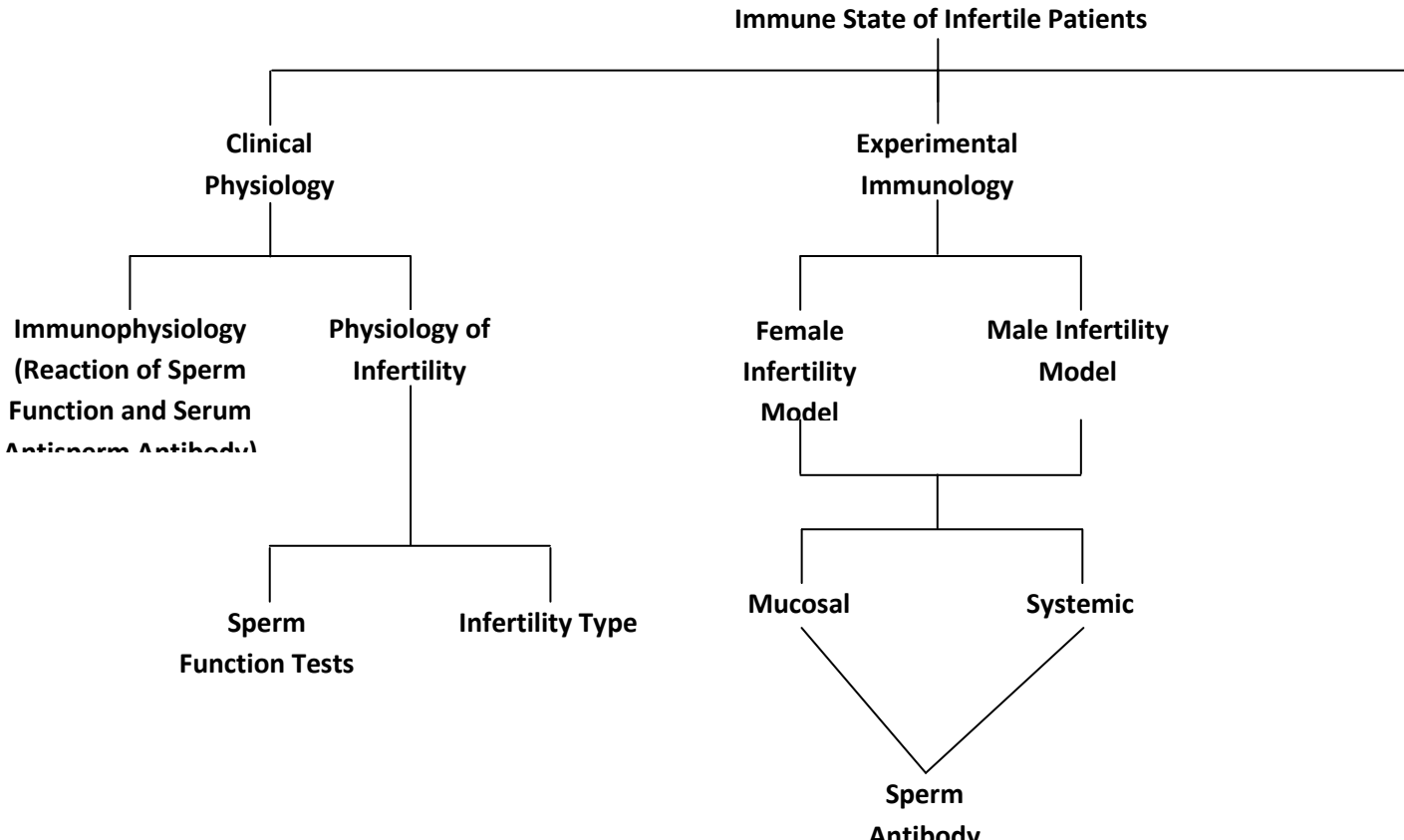


Figure: (III)

The Study Menu

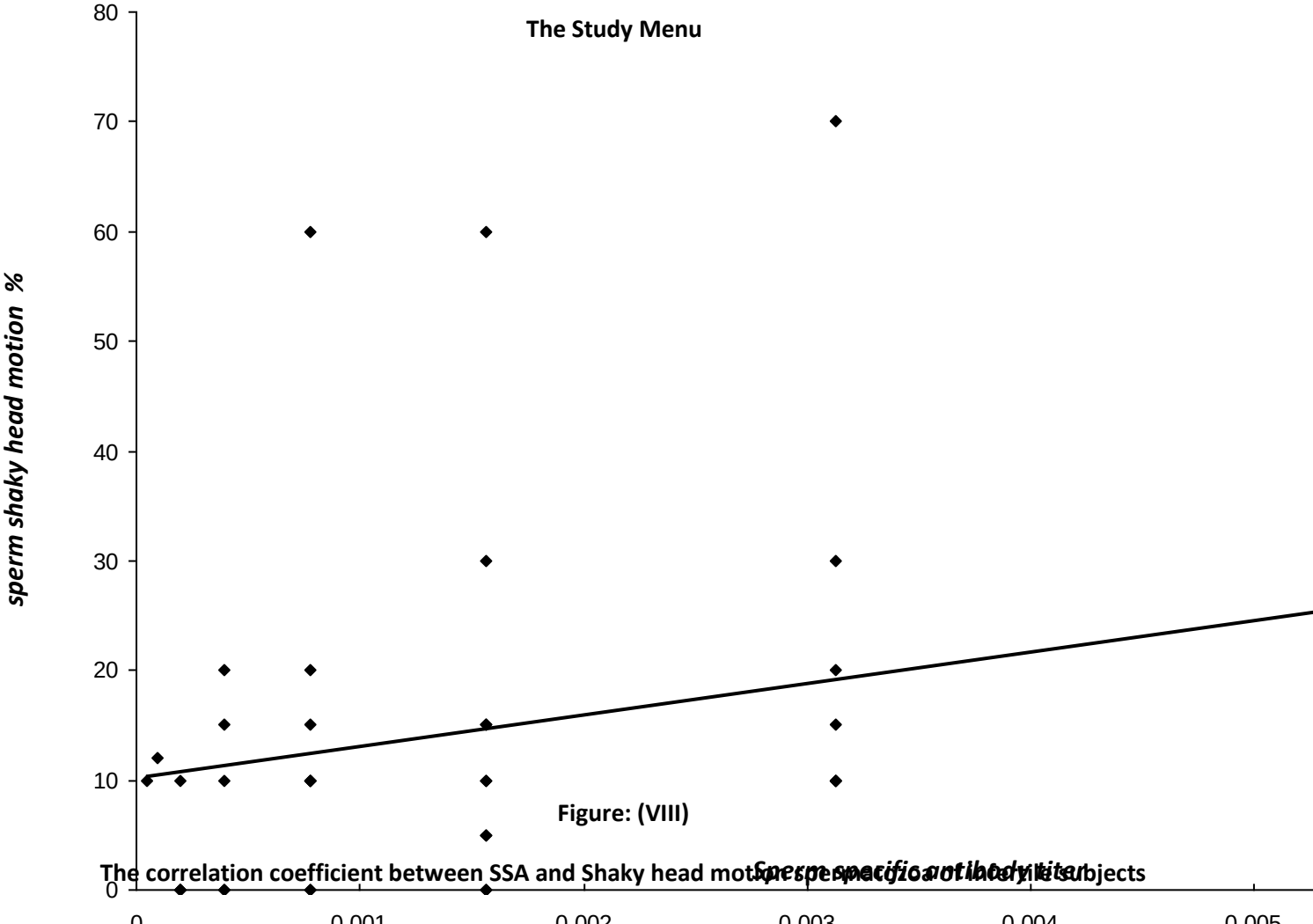


Figure: (VIII)

The correlation coefficient between SSA and Shaky head motion spermatozoa of infertile subjects

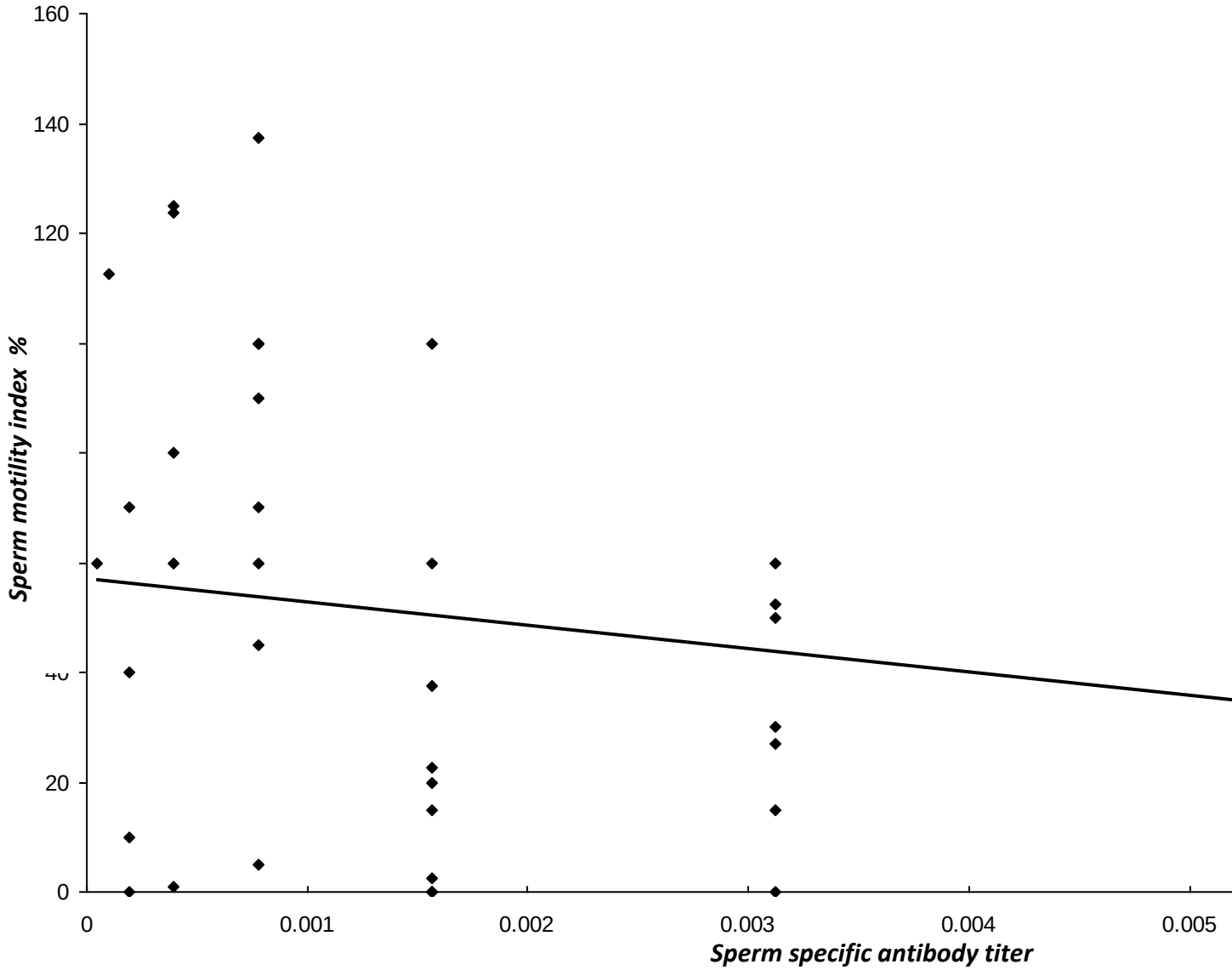


Figure: (IX)

The correlation coefficient between SSA and sperm motility index of infertile subjects

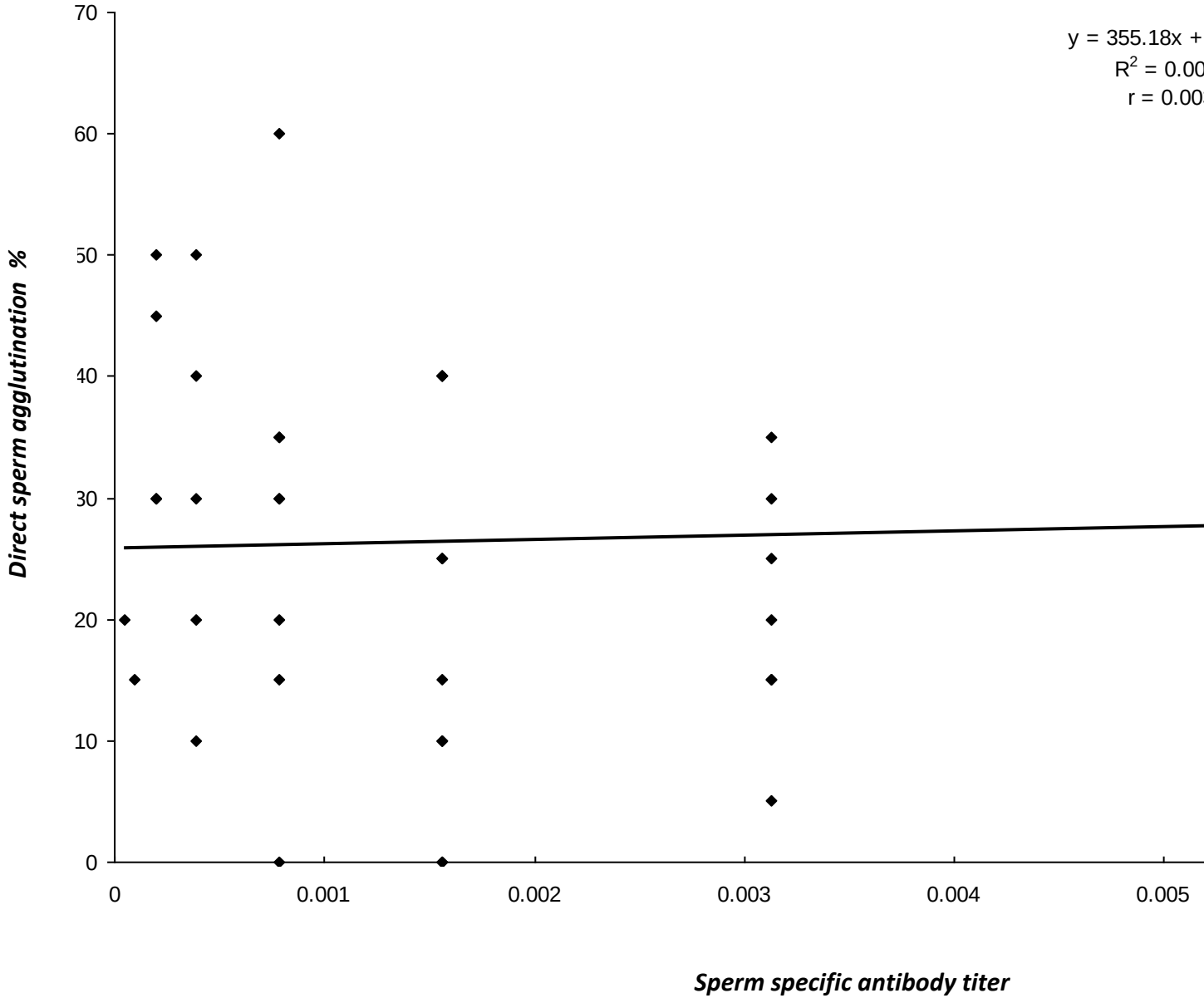


Figure: (X)

The correlation coefficient between SSA and the direct sperm agglutination of infertile subjects

Table (٤ – ١٢)

The Immune status of Athenozoospermic Infertile Couple

eucocytospermia ×١٠٦ / ml	Direct semen agglutination (%)	LIF		Antigenemia				Passive haemagglutination				Semiqua agglu	
				Male		Female		Male		Female		Male	
		Male	Female	M	S	M	S	M	S	M	S	M	S
٣	٤٠	١.٠	٠.٥٠	_	_	_	_	١٢٨	٦٤٠	٣٢	٣٢٠	١٦٠	١٦٠٠
٥	٣٠	٠.٥٨	١.٠	_	_	٤٠	٤٠	٥١٢	٢٥٦٠	-ve	-ve	٣٢٠	٣٢٠٠
٣	٤٠	١.٠	٠.٤٠	_	_	_	_	٢٥٦	٦٤٠	١٢٨	٦٤٠	٣٢٠	١٦٠٠
٢٠	٣٥	٠.٦٢	٠.٩٦	_	_	_	_	٣٢	٣٢٠	٢	٢٠	٤٠	٤٠٠
١	٤٠	٠.٤٠	١.٠	_	_	_	_	١٢٨	١٢٨٠	١٢٨	١٠٢٤	١٦٠	٣٢٠٠
١٤	٦٠	٠.٧٠	٠.٦٢	_	_	_	_	١٢٨	١٦٠	٣٢	١٦٠	١٦٠	٢٠٠
٤	٥٠	٠.٥٦	١.٠	_	_	_	_	٢٥٦	٢٥٦٠	١٢٨	٦٤٠	٣٢٠	٣٢٠٠

١٠	٣٥	٠.٩٥	٠.٩٦	–	–	٤٠	٢٠	١٢٨	١٢٨٠	-ve	-ve	٣٢٠	٣٢٠٠
٧	٣٠	١.٠	٠.٩٦	–	–	٨٠	٤٠	٢٥٦	٥١٢٠	-ve	-ve	٣٢٠	٣٢٠٠
٦	٤٥	٠.٣٥	٠.٦٠	–	–	–	–	١٢٨	٥١٢٠	٣٢	٣٢٠	١٦٠	٣٢٠٠
٤	٣٠	٠.٦٦	١.٠	–	–	–	–	١٠٢٤	٢٠٤٨	١٢٨	٥١٢٠	٣٢٠	٣٢٠٠
٣	١٥	٠.٥٠	٠.٨٣	–	–	–	–	١٢٨	١٠٢٤	٥١٢	٢٥٦٠	٣٢٠	٣٢٠٠
٥	٢٥	٠.٨٣	٠.٩١	–	–	–	–	٦٤	٦٤٠	٣٢	١٦٠	٨٠	٨٠٠
٩	٢٥	١.٠	٠.٦٠	–	–	–	–	١٠٢٤	٢٥٦٠	١٢٨	٦٤٠	٣٢٠	٣٢٠٠
٥	٣٠	٠.٥٤	٠.٣٩	–	–	–	–	٥١٢	١٢٨٠	٢٥٦	١٢٨٠	٣٢٠	٣٢٠٠
١٤	٦٠	٠.٣٨	٠.٤٧	–	–	–	–	١٢٨	١٢٨٠	١٢٨	٦٤٠	١٦٠	٣٢٠٠
٧	١٠	١.٠	٠.٣٣	–	–	–	–	٦٤	٢٥٦٠	٦٤	٣٢٠	١٦٠	٣٢٠٠
٦	٢٠	٠.٥٣	٠.٦٠	–	–	–	–	٣٢	١٢٨٠	١٢٨	٢٥٦٠	٤٠	١٦٠٠
٣	١٥	٠.٣٨	٠.٣٥	–	–	–	–	١٢٨	٣٢٠	٦٤	١٢٨٠	١٦٠	٤٠٠
٤	٦٠	٠.٦٢	٠.٩٦	٦٤	٦٤٠	–	–	-ve	-ve	١٦	٣٢٠	–	–
٣	٤٠	٠.٩٠	٠.٦٦	٦٤	٣٢٠	–	–	-ve	-ve	١٢٨	٢٥٦٠	–	–
٣	٤٠	٠.٤٢	٠.٨٦	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	–	–
٨	٣٥	١.٠	١.٠	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	–	–
٤	١٠	٠.٩٢	١.٠	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	–	–
١	١٠	٠.٦٦	٠.٩٥	٣٢	١٦٠	–	–	-ve	-ve	٢٥٦	٢٥٦٠	–	–

M = mucosal S = serum LIF = leucocyte inhibition factor

Table (٤ – ١٣)

The Immune status of Oligozoospermic Infertile Couple

Sequence	Leucocytospermia ×١٠^٦ /ml	Direct semen agglutination (%)	LIF		Antigenemia				Passive haemagglutination				Semiquantitave slid agglutination			
			Male	Female	Male		Female		Male		Female		Male		Female	
					M	S	M	S	M	S	M	S	M	S	M	S
١	٦	١٥	٠.٤٠	٠.٩١	–	–	-ve	-ve	٢٥٦	١٢٨٠	-ve	-ve	٣٢٠	٣٢٠٠	–	–
٢	٣	١٥	٠.٣٣	٠.٧٦	–	–	-ve	-ve	٥١٢	٦٤٠	-ve	-ve	٣٢٠	١٦٠٠	–	–
٣	٦	١٠	٠.٥٠	٠.٨٣	–	–	–	–	٥١٢	٦٤٠	١٦	٣٢٠	٣٢٠	١٦٠٠	٨٠	٤٠٠
٤	٧	٢٠	٠.٦٦	٠.٩٧	–	–	–	–	١٢٨	١٢٨٠	١٢٨	٦٤٠	١٦٠	٣٢٠٠	١٦٠	١٦٠٠
٥	٥	١٥	٠.٤٤	٠.٨٤	–	–	–	–	٢٥٦	٣٢٠	١٢٨	٣٢٠	٣٢٠	٨٠٠	١٦٠	٤٠٠
٦	٦	٣٠	٠.٨٣	٠.٤٢	–	–	–	–	٦٤	٥١٢٠	٦٤	١٦٠	٨٠	٣٢٠٠	١٦٠	٢٠٠
٧	٢	١٥	٠.٩٢	٠.٩١	–	–	-ve	-ve	١٢٨	١٢٨٠	-ve	-ve	١٦٠	١٦٠٠	–	–
٨	٥	٢٠	٠.٥٠	٠.٥٠	–	–	–	–	٦٤	٣٢٠	٢٥٦	٣٢٠	٨٠	٤٠٠	٣٢٠	٨٠٠
٩	٥	٢٠	٠.٥٣	٠.٧١	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	–	–	–	–
١	٨	٢٠	٠.٧٧	٠.٩١	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	–	–	–	–
١	٣	١٥	٠.٥٠	٠.٤٧	٣٢	٣٢٠	–	–	-ve	-ve	٣٢	٣٢٠	–	–	٤٠	٤٠٠
١	٨	٢٠	٠.٦٠	٠.٩١	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	–	–	–	–
١	١٦	١٥	٠.٦٢	٠.٩١	٨	١٦٠	-ve	-ve	-ve	-ve	-ve	-ve	–	–	–	–
١	٤	١٠	٠.٥٠	٠.٧٧	٨	٤٠	-v	-v	-ve	-ve	-ve	-ve	–	–	–	–

٨٠٠	٨٠	١٦٠٠	١٦٠	٨٠	٣٢	٦٤٠	١٢٨	–	–	e	–	–	٠.٦٦	٠.٤٨	٢٥	٤	١٠

Table (٤ – ١٤)

The Immune status of Azoospermic Infertile Couple

Sequence	Leucocytospermia ×١٠^٦ / ml	Direct semen agglutination (%)	LIF		Antigenemia				Passive haemagglutination				Semiquantitave slid agglutination			
			Male	Female	Male		Female		Male		Female		Male		Female	
					M	S	M	S	M	S	M	S	M	S	M	S
١.	٠	–	١.٠	١.٠	–	–	-ve	-ve	٦٤	٣٢٠	-ve	-ve	٨٠	٤٠٠	–	–
٢.	٠	–	١.٠	٠.٩١	–	–	–	–	٢٥٦	٢٥٦٠	١٦	١٦٠	٣٢٠	٣٢٠٠	٢	٢٠٠
٣.	٢	–	٠.٩٦	٠.٩٦	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	–	–	–	–
٤.	٠	–	١.٠	٠.٨٧	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	–	–	–	–
٥.	١	–	١.٠	٠.٨٣	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	–	–	–	–
٦.	٢	–	٠.٧٥	٠.٨٦	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	–	–	–	–

Table (٤ – ١٥)

The Immune status of Fertile Couples (Controls)

Sequence	Leucocytospermia ×١٠^٦ / ml	Direct semen agglutination (%)	LIF		Antigenemia				Passive haemagglutination				Semiquantitave slid agglutination			
			Male	Female	Male		Female		Male		Female		Male		Female	
					M	S	M	S	M	S	M	S	M	S	M	S
١.	٠	٠	١.٠	٠.٩١	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	–	–	–	–
٢.	٢	٠	٠.٨٣	١.٠	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	–	–	–	–
٣.	١	٠	٠.٩٦	١.٠	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	–	–	–	–
٤.	٠	٠	١.٠	٠.٩٦	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	–	–	–	–
٥.	٢	٠	١.٠	٠.٩٢	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	–	–	–	–
٦.	١	٢	١.٠	١.٠	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	–	–	–	–
٧.	٠	٢	٠.٨٨	١.٠	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	–	–	–	–
٨.	١	٠	٠.٩٢	٠.٨٦	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	–	–	–	–
٩.	١	٤	٠.٨٦	١.٠	-ve	-ve	-ve	-ve	٢	٢٠	-ve	-ve	-ve	-ve	–	–
١٠.	١	٠	١.٠	١.٠	-	-	-	-	٢	٢٠	-	-	-	-	–	–

					ve	ve	ve	ve			ve	ve	ve	ve		

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Conclusions:

١. Leucocytospermia was evident among infertile subjects.

٢. Leucocyte inhibitory factor (LIF) was mostly significant among infertile couples indicating the involvement of cell mediated immunity in aetiogenesis of infertility.

٣. Sperm specific mucosal and systemic agglutinins and haemagglutinins were noted in clinical titer among infertile couples.

٤. Sperm specific antigenemia was noted at mucosal and systemic levels among some of the SSA negative couples.

٥. Rabbit cellular and humoral immune responses to sperm antigens were simulating that noted among human infertile couples.

٦. The applied immune function test battery including LIF, agglutinins, haemagglutinins and antigenemia was statistically feasible for use diagnostic and follow up of infertile subjects.

٧. The infertility of these subjects were of primary and secondary types. The semenologic entities of those subjects were asthenozoospermia, oligozoospermia and azoospermia.

٨. Direct sperm agglutination was not statistically significant with SSA.

٩. Sperm motility index and shaky head motion are correlated with SSA.

Recommendations:

١. The investigation of role of cytokines in immune infertility and evaluation for their possible role as diagnostic probe.

٢. The use of lymphocyte transformation test in the diagnosis of immune infertility.

٣. The use of SSA titers and/or cytokines concentrations as pre and postoperative investigation in IVF and embryo transplantation.

٤. The use of immunoglobulin splitting enzymes and cleave SSA before IVF.

٥. The use of interacytoplasmic sperm injection for curing cases of immune infertility which were irresponding to IVF.