SPERM ANTIBODY

AS A FACTOR OF INFERTILITY IN NIGERIAN MALES

BY

MABEL AYEBATONYO CHARLES-DAVIES

B.Sc. Biochem. (Port Harcourt)

M.Sc. Chem. Path. (Ibadan)

A thesis in the Department of CHEMICAL PATHOLOGY

Submitted to the Faculty of Basic Medical Sciences in partial

fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

UNIVERSITY OF IBADAN

April, 1999.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

ABSTRACT

Sperm antibodies (SA) have been observed as a cause of infertility. The production of the SA have in some circumstances been attributed to chronic infection of the genital tract. In Nigeria, sexually transmitted diseases (STDs) and infection related infertility are reportedly highly prevalent. The minimal studies that have been conducted in the male population would suggest that 40% of clinical infertility is male factor dependent. This study was therefore designed to define the role of SA in male infertility as well as determine the involvement of STDs in the production of such SA among Nigerians.

Hypothetically, STDs may lead to the damage of the blood-testis barrier resulting in the exposure of sperm antigens to the immune system with the subsequent formation of sperm antibodies. This may be associated with reduced fertility in the long term.

In this study, 182 adult males aged 18-56 years were investigated. 85 were normospermic with no evidence of STDs and served as controls, 50 were infertile while 47 had proven STDs.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Demographical characteristics were obtained through the administration of questionnaires. Anthropometric measurements such as height and weight were also obtained and body mass index was calculated from these indices. Biophysical analysis of semen was performed according to WHO guidelines while seminal zinc was analysed using atomic absorption spectrophotometer. These were carried out in order to ascertain the fertility status of the males. Fertility was confirmed using endocrinological measurements such as luteinizing hormone, follicle stimulating hormone, prolactin and testosterone. These were estimated by a double antibody radiommunoassay technique. Direct microscopy, culture, and serology were performed on urine, semen, urethral swab and plasma in order to detect the presence or absence of STDs.

Chlamydia IgG antibody and antigen were detected in plasma and urethral swab samples respectively using enzyme linked immunosobent assay (ELISA) kits since this set of organisms have been specifically associated with the production of sperm antibodies. Sperm antibodies-IgG, IgA and IgM were detected in plasma, seminal plasma and on spermatozoa using the currently used immunobead binding technique. Appropriate statistical tests were carried out to indicate which variables are associated with SA.

Result in this study showed that SA – IgG, IgA and IgM are present in plasma and semen. However, percentage binding of these SA on motile spermatozoa to immunobeads is low and comparisons of these SA in plasma and semen between infertile/STDs group and fertile control were not significantly different ($p>0.05$). The spermatozoan tail was observed as the most predominant region of binding of

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

These SA in plasma and semen Chlamydial IgG antibody was significantly associated with past history of STDs but was found significantly higher in fertile controls than both infertile and STDs groups ($p<0.05$). Gonococcal urethritis and non-specific urethritis were the most common infections in the STDs group. Biophysical parameters like sperm count, percentage motility and morphology were significantly lower in infertile than control ($p<0.001$). However, only percentage motility was significantly lower in STDs group than fertile controls ($p<0.05$) of all the seminal indices tested. Zinc and leukocytospermia were not significantly different between infertile men, men with STDs and fertile controls ($p>0.05$). Reproductive hormones- FSH, LH, prolactin and testosterone were also not significantly different ($p>0.05$) in comparisons between infertile and fertile groups. Similar observations were made between STDs and fertile groups except in prolactin which was significantly lower in STDs than fertile group ($p<0.05$).

Results suggest that sperm antibodies are present but may not be associated with STDs or infertility in Nigerian males. Thus sperm count, percentage motility and percentage morphology are still the important indices in the assessment of male fertility status.

KEY WORDS: Sperm antibodies, male infertility, sexually transmitted diseases, chlamydiatrachomatis, hormones.

UNIVERSITY OF IBADAN LIBRARY

13402

UNIVERSITY LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

ACKNOWLEDGEMENT

This thesis owes its final shape and form to the assistance and hard work of many people. It is not possible to mention all their names because of limited space.

My sincere and profound gratitude goes to my supervisor, Professor B. O. Osotimehin for his keen interest, guidance and understanding throughout the duration of this project. This project would still have been a dream but for his generosity. He provided financial and material support. He also provided a suitable laboratory, academic and social environment especially the famous 'Room 9' meetings for the successful completion of the work. The glee on his face each time success was reported encouraged me so much.

I wish to acknowledge the invaluable assistance of Professor Oduola who flung open his laboratory, staff and equipment and made himself accessible for me to tap from his wealth of knowledge and experience. His encouraging remarks especially during very depressing moments with thoughts of failure, helped me so much.

I am also grateful for the care and concern shown for the speedy completion of this project by the past heads of department - Professors Osifo and Taylor and the present head of the department- Professor L.S. Salimonu. I must also not fail to thank my lecturers, Professor Agbedana and Drs Adejuwon,

UNIVERSITY OF IBADAN LIBRARY

IBADAN UNIVERSITY LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Adeniyi and Bolodeoku for their concern and encouragement. The lecturers and staff of Chemical Pathology department make it a department I am proud to be associated with.

I am also indebted to the Head of department and staff of Microbiology department especially those of the Sexually Transmitted Disease Clinic, as well as those of Pathology department, Obstetrics and Gynaecology laboratory, Surgery laboratory and Postgraduate Institute for Medical Research (P.I.M.R&T) for their assistance. The stimulating and conducive atmosphere made possible by Drs Adeyefa and Abbiyesuku, Mr. Oduwole and members of the famous 'Room 9' is highly appreciated. I want to also appreciate the efforts of Drs Okeke, Kotila, Oni, Ekweozor, Onyemenem and Agomo, Messrs Omoriah, Diran Oluwole, Yomi, George, Tayo and Miss Margaret Ehinju.

I am particularly grateful to Dr. and Mrs. Kejeh and Dr. and Dr. (Mrs) Arowojolu who flung their homes open to me. I am also grateful to Dr. Sanusi for going an extra mile in ensuring that I had enough samples to work with during the period of industrial unrest in the nation.

My deep love and appreciation go to my dear husband Dr. D. Charles-Davies for his love, moral and financial support. I am also grateful to my loving children - Tamunoibi, Tamunomiete and Tamunodiepriye (jointly called Tamboys) for their love and understanding especially when I had to leave them for long periods. They have been a wonderful upliftment to me with their question "mummy did your teacher give you homework?"

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

I will at this point thank my parents, brothers and sisters for their love, understanding and moral support. Unfortunately my father who stimulated my interest in academics and aspiration to such height and my brother - Reginald, who supported me at home and could pass for my son, did not live long enough to see the end of this work. Though late, their efforts and works still live on. "We shall be remembered by the works we have done". May their gentle and loving souls continue to rest with the Lord Jesus. Amen.

To my mother who continually encouraged me even after the loss of my father and continued with the spirit of ensuring the education of all my brothers and sisters, I say thank you. Yet there are those who prayed and wished openly and secretly for this programme to come to an end. May the Almighty God bless you severally.

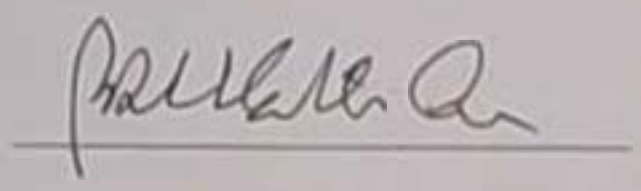
Finally I give glory to the Almighty God. He has indeed been my sufficiency.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

CERTIFICATION

I certify that this work was carried out by Mrs. M. A. Charles-Davies in the Department of Chemical Pathology, University of Ibadan.



(Supervisor)

B. O. Osotimehin, M.B.B.S. (Ibadan), M.D. (Birmingham),
F.R.C.P. (London),
Professor in the Department of Chemical Pathology,
University of Ibadan

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

DEDICATION

This work is dedicated to the Lord Jesus Christ, the citadel of wisdom, my loving husband, Domimon and my wonderful boys - Tamunoibi, Tamunomiete and Tamunodiepriye.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

TABLE OF CONTENTS

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

LIST OF TABLES

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

LIST OF FIGURES

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

ABBREVIATIONS

ANOVA	=	Analysis of Variance
AIDS	=	Acquired Immune Deficiency Syndrome
BSA	=	Bovine Serum Albumin
C. Trachomatis	=	Chlamydia Trachomatis
ELISA	=	Enzyme Linked Immunosobent Assay
FSH	=	Follicle Stimulating Hormone
H	=	Head of Sperm
H/T	=	Head/Tail (entire sperm surface)
HLA	=	Human Leucocyte Antigen
IBT	=	Immunobead Test
IRMA	=	Immunoradiometric Assay
IUI	=	Intrauterine Insemination
IVF	=	*In vitro* Fertilization
LDH	=	Lactate Dehydrogenase
LH	=	Luteinizing Hormone
MAR	=	Mixed Agglutination Reaction
MI	=	Immotility
NPM	=	Non-progressive Motility
NSU	=	Non-Specific Urethritis
P	=	Probability
Prl	=	Prolactin
RIA	=	Radioimmunoassay
RPL	=	Rapid and Linear Progressive Motility
SA	=	Sperm Antibodies

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

SLP	=	Slow Linear Progression
STD(s)	=	Sexually Transmitted Disease(s)
t	=	Student's t-test
T	=	Testosterone
tt	=	Tail Tip
T-BSA	=	BSA in Tyrode's Solution
T-Buffer	=	Tyrode's-buffer
WBC	=	White Blood Cells
WHO	=	World Health Organisation
X^2	=	Chi-Square Test

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

CHAPTER ONE

INTRODUCTION

Africa has a strong traditional heritage and marriage in the Nigerian socio-cultural context is primarily for procreation (Adekunle, 1986, Leke *et al.*, 1993). There is therefore, a great deal of pressure on married couples to perform this social obligation fairly early in matrimony. Failure to achieve this is a social stigma often associated with considerable emotional stress, marital instability, divorce, separation , higher risk of having more sexual partners or acquisition of other wives (Ladipo and Osobu, 1978, Favot *et al.*, 1997). Consequently, couples intending to get married ensure that their partners can procreate before marriage is conducted. This is evident in the incidence of 65.5% premarital conception in married couples in the Nigerian Igbo experience - in a society where chastity before marriage was 'highly priced' (Meniru, 1996).

Infertility is common in Africa and affects 10% of couples (Adegoke, 1986, Favot *et al.*, 1997). It is defined as the inability to conceive after one year of unprotected intercourse (Gilbaugh and Lipshultz, 1994) and is regarded as one of the most important health problems in sub-Saharan Africa (Leke *et al.*, 1993). As a medico-social problem in Nigeria, it accounts for 40% of out-patient gynaecological consultations at the University College Hospital, Ibadan (UCH) (Ilesanmi, 1995). This is inspite of the global problem of over-population and efforts at its control (Dickman and Herr, 1997). The evaluation of the infertile

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

couple hence, remains a continuing challenge to the practising doctor in this part of the world (Ilesanmi *et al.*, 1995).

The problem of infertility is not exclusively African. Infact it is a global problem. The incidence of infertility in the newly married couple has increased in industrialized countries from 7% to 8% in the early 1960s to 20-35% in 1994 (Thompson, 1994). Approximately 15% of American couples are affected and incidence appears to be increasing (Sigma and Vance, 1987; Gilbaugh and Lipshultz, 1994).

In most societies, infertility is perceived as a female issue. Thus, investigations and treatment modalities have centred mainly on the female partner (Kaufman and Nagler, 1987, Bomman *et al.*, 1994). The male role is often neglected as most cultures and particularly in Nigeria equate sexual potency with normal male fertility. Only recently has the potent male been considered a possible cause of infertility in a couple (Ladipo and Osoba, 1978; Awojobi *et al.*, 1983). Although the female is still the perceived culprit, increasing attention is focused on the role of the males in infertile marriages (Obafunwa *et al.*, 1993; Silber, 1994; Adejuwon *et al.*, 1996).

In the past, the contribution of the males to infertile marriages was assessed to be about 16.4% (Ojo, 1968). It is now estimated from clinical reports that 40-45% of clinical infertility is male factor dependent (Omoniah *et al.*, 1984; Marinho, 1986; Ilesanmi *et al.*, 1996). Bomman *et al.* (1994) observed as much as 70% male factor contribution in their study of 1726 infertile African men in a South African andrology clinic. An increase in male infertility has been reported in several parts of the world. A worldwide decline in sperm count and semen

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

volume in the last 50 years is of growing concern. A recent study in England showed that the average sperm density has decreased significantly from 113 million/ml in 1940 to 66 million in 1990. The mean seminal volume has also declined from 3.40ml to 2.75ml, indicating that the decrease in the total sperm count is even more pronounced than the fall in sperm density would indicate (Carlsen *et al.*, 1992, Giwercman *et al.*, 1993). In Britain and America, the male factor is present in nearly half (50%) of the couples consulting for infertility (Comhaire, 1992, Gilbaugh and Lipshultz, 1994). It is believed that 30% is solely secondary to male factors, while in another 20% both partners have detectable abnormalities (Sigma and Vance, 1987).

Male infertility is the most commonly investigated testicular disorder (Mcclure, 1987) and has multiple causes which may be pre-testicular, testicular and post testicular (Sigma and Vance, 1987, Obafunwa *et al.*, 1993). In male infertility, dysfunction can also be at the level of the central nervous system or in the genital tract (Saeed *et al.*, 1994). In this geographical subregion, male infertility results from low concentrations of sperm in semen (low sperm count or abnormal spermatozoa). 16% of infertile patients with oligospermia was observed while idiopathic oligoasthenozoospermia was the most frequent cause of infertility (Akande, 1986, Saeed *et al.*, 1994, Chavarria *et al.*, 1995). Awojobi *et al.* (1983) studied 646 infertile males at the UCH and noted that testicular failure of various types was the aetiological factor in most of the cases (61%). Chronic epididymitis was found in 32% while varicocoele was observed in 25%. In a World Health Organisation (WHO) sponsored multi-centre, collaborative investigation of 5800 infertile couples, varicocoele was diagnosed in 20% of

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

African men seeking evaluation compared with less than 12% in other areas except Latin America. Accessory gland infection was also commoner in African and Latin American men (Cates *et al.*, 1985). In the study of Bornman *et al.* (1994), 49% of 1726 infertile African men were secondarily infertile. 36% had previously received treatment for urethral discharge, varicocoeles were present in 11%, 11% had serological evidence of previous exposure to syphilis. 9% had azoospermia, 5% had polizoospermia, 45% had hypospermia while 7% had hyperspermia. 5 Patients had bilateral absence of the vas deferens while multiple factors were present in 16.9%. Obafunwa *et al.* (1993) in their study of testicular biopsies done for infertility over a 5 years period, suggested that hypospermatogenesis and chronic non-specific orchitis represent the two most common anomalies in their series. Majority of cases of non-specific chronic orchitis were seen in the third and fourth decades of life which coincides with the period of maximum sexual activity. They however, suggested that gonorrhoea and non-gonococcal urethritis may be the underlying cause.

In developing countries, poverty and infections are common place (Leke *et al.*, 1993). Sexually transmitted diseases (STDs) are said to be highly prevalent in sub-Saharan Africa (Ekwere, 1995). Osoba *et al.* (1975) observed in Nigeria that complications of gonorrhoea were common in both males and females but non specific urethritis (NSU) was commoner in hospital practice than gonorrhoea. Bello and Lombin (1990) observed 27.7% and 27% of patients with gonococcal infection attending venereal disease clinic in Zaria, Nigeria were positive for Ureaplasma Urealyticum and Mycoplasma hominis respectively. Analysis of aetiological diagnosis of 308 male patients at the STDs clinic, UCH showed that

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

50.2% had NSU, gonorrhoea accounted for 33.4%, syphilis (early) (5%), trichomonas (4.1%) and lymphogranuloma venereum (3.6%) (Alausa and Osoba, 1978).

Infection related infertility is common in Africa (Cates *et al.*, 1985). Ogunbanjo *et al.* (1989) on examination of 782 infertile males, recovered various infective agents from 54(7%) of the patients while in 25% of the remaining patients, a significant number of pus cells was present with abnormal seminal indices. Gonococcal epididymorchitis and urethral stricture were the commonest complications encountered when patients with STDs were investigated (Alausa and Osoba, 1978). Urethral stricture was observed as producing the highest number of infertility states in Nigerian males while NSU was often complicated with prostatitis. In Accra, Ghana, Yeboah and Manna (1994) in their studies of the relationship between urethral stricture and infertility, observed that STDs accounted for 79% of the stricture within this group. They suggested that urethral strictures could be responsible for infertility. Complications of gonococcal urethritis (GU) such as epididymitis and epididymorchitis are commonly seen in Nigerians, as these could lead to both testicular damage as well as tubal blockage and consequently oligospermia or azoospermia. Similarly, secretions from infected prostate and seminal vesicles could create a hostile medium for spermatozoa although usually secondary invaders, gram-positive and gram-negative micro organisms could become pathogenically important in previously damaged mucosal epithelium and could give rise to similar sequelae as above (Ogunbanjo *et al.*, 1989). Ureaplasma urealyticum has been implicated as having a potential role in reproductive failure (Osoba *et al.*, 1975). Ladipo and Osoba

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

(1978) observed that 92.3% with positive culture were infertile. In Ibadan, Ladipo (1979) observed that the prevalence of Ureaplasma Urealyticum was significantly higher in infertile couples but their co-existence with gonorrhoea and other infections make it difficult to define their role in tubal disease. Infections from the male or from other sources also play a major role in female infertility particularly caused by tubal blockage which is the main female factor (Marinho *et al.*, 1986). In sub-Saharan Africa, Neisseria gonorrhoea (N. gonorrhoea) and Chlamydia trachomatis (C trachomatis) are common infections. A mathematical model recently devised to estimate the effect of gonococcal infection on population growth of society, predicts that N.gonorrhoea produces a steeper reduction in population growth than C.trachomatis because of its transmission dynamics which results in a higher force of infection (Brunham *et al.*, 1993).

The contribution of the male to a couple's infertility in majority of cases can be assessed from analysis of the semen (Nkposong *et al.*, 1982; Overstreet and Katz, 1987; Bornman *et al.*, 1994). Endocrine causes are uncommon (Mcclure, 1987). Estimation of circulating level of follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone, although desirable, have been found to be normal in most infertile males (Nkposong *et al.*, 1982). The incidence of primary endocrine defects in subfertile men is less than 3% and is rare in men with sperm concentrations greater than 5 million per millimetre (Mcclure, 1987). However, the traditional semen analysis does not provide complete diagnostic information since many infertile men demonstrate normal parameters on standard semen analysis (Bar-Charma and Lamb, 1994). Adejuwon *et al.* (1996) suggested

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

that semen values may not be an objective index of male infertility. Despite the growing interest and research in the field of infertility, treatment for infertility can often be a frustrating problem because no identifiable cause can be found in approximately 25% of subfertile males (Gilbaugh and Lipshultz, 1994). These are classified as having idiopathic infertility (Sigma and Vance, 1987). Cates *et al.*(1985) found 46% of couples with no demonstratable cause in male diagnosis. Marinho (1986) observed no abnormality in 19.3% of infertile couples. Obafunwa *et al.*(1993) observed that 20% of infertile adult testicular biopsies were normal. These have primary infertility with no abnormality whatsoever in either of the partners. This was attributed to immune mechanisms (Ojo, 1968; Ladipo, 1986).

It is probable that all humans are endowed with the ability to produce antibodies against virtually every antigen with which they come in contact. The subsequent strategy of immunologic defence then depends on the body's potential to initiate cell-mediated and humoral immunologic responses to non-self antigens. A man's mature spermatozoa are foreign to his immune system because they first appear at puberty, long after the process of self recognition has occured. A man's reproductive success therefore depends upon the prevention of immune response to sperm antigens (Haas, 1987).

Sperm antibodies have been implicated as a factor of infertility especially in normospermic infertile males or couples with unexplained infertility (Lehman *et al.*, 1987; El-Roeiy *et al.*, 1988; Unlu *et al.*, 1990; Meisel *et al.*, 1994). The role of antibody mediated infertility in patients with primary/secondary infertility is a subject of current interest world wide (Hameed *et al.*, 1995). In Nigeria, Ojo

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

(1968) observed that some couples have excellent profiles - the wife ovulates regularly, the sperm count of the man is normal - but somehow they do not seem to be able to procreate. After divorce and second marriage, both man and woman occasionally become fertile. He suggested that there could be a little problem which could be immunological. Similar findings were observed by Adegoke (1986). In his report, a man and his wife were found normal after fertility investigation except for mixed agglutination reaction (MAR) test which showed that sperm antibodies present in the serum of the wife immobilizes her husband's spermatozoa when in contact with her cervical mucus. When artificially inseminated, the woman became pregnant and had a baby. The finding of Cates *et al* (1985) that 5% of Africans have unexplained infertility also raises a question as to the possibility of sperm antibodies being responsible. Mazzolli and Berrera(1989) showed that 50% of azoospermic patients showed autoimmunisation. Hameed *et al.* (1995) also showed that immunomodulation may be responsible for some cases of infertility. Ouahes *et al.* (1997) observed that autoimmune disease may adversely affect reproductive function and could result in infertility. A case was presented of secondary infertility due to the occurrence of sperm autoimmunity and hyperprolactinemia. Treatment of hyperprolactinemia with bromocriptine did not improve his fertility, however, concurrent treatment of both conditions improved semen quality conception and birth of a healthy baby girl (Fuller *et al.*, 1992).

The testicle is well suited to act as a citadel that prevents egress of spermatozoal antigens and ingress of circulating immunoglobulins and immunologically active cells. The multiple tight junctions between the sertoli

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

cells provide one of the strongest barriers between the circulation and the seminiferous tubules. As the process progresses from spermatogonia to spermatocyte to spermatid to mature spermatozoa, intersertoli cell junctions from behind the developing spermatozoa isolate its antigens from the extratubular environment. By these mechanisms, sperm within the seminiferous tubules are separated from the immune system of the host. Any event or circumstance that would breach these protective mechanisms would result in the formation of sperm antibodies (Haas, 1987).

There are multiple insults to the male genital tract that have been associated with an increased risk of sperm antibody formation. The most common of these is vasectomy, infection or obstruction of the male genital tract, cryptorchidism, varicocoele, testicular biopsy, trauma, torsion, cancer, homosexuality with rectal intercourse and genetic predisposition have all been thought to harbour a possible increased risk of circulating sperm antibodies (Haas, 1987).

It is not known what antigenic determinants stimulate immune response. However, several antigens have been identified and many more are being defined by sperm monoclonal antibodies (Alexander and Anderson, 1987). Some human antigens of possible immunologic significance include sperm enzymes such as Lactate dehydrogenase ($LDH\text{-}C_4$), Acrosin and Hyaluronidase (Jones, 1980; Alexander and Anderson, 1987; Gupta *et al.*,(1992). Diekman and Herr (1997) observed that active immunization with $LDH\text{-}C_4$ suppressed fertility in a variety of mammalian species.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Genital tract inflamnation facilitates the formation of sperm antibodies (Wolf, 1995). Patients with genital infection or sperm duct obstruction exhibited positive antisperm autoimmunity tests (Mazzoli and Barrera, 1989). High incidence of humoral sperm reactive antibodies was found in association with genital tract infection and reduced fertility in men (Witkin and Toth, 1983; Soffer *et al.*, 1990). Recently, Ekwere (1995) working in Calabar, Nigeria, reported a high (44%) incidence of sperm antibodies among infertile men in Nigeria. This he related to the high prevalence of STDs in sub-Saharan Africa.

Clinically, sperm antibodies are found in 3% to 12% of men who undergo infertility evaluation (Turek and Lipshultz, 1994). Sperm antibodies - IgG, IgA and IgM have been detected (Bronson *et al.*, 1984) although IgG and IgA are reportedly most predominant (Bronson *et al.*, 1992, Gonzales *et al.*, 1992). These antibodies can be found in three locations in males-serum, seminal plasma and bound to sperm in males (Koksal *et al.*, 1991; Castle *et al.*, 1997). Binding of sperm antibodies to different regions of the sperm cell-head, tail and tail-tip have been observed. Head binding has been found correlating with infertility. However, antibodies attached to sperm head with binding rate of less than 40% were not considered to be a causative factor in male infertility (Takahashi *et al.*, 1992). Tail bound antibodies are said to weakly affect motility (Barlow, 1988, Broderick *et al.*, 1989, Carson *et al.*, 1988). Bronson *et al.* (1984a) suggested that these antibodies may interfere with fertilization by affecting sperm transport and gamete interaction. In cases of severe immunological male factor infertility, impairment of spermatozoal motility and acrosome reaction resulting in reduced fertilization capacity have been described (Verheyen *et al.*, 1994)

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

1.1 RATIONALE OF STUDY

1) Infertility is a medico-social problem that has gained prominence in the world over including sub-Saharan Africa (Leke *et al.*, 1993, Ilesanmi, 1995). Sperm antibodies have been observed as a cause of infertility in some cases of infertility (Hameed *et al.*, 1995). The role of sperm antibodies in male infertility remains controversial to date (Heidanreich *et al.*, 1994) and is a subject of current interest worldwide (Hameed *et al.*, 1995).

2) Several risk factors including genital tract infections have been defined for the development of sperm antibodies (Haas, 1987). Genital inflammation facilitates the formation of sperm antibodies (Wolff, 1995). In sub-Sharan Africa, STDs especially N. gonorrhoea and C.trachomatis are common infections (Brunham *et al.*, 1993).

3) Infection related infertility is alarmingly high (Cates *et al.*, 1985, Marinho, 1986). Many infertile men have STDs especially gonorrhoea and NSU (Obafunwa *et al.*, 1993). Past or chronic infection of the genital tract especially 'silent' infection of C. trachomatis has been implicated (Ruijs, 1990). Recently, Ekwere (1995) in Calabar, Nigeria, reported high (4.4%) incidence of sperm antibodies among infertile men in Nigeria which may be related to the high prevalence of STDs.

4) Studies suggest that male autoimmunity is more prevalent than female isoimmunity. In addition, a woman's isoimmunity is often associated with

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

her husband's auto immunity (Mellinger and Goldstein, 1987). The high (40-45%) contribution of males to infertility is now widely known (Marinho, 1986; Hesannii *et al.*, 1996). Decline in male fertility in the past 50 years has been reported (Carlsen *et al.*, 1992).

5) All is not known about male infertility. The use of semen biophysical and biochemical assessment as an objective index of fertility and the choice of sperm motility as by far the most important parameter in determining semen quality is in doubt (Adejuwon *et al.*, 1996). The frustrating experience of treating a large percentage of infertile men with no identifiable cause (Gilbaugh and Lipshultz, 1994) is of great concern. Minimal studies have been conducted in the male population and parallel studies on the immunological basis of infertility are rare in this geographical sub-region despite its reported increasing significance in other parts of the world.

1.2 AIMS AND OBJECTIVES

1) To confirm the occurrence of sperm antibodies and their possible significance in Nigerian males.

2) To determine the incidence of sperm antibodies in infertile men, men with STDs and 'normal' population of men.

3) To comprehensively assess the current sperm antibody status and show what factor(s) predispose to the development of sperm antibodies.

4) To examine the role of sperm antibodies in the pathogenesis of male infertility.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

(5) To define the involvement of STDs in the production of sperm antibodies and how this either singly or together with past STD infection influence the fertility of the male

(6) To identify sperm antibody specifications in terms of Immunoglobulin isotype, proportion and pattern (or region) of binding to the sperm cell

(7) To describe relationship of sperm antibodies in different body compartments - spermatozoa, seminal plasma and blood plasma

(8) To evaluate what body compartment could be more appropriate and clinically relevant when testing for sperm antibodies and what immunoglobulin isotype is most appropriate to aid in diagnosis of antibody mediated infertility

(9) To study the relationship between semen quality (biophysical and biochemical indices) and sperm antibodies

1.2 SIGNIFICANCE OF STUDY

1) Comprehensive evaluation of biochemical and biophysical indices in semen in Nigerian males - fertile men, men with STDs and infertile males may improve the currently limited understanding of male infertility in Nigeria and shed new light on what factor best correlates with infertility. This may augur well for prospective studies or clinical interventions as well as aiding in the reduction of incidence of infertility among Nigerian males

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

2) Detection of sperm antibodies in these men may reveal the level of immune responsiveness to sperm antigenic challenge. Relationships of sperm antibodies and infertility will establish and resolve its role as a causative factor in infertility in this geographical sub-region. It may also stimulate research interest in immune-mediated infertility or redirect attention and resources to other possible causes of unexplained infertility. Correlations between sperm antibodies and semen characteristics may reveal some other underlying factors that may not be known. Moreover, it may aid in the development of safe and reversible male contraceptives in control of population growth.

3) Relationships between past STD infection especially chlamydia, current STD infection and sperm antibody levels is an approach towards a better understanding of the pathogenesis of immune infertility and inform new strategies in infertility management. Furthermore, such knowledge may help explain the increasing rise in male infertility.

4) Research on the relationship between sperm, semen and the immune system may provide novel and rational approaches to treating immune related infertility. Sperm antibody specifications may be important for therapeutic influence upon immunocompetent cells. Identification of clinically relevant sperm-reactive immunoglobulin isotype, binding rate and pattern, and body compartment (i.e serum, seminal plasma or sperm cell) will aid in the diagnosis of immune mediated infertility.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

5) These studies are expected to form a basis for further research into immune-mediated infertility in Nigeria and Africa in general.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

1.4 HYPOTHESIS

Sexually transmitted diseases (STDs) in men can lead to the violation or damage of the blood-testis barrier, which normally prevents plasma proteins from contact with the seminiferous tubules. As a result of these inflammatory lesions, there is a possibility of extravasation and exposure of spermatozoan antigens to immunocompetent cells in the lymphatics and capillaries, with subsequent formation of sperm antibodies. These antibodies may in the long term be associated with reduced fertility.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

CHAPTER TWO

LITERATURE REVIEW

2.1 Infertility

Infertility is a profound and widespread problem affecting an estimated 15% - 20% or more of couples in the United States who are trying to conceive (Lipshultz, 1994; Skakkeback *et al*., 1994). Whether the problem lies with the female or male, for many patients infertility may mean the dissolution of a couples entire life span (Lipshultz, 1994).

Infertility is defined as the failure of a couple to achieve a pregnancy despite one year of regular unprotected sexual intercourse (Beastall, 1993). It may be regarded as an indicator of more general systemic effects (Skakkeback *et al*., 1994) and may result from abnormalities in either or both partners or from their incompatibility. Of the many specific causes of infertility, some have genetic or anatomical basis, some result from a previous disease or treatment and some are endocrine in origin (Hull *et al*., 1985). The study in England by Hull *et al* (1985) indicated that unexplained infertility accounted for 28% of infertile couples, sperm problem 21%, ovulatory failure 18%, tubal damage 14%, endometriosis 6%, coital problems 5%, cervical mucus 30% while other male problems accounted for 2%. Marinho (1986) in Nigeria observed that the male factor was a problem in 44.6% of couples, tubal factor was found in 9.1% of couples 8.7% had uterine factor, ovulation factor was found in 12.5% of couples.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

In about half of the cases of infertility, there is a contributing male factor infertility. However, the distinction between a man with normal fertility and one with reduced fertility may be difficult (Lipshultz, 1994, Skakkebaek *et al*., 1994). In Nigeria, 40-45% of infertility is male factor related (Ilesanmi *et al*., 1996). Efforts therefore, have been intensified recently on the elucidation of the actiological factors responsible for male infertility in Nigeria (Alemnji and Thomas, 1997).

There are basically two types of infertility - primary and secondary infertility (Ladipo, 1986). A male has primary infertility if he has never impregnated any woman but has secondary infertility if he had in the past impregnated at least one woman irrespective of the outcome of the pregnancy (Alemnji and Thomas, 1997). From the female view-point, in primary infertility, the woman has never conceived, despite cohabitation and exposure to pregnancy for a period of one year while in secondary infertility, the woman has previously conceived but is subsequently unable to conceive despite cohabitation and exposure to pregnancy for the same length of time. If the woman has previously breastfed her infant, then exposure to pregnancy is calculated from the end of period of lactational amenorrhoea (Ladipo, 1986; Adekunle, 1986). However, these two types of infertility are said to have no relevance in the African fertility concept (Adekunle, 1986). However, differences were recently observed by Alemnji and Thomas (1997). Their findings indicated that a higher proportion of husbands in infertile couples had secondary infertility than those with primary infertility. Infection of the genital tract was implicated as a causative factor of

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

secondary infertility in this environment. Similar observations were made by Cates *et al.* (1985).

2.1.1 Aetiology of male infertility

The causes of male infertility generally fall into three categories- pretesticular, testicular and post-testicular. Pre-testicular cause is secondary testicular failure and causes are extragonadal endocrine disorders such as those originating in the pituitary or adrenals, which have an adverse effect on spermatogenesis. Testicular cause is primary testicular failure and includes poor semen quality, abnormal testicular histology, hypoplastic testis, cryptorchidism, torsion of the testis and mumps orchitis while post testicular causes include obstructions of the excurrent ducts of the testes, severe hypospadias, epispadias, potency or ejaculatory difficulties, impaired sperm motility, morphological abnormalities of the spermatozoa and biochemical abnormalities of the seminal fluid (Nkposong *et al*, 1982; Awojobi *et al*., 1983; Obafunwa *et al*., 1993). Male infertility is not an entity but reflects a variety of different pathogenic mechanisms. Known causes of male infertility were summarised from the functional view point (Skakkebaek, 1994).

Known causes of male infertility

Mechanism	*Cause/Pathogenesis*
Absent testicular tissue	- anorchia
	bilateral castration

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Impaired sperm production or function	-	hypogonadotropic hypogonadism
		Klinefelters syndrome variants
		47, XXY males, 46, XX males
		Partial androgen insensitivity
		Autosomal rearrangements
		AZF-gene deletions
		Spermatocystic arrest
		Cryptorchidism
		Cancer of testis
		Varicocoele
		Irradiation
		Cytotoxic drugs
		Other drugs
		Environmental agents
Impaired sperm transport	-	Autoimmune infertility
		Epididymal blockage of vas deferens and other parts in seminal pathways
		Ejaculatory failures
		Previous vasectomy
		Impotence
		Kartagener's syndrome
Disturbances in sperm oocyte fusion	-	Abnormal egg-binding proteins

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Recurrent miscarriage - Chromosomal aberrations

(Skakkebaek *et al*, 1994)

In the African experience, hypospermatogenesis (Akande, 1986) and chronic non-specific orchitis represent the two most common causes of infertility in males (Obafunwa *et al*., 1993). Awojobi *et al*. (1983) studied infertile males and noted that testicular failure of various types was the aetiologic factor in 61% of cases, chronic epididymitis in 32% and varicoceles in 25%. Five patients had bilateral absence of the vas deferens. Multiple factors were present in eighty-six patients (16.9%). In studying testicular biopsies done for infertility investigations over a five-year period in Nigeria, Obafunwa *et al*. (1993) also observed varying degrees of hypospermatogenesis in 49% while 19% had non-specific orchitis. 20% had normal histological pictures while the remaining 12% had various other pathological changes.

Majority of the cases of non-specific chronic orchitis were seen in the third and fourth decades of life which coincides with that of maximum sexual activity. Gonorrhoea and non-gonorrhoea urethritis especially C. trachomatis were thought to be the underlying cause (Obafunwa *et al*., 1993; Brunham *et al*., 1993). Osoba *et al*. (1975) observed that complications of gonorrhoea were found to be common in both males and females while NSU was commonest in hospital practice than gonorrhoea. Syphilis was rarely encountered while lymphogranuloma venereum with its sequelae was more frequently observed in both sexes. Gonococcal epididymo-orchitis and urethral stricture were the commonest complications encountered, the later complication producing the

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

highest number of infertility state in Nigerian males. Epididymitis and epididymorchitis could lead to both testicular damage as well as tubal blockage and consequently oligospermia or azoospermia. NSU was observed often complicated with prostatitis. Secretions from the infected prostate and seminal vesicle could create a hostile medium for spermatozoa. Although usually secondary invaders, gram-positive and gram-negative organisms could become pathogenetically important in previously damaged mucosal epithelium and could give rise to similar sequelae as above (Alausa and Osoba, 1978, Ogunbanjo *et al.*, 1989, Yeboah and Marma, 1994).

On examination of 782 infertile males, Ogunbanjo *et al.* (1989), recovered various infective agents from 54 (7%) of the patients while in 25% of the remaining patients a significant number of pus cells was present with associated abnormal seminal fluid indices. 92.3% of patients with positive culture of Ureaplasma Urealyticum were infertile, hence implicating the organism as having a potential role in reproductive failure (Ladipo and Osoba, 1978).

2.1.2 Aetiology of Female Infertility

Tubal Factor: The most important causes of female infertility are summarised below. Tubal abnormalies could be due to previous pelvic inflammatory disease - an infection orginating in the lower genital tract that ascend to the upper reproductive organs. The sequelae of such inflammatory process is pelvic inflammatory diseases, tubal blockage, hydrosalpinges and periadnexial adhesions. The major causes of pelvic inflammatory disease are sexually transmitted disease, post partum infection and post abortal infection. Other

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

causes of tubal blockage are tuberculosis, schistosomiasis, tubal disease following burst appendicular abscess or abdomino-pelvic surgery complicated by pelvic inflammatory disease, endometriosis, psychosomatic factors and congenital tubal aplasia or convoluted tubes (Ladipo, 1986, Akande 1986, Mannho 1986)

Ovulation Factor: Ovulation disorders generally arise from disruption of the hypothalamic-pituitary-ovarian axis. The causes of ovulation disorders include the following

a) Hypothalamic dysfunction due to environmental, physical or emotional stress

b) Pituitary adenoma with or without hyperprolactinaemia Hyperprolactinemia can inhibit ovulation possibly by blocking various ovarian hormone receptors and thus causing hormonal imbalance

c) Pituitary hypofunction or Shehans syndrome

d) Hypogonadism and or corpus luteum insufficiency with lutcal phase deficiency

e) Primary ovarian failure e g Turner's syndrome

f) Premature ovarian failure

g) Polycystic ovarian disease or Stein-Leventhal syndrome, characterised by enlarged ovaries with follicular cysts, amenorrhoea, abnormal hair growth and obesity Almost all women with this disease have anovulatory infertility

h) Oral or injectable contraceptives

UNIVERSITY OF IBADAN LIBRARY

IBADAN UNIVERSITY LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Uterine Factors: Congenital uterine abnormalities although rare can be a cause of infertility e.g developmental abnormalities such as separate uterus and hypoplastic uterus. Acquired uterine abnormalities are often associated with infertility or reproductive failure e.g

a) Uterine fibroids and polyps

b) Postpartum or post abortal endometritis resulting in uterine synechae

c) Uterine adhesions following uterine surgery or curettage of the endometrium

d) Tuberculous endometritis

e) Insufficient transformation of the endometrium due to hormonal insufficiency or rarely lack of receptor sites at the level of the endometrium (congenital)

Cervical factors: Abnormalities of the cervix that can cause infertility are not uncommon. These include

a) Hormonal imbalance such as low oestrogen level which cause inadequate cervical mucus or thick mucus that is impervious to sperm

b) Cervical infection and immobilising sperm antibodies

c) Damage of cervical glands by infection or surgery

d) Cervical stenosis due to congenital defect or extensive cauterisation or cone-biospy

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Vaginal Factors: These are not a frequent finding among infertility patients. However causes of severe vaginitis that may result in dyspereunia can be identified. Other vaginal factors worthy of note are developmental defects such as transverse or longitudinal septums or imperforated hymen and stenosed forchette and occasionally, patients with vaginal stenosis following chemical vaginitis.

Other causes of female infertility: A variety of other factors that may occassionaly cause female infertility are relevant to public health programs because they are predictable and preventable. These include alcohol, tobacco, certain drugs such as barbiturates, narcotics, antidepressants, certain environmental pollutants, severe malnutrition, female genital mutilation, syphilis, schistosomiasis, filariasis, trypanosomiasis. The effect of these disorders is usually pregnancy wastage rather than failure of conception. Other endocrine and metabolic factors such as thyroid disorders, diabetes mellitus and renal disorder could also contribute to female infertility (Ladipo, 1986, Akande, 1986, Marinho, 1985, Cates *et al*., 1985).

The most important causes of female infertility are tubal, ovulation, uterine and vaginal factors especially in sub-saharan Africa (De Muylder, 1995). Failure to ovulate is the major problem in approximately 40% of women with infertility in developed countries, 30%-50% have tubal pathology while 10% or less have a cervical barrier to fertility (Speroff *et al*., 1989). A great number of women in black Africa are affected by infertility and tubal obstruction seems common (Ladipo, 1986; De Muylder, 1995). Ladipo (1986) observed that 56%-73% of women attending infertility clinic have tubal anomalies while anovulation

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

and luteal phase deficiency syndrome account for about 10% Marinho (1986) also observed tubal infertility in 46.7% of infertile couples while 12.3% had ovulation disorders. Cates *et al.* (1985) reported a figure of 49 percent with tubal abnormality and 21% with ovulation problem. Anate and Akeredolu (1995) observed over 50% of female infertility in sub-saharan Africa as due to tuboperitoneal disorders and pelvic disease while tubal damage and endometriosis were related to infertility in the study of Guzick *et al.* (1994). Nearly 15% of infertile women had previous episodes of pelvic inflammatory disease and 26% had had induced abortions (Okonofua *et al.*, 1995). 16.7% prevalence rate for genital tuberculosis was revealed in 114 infertile patients in Northern Nigiera. The presenting features were indistiquishable from those of chronic pelvic inflammatory disease (Emembolu *et al.*, 1993).

Infection related infertility is common in Africa as over 85% of African infertile women had diagnosis which could be annbuted to infection (Cates *et al.*, 1985). STDs particularly N gonorrhoea are important factors contributing to female infertility in Nigeria (Okonofua *et al.*, 1997). Women with fertility problems appear to have higher human immunodeficiency virus (HIV) prevalence (Favot *et al.*, 1997). In sub-Saharan Africa, Brunham *et al.* (1993) observed that N Gonorrhoea and C. Trachomatis are common infections and these pathogens are the major causes of post-salpingitis tubal infertility. Infertile women have higher prevalence of antigonococcal antibodies compared with fertile controls (Okonofua *et al.*, 1997). Infections from the male or female or from other sources according to Marinho (1986) play a major role in female infertility particularly caused by tubal blockage which is the main female factor.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

2.1.3 Other Causes of Infertility

The physical, psychological and behavioural aspects of coitus and systemic disease can have a profound effect on the ability to conceive (Thompson, 1994). Indeed, in about 15% of couples who seek help, no apparent cause can be found: semen quality fulfils the criteria for normalcy, and no defect in the woman's reproductive system can be found. It is common to use the term unexplained infertility in such cases. (Skakkebaek *et al.*, 1994). Cates *et al.* (1985) observed 16% and 46% of African couples with no demonstrable cause in female and male diagnosis respectively with 5% of couples having unexplained infertility. Marinho (1986) observed no abnormality in 19.3% of infertile couples while Obafunwa *et al.* (1993) observed that 20% of adult testicular biopsies were normal. These perhaps may have some immune mechanisms responsible for their infertility. Ojo (1968) and Ladipo (1986) stressed the importance of sperm reactive antibodies as responsible for some unexplained infertility.

2.2 Immunologic Infertility

The testis is an immunologically privileged site and spermatozoal antigens are effectively shielded from being recognised by the cells of the immune system (Skakkeback *et al.*, 1994). However the concept of immunologic infertility was opened in 1899 with the independent pioneering works of Landsteiner and Metchnikoff who demonstrated that sperm was antigenic when injected into foreign species. It was later discovered that sperm was antigenic even in the same species (Metalnikoff, 1990). As early as 1950s, an immunologic

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

basis for some cases of infertility was demonstrated (Gilbaugh and Lipshultz, 1994). Wilson and Rumke in 1954 implicated antisperm antibodies clinically in male infertility while reports from animal studies suggest that immunisation of animals by sperm could result in lowering fertility (Isojima *et al.*, 1959, Edwards, 1964, Menge, 1971). More studies by Tung *et al.* (1980) and Yanagimachi *et al.* (1981) have shown that autoantibodies induced in male guinea pigs impaired the acrosome reaction *in vitro* and prevent zonal penetration by acrosome - reacted sperm.

Rumke and Hellinger (1959) found that a significant number (3.3%) of men with infertility manifested an autoimmunity to spermatozoa the incidence of fertile men with autoimmunity was zero. Their study suggests that sperm antibodies at a significant titre may lower male fertility. Fjallbrant (1968) found agglutination in 6.8 percent of infertile men compared with 0.33% of normal men subsequently Rumke (1974) published a 10- year follow - up. In this study, no man who had original titres of greater than 1:512 fathered a child. Between titres of 1:32 and 1:12, the chances of fertility decreased. These studies brought to the forefront the possible role of immunologic factors in male infertility (Kaufman and Nagler, 1987). Although immune mediated aetiologies are suspected as a contributing factor in significant number of infertile men (Mellinger and Goldstein 1987) others did not find any significant difference in percentage binding of antibodies between fertile and infertile patients (Criser *et al.*, 1989; Guzick *et al.*, 1994; Meisel *et al.*, 1994).

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

basis for some cases of infertility was demonstrated (Gilbaugh and Lipshultz, 1994). Wilson and Rumke in 1954 implicated antisperm antibodies clinically in male infertility while reports from animal studies suggest that immunisation of animals by sperm could result in lowering fertility (Isojima *et al.*, 1959, Edwards, 1964, Menge, 1971). More studies by Tung *et al.* (1980) and Yanagimachi *et al.* (1981) have shown that autoantibodies induced in male guinea pigs impaired the acrosome reaction *in vitro* and prevent zonal penetration by acrosome - reacted sperm.

Rumke and Hellinger (1959) found that a significant number (3.3%) of men with infertility manifested an autoimmunity to spermatozoa the incidence of fertile men with autoimmunity was zero. Their study suggests that sperm antibodies at a significant titre may lower male fertility. Fjallbrant (1968) found agglutination in 6.8 percent of infertile men compared with 0.33% of normal men subsequently Rumke (1974) published a 10- year follow - up. In this study, no man who had original titres of greater than 1:512 fathered a child. Between titres of 1:32 and 1:12, the chances of fertility decreased. These studies brought to the forefront the possible role of immunologic factors in male infertility (Kaufman and Nagler, 1987). Although immune mediated aetiologies are suspected as a contributing factor in significant number of infertile men (Mellinger and Goldstein 1987) others did not find any significant difference in percentage binding of antibodies between fertile and infertile patients (Criser *et al.*, 1989, Guzick *et al.*, 1994, Meisel *et al.*, 1994)

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

2.3 SPERM ANTIGENS

Jones (1980) described human semen as an antigenic nightmare. Different types of sperm antigens can be found in the seminal fluid and on spermatozoa. Koyama *et al.* (1991) observed that the majority of sperm immobilizing antibodies in infertile patients might be generated to carbohydrate structures of the sperm coating antigens or sperm - membrane antigens. Seminal plasma contains a vast array of antigens, many of which are common to other tissues. Those that are unique to seminal plasma have an uncertain relevance to the induction of immune infertility. The intrinsic cell surface antigens of the spermatozoa itself are of more importance in this context. With the use of antibody localization techniques, the spermatozoon has been shown to possess intrinsic antigens on the acrosome, midpiece, and tail, some of which may provoke immunologic infertility (Jones, 1980, Alexander and Anderson, 1987, Weiss, 1987). Thaler *et al.* (1990) observed that during ejaculation, the iron binding lactoferrin binds to sperm and forms a major component of sperm-coating antigens.

The mammalian spermatozoa is covered with a plasma membrane that contains specific antigens that function in the recognition of the zona pellucida of the egg and participate in events involved in capacitation and acrosome reaction (Yanagimachi, 1988). These sperm surface components have not been clearly defined (Kaplan and Naz, 1992, Koyama *et al.*, 1991). Shulman (1986) reported that there are several antigens of the human sperm cell that can stimulate the production of autoantibodies in certain individuals. Certain proteins of epididymal origin are said to bind to the sperm plasma membrane (Ross *et al.*

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

1990). The results of Naz (1990) indicated that mammalian sperm have several fertilization related antigens that are evolutionarily conserved. Their data also indicate that the rabbit can provide an animal model for studying antibody-mediated human infertility.

The significance of sperm-coating antigens derived from seminal plasma is uncertain. They may provide a degree of immunologic protection by masking the possibly more important immunogenicity of instrinsic sperm surface antigen. On the other hand, sperm-coating antigens themselves may-theoretically, at least-provoke immunity in the female, but the clinical significance of this response requires further study (Jones, 1980).

Shai and Naot (1992) observed that the major sperm antigens reacting with systemic antibodies differ from the antigens recognized by local antisperm antibodies. Sperm antigens exhibiting relative molecular weights of 62kd are major antigens reactive with local antisperm antibodies from infertile human.

Sperm enzymes also contribute to the antigenicity of sperm. Hyaluronidase, acrosin and lactic acid dehydrogenase - X (LDH-X) have been studied extensively, but of these only the last enzyme shows any evidence of an association with immunologic infertility (Jones, 1980). Gupta *et al.* (1992) studied immune responses to a well defined sperm-specific isogenic lactate dehydrogenase C4 (LDH-C4) in C57BIKs (H-2) mice after immunization through intra-rectal route. Their results suggest that males are more susceptible to immune suppression in T-cell functions through generation of T suppressor cells.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Humpheys-Beher (1990) reported the antigenic presence of galacotosyl transferase in human sperm which has been implicated as a macromolecule involved in sperm to egg binding interactions during fertilization.

Several antigens have been identified and many more are being defined by sperm monoclonal antibodies (Alexander and Anderson, 1987). Studies have revealed sperm antigens with limited expression in other tissues including the embryo, placenta, ovary, T.lymphocytes, brain and variety of neoplastic tissues. Some human sperm antigens of possible immunologic significance include:

LDH	-	C-12 KD testicular isoenzyme o flactate dehydrogenase, produced abundantly by testicular germ cells, expressed on surface of mature sperm
Acrosin	-	Sperm acrosomal enzyme, facilitates penetration of zona pellucida
Hyaluronidase	-	Sperm acrosomal enzyme, facilitates penetration of cumulus mass surrounding egg
RSA-1, MA-29, FA-1	-	Sperm surface antigens identified by heterologous antibodies
SO_3, S_{37}, S_{61}, S_{70}	-	Sperm antigens identified by WHO monoclonal antibody workshop

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

ABO group antigens	-	Not intrinsic sperm membrane antigens; present in seminal plasma of secretors, can coat the surface
HY	-	Expression on sperm is controversial
HLA antigens	-	Not expressed on human sperm

(Alexander and Anderson, 1987)

Primakoff *et al.* (1990) and D'Cruz *et al.* (1993) observed that sera from different infertile males, different infertile females and vasectomized males were remarkably similar in their surface antigen recognition

Mathur *et al.* (1988b) in their study suggested that cytotoxic antibodies against sperm from autoimmune infertile men in the presence of native complement have more potent sperm -immobilizing and - killing properties than those directed against fertile men' sperm antigen

Some antigens found in native autoimmune spermatozoa was strongest in infertile couples with sperm antibodies This reactivity significantly increased against capacitated autoimmune spermatozoa Native spermatozoa from few fertile men have these antigens but they appeared after capacitation (Wingate *et al.*, 1993) Yanagimachi (1988) states that capacitation is a series of changes that involve removal or alteration of substances absorbed on or integrated in the sperm membrane surface Okabe et al (1986a, 1986b) concluded that during capacitation, some antigens of sperm plasma membrane are removed or altered, whereas others hidden or masked in fresh epididymal spermatozoa are exposed Margalioth *et al.* (1992) reported that major antigenic difference may exist

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

ABO group antigens	-	Not intrinsic sperm membrane antigens; present in seminal plasma of secretors, can coat the surface
HY	-	Expression on sperm is controversial
HLA antigens	-	Not expressed on human sperm

(Alexander and Anderson, 1987)

Primakoff *et al.* (1990) and D'Cruz *et al.* (1993) observed that sera from different infertile males, different infertile females and vasectomized males were remarkably similar in their surface antigen recognition

Mathur *et al.* (1988b) in their study suggested that cytotoxic antibodies against sperm from autoimmune infertile men in the presence of native complement have more potent sperm -immobilizing and - killing properties than those directed against fertile men' sperm antigen

Some antigens found in native autoimmune spermatozoa was strongest in infertile couples with sperm antibodies This reactivity significantly increased against capacitated autoimmune spermatozoa Native spermatozoa from few fertile men have these antigens but they appeared after capacitation (Wingate *et al.*, 1993) Yanagimachi (1988) states that capacitation is a series of changes that involve removal or alteration of substances absorbed on or integrated in the sperm membrane surface Okabe et al (1986a, 1986b) concluded that during capacitation, some antigens of sperm plasma membrane are removed or altered, whereas others hidden or masked in fresh epididymal spermatozoa are exposed Margalioth *et al.* (1992) reported that major antigenic difference may exist

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

between capacitated and non- capacitated sperm Capacitated sperm absorb serum antibodies different from those absorbed by freshly ejaculated sperm as a result of exposition of new sperm surface antigen during capacitating procedure. In some women, sperm antibodies were reactive against capacitated sperm only Hence, it seems that certain antigens normally appearing or enhanced after capacitation are already present in native spermatozoa from infertile men, probably due to an inherent aberration or premature capacitation This might account for the observed enhanced immune responses in infertile couples to sperm antigens from infertile husbands (Wingate *et al*., 1993)

HLA class I and II are reportedly weakly expressed on sperm cells (Ogbimi *et al*., 1986/87) Blood group susbtances A and B, which are present in the seminal plasma of secretors, appear to adhere to sperm rather than being instrinsic components of human sperm (Hekman and Rumke, 1976) Ogbimi *et al.* (1986/87) and Martin-Villa *et al.* (1993) failed to reveal any significant differences between infertile and fertile couples with respect to ABO compatibility between reproductive partner or HLA antigens

2.4 Sperm Antibodies

2.4.1 Incidence

Clinically antibodies to sperm are found in 3% to 12% of men who undergo evaluation for infertility (Turek and Lipshultz, 1994) Skakkebaek *et al.* (1994) reported autoimmune reaction against spermatozoa in 5-10% of men being treated for infertility 7.8% of 813 unselected infertile men were seen with antibodies coating their motile sperm and significant interference in sperm motility was

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

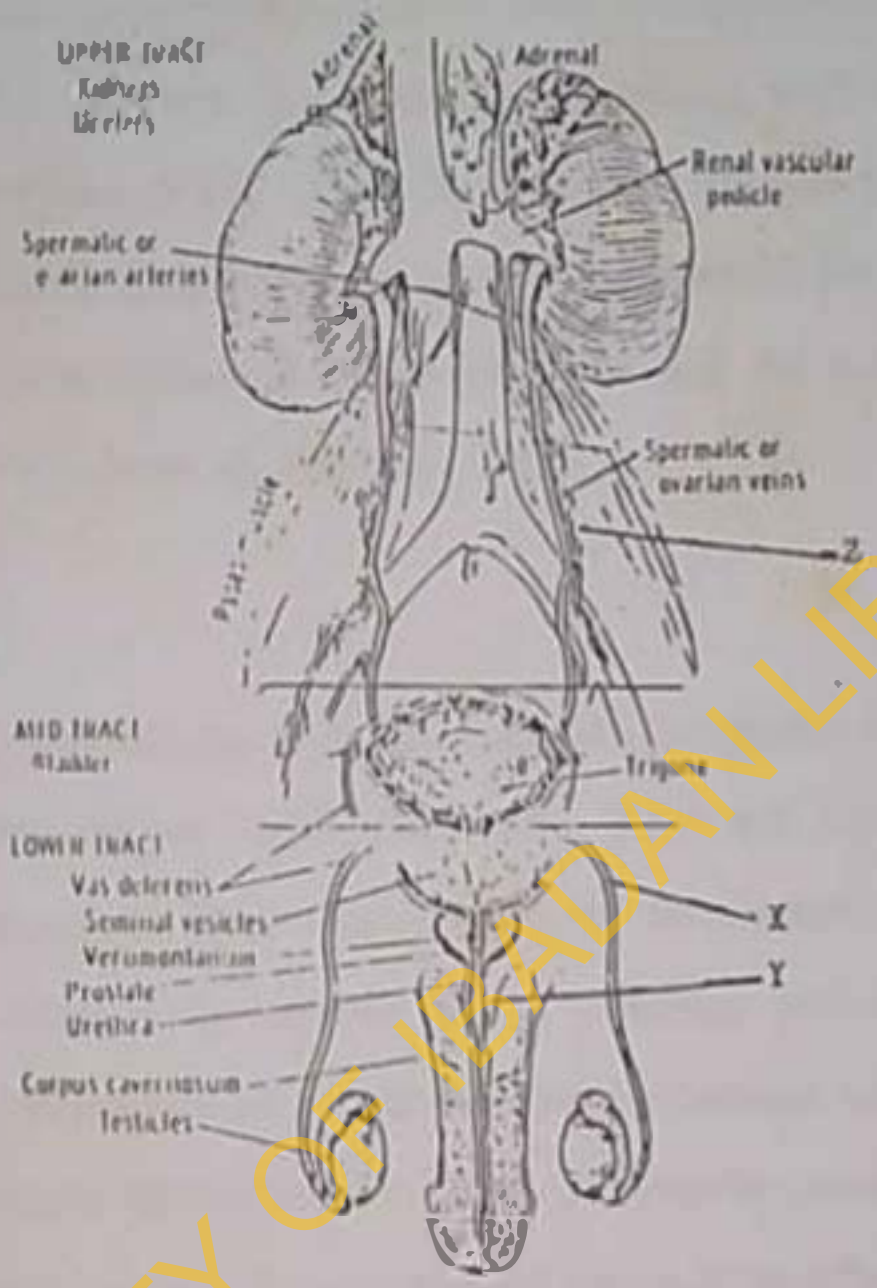
observed in 6% (Clarke *et al.*, 1985) 616 couples evaluated by Witkin and Chaudhry (1989) showed 12.4% incidence of sperm-surface antibodies in men whose wives had antisperm antibodies in their sera, but only a 6.5% incidence in partners of women who lacked these antibodies. It is established that 50% to 80% of vasectomised men have circulating sperm antibodies (Haas, 1987, Turek and Lipshultz, 1994).

2.4.2 Location

Sperm antibodies can be found in three sites in men: serum, seminal plasma and directly on the sperm surface (Turek and Lipshultz, 1994, Haas, 1987, Kaufman and Nagler, 1987). Many studies regarding antisperm antibodies in men are based on measurement of antibodies in circulation because of the convenience of assaying blood (Haas, 1987). However, these antibodies are considered clinically less important than sperm-bound antibodies because serum antibodies cannot logistically bind to sperm unless they transudate into semen (Turek and Lupshultz, 1994). It is believed that antisperm antibodies should be assessed in seminal fluid and more specifically on the sperm surface as 'surface bound antibody' (Kaufman and Nagler, 1987). Seminal plasma antisperm antibodies not attached to the sperm surface are probably of little clinical significance since seminal plasma components do not ascend past the vagina. In addition the adsorptive effect of sperm on these antibodies may result in false negative assays for sperm antibodies (Haas, 1987). Hendry (1992) observed that antisperm antibody will preferentially bind to the spermatozoa, leaving only the excess in seminal plasma to be measured indirectly : how much this is depends on the total

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT



X Spermatozoal sperm antibodies (passing through the vas deferens)

Y Seminal plasma sperm antibodies (passing through the urethra)

Z Blood sperm antibodies (in circulation in blood vessels)

Fig 1 Anatomy of the male genitourinary tract (Smith, 1972) showing the location of sperm antibodies.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

antibody present and how many spermatozoa are in the ejaculate to absorb it It is therefore probable that antisperm antibodies identified directly on a man's sperm surface correlate best with the presence of antibody-mediated infertility in males (Haas, 1987) However, studies on vasectomy - the most common and thoroughly studied association with sperm antibodies showed that vasectomised men have circulating sperm antibodies and not seminal plasma antibodies (Haas. 1987, Turek and Lipshultz, 1994)

2.4.3 Class:

Sperm antibodies belong to the IgA, IgG and IgM classes (Bronson *et al.*, 1984a, Kaufman and Nagler , 1987, Haas, 1987, Turek and Lipshultz, 1994) IgG and IgM classes are found circulating in the serum, and IgG and IgA antibodies predominate in the seminal plasma (Kaufman and Nagler, 1987) Broderick *et al* (1989) observed that IgG was the predominant immunoglobulin class in sera and semen while Combaire (1993) observed that secretory IgA does not occur in serum but semen In evaluating the nature of the different classes of immunoglobulins covering sperm cells, Clarke *et al* (1985a) and Shulman *et al* (1985) found IgG and IgA more commonly than IgM Although sperm antibodies of IgM class have been reported, these large molecules are almost uniformly confined to serum and only rarely gains access to organs or secretions of the male genital tract without a significant lesion in the reproductive tract (Bronson *et al* 1984a, Turek and Lipshultz, 1994, Haas, 1987) IgM antibodies are not routinely measured in detection systems because their role in antibody mediated infertility is limited (Turek and Lipshultz, 1994)

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

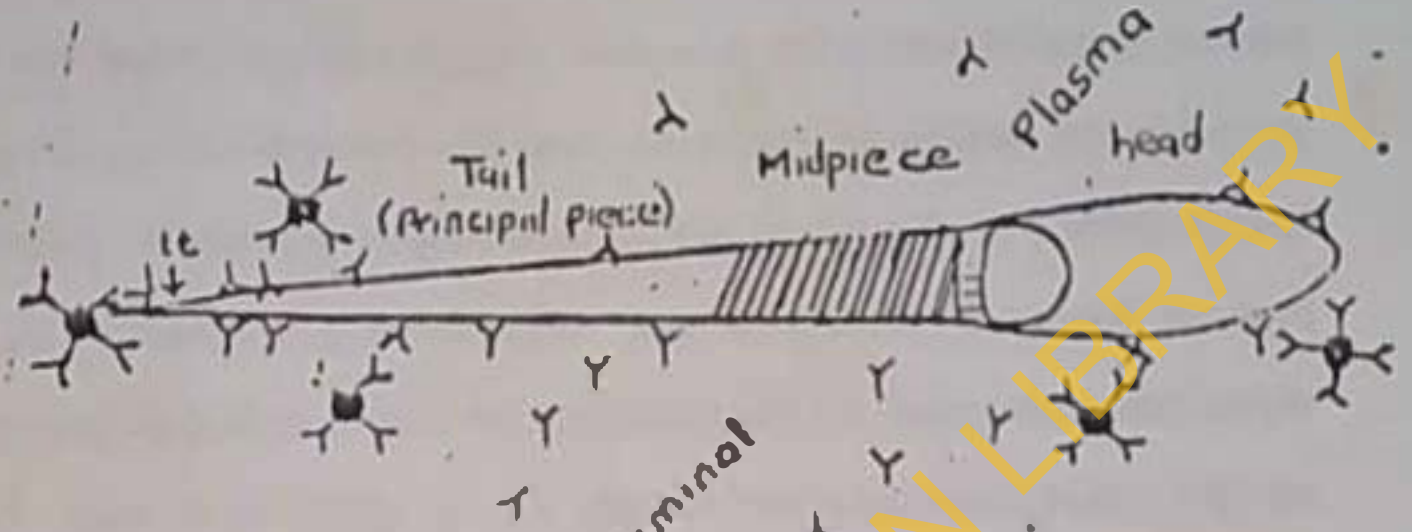
Only 1% of serum IgG content is found in male genital tract secretions IgG in seminal plasma is probably derived from two sources local antibody production as well as transudation from circulation Often sperm bound IgG can be measured in the absence of assayable serum IgG antibody levels (Turek and Lipshultz, 1994, Haas, 1987) Along with IgG, IgA is found in semen and bound to sperm, but it is thought to be mainly locally derived and secreted into seminal plasma (Turek and Lipshultz, 1994) Local production is assumed because the seminal plasma IgA is of the secretory IgA type (Haas, 1987) The presence of IgA antibodies in seminal fluid is not associated with serum antisperm antibodies and does not seem to prevent fertilization (Kaufman and Nagler, 1987) However, Skakkebaek (1994) observed that in vasovasostomy patients, when there is a close association between the presence of antibodies and fertility potential, IgA in semen appears to have an important role than does IgG Systemic inoculation of sperm antigen is thought to stimulate IgG production, whereas local reproductive tract antigens are stimuli for IgA production The sources for local production of these antibodies in the genital tract are unclear (Turek and Lipshultz, 1994)

2.4.4 Pattern of binding

The effect of sperm antibodies on sperm function depends on the area to which the antibodies are directed (Kaufman and Nagler, 1987) Different patterns of sperm agglutination were observed with different sera either head to head or tail to tail, or tail tip to tail tip Differences have been detected between the 'clinically relevant' antibodies in spontaneously infertile males and the less

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT



tt = tail tip
Ig = Sperm antibody (IgG, IgA or IgM)

Motile sperm washed free of seminal fluid are mixed with a suspension of immunobeads which act as antibody detectors by binding to regions of the sperm surface to which immunoglobulins have bound. In this manner, the regional specificity of binding of antisperm antibodies, as well as the proportion of sperm in the ejaculate bound by immunoglobulins may be determined. As illustrated here, immunobead binding detected Igs bound to sperm head, tail and tail tip (Bronson, 1984a)

Fig. 2 Immunobeads detect membrane-bound antibodies

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

important antibodies in males who have undergone reversal of vasectomy. It has been suggested that the variations in agglutination patterns are due either to different classes of antibody or to binding of antibody to different antigens. The results of Parslow *et al.* (1987) showed that although antisperm antibodies bind to discrete and sperm associated antigens, there is no substantial difference between the antigenic patterns observed with antibodies producing different types of sperm agglutination. Neither the antigens detected, nor the intensity of reaction showed significant differences although there was a tendency for head to head agglutinating antibodies to react more strongly with the higher molecular weight antigens. Ross *et al.* (1990) revealed that the antibodies react mainly with the head region of ejaculated spermatozoa.

Many head-directed antisperm antibodies are of the IgG class while those that are tail directed are of the IgA class. Penetration into cervical mucus is inhibited by antibodies bound to the sperm head and antibodies directed against sperm head appear to affect all the functions contributing to impaired fertility. These functions include immobilization of sperm in mucus, stimulation of complement mediated cell lysis and phagocytosis by macrophages, interference with capacitation or acrosome reactions and defective interaction with ovum. Antibodies against tails only weakly affect cervical mucus interaction. Antibodies to tail tip piece rarely have any effect on sperm function (Bronson *et al.*, 1982a, Bronson *et al.*, 1984a, Barlow, 1988).

Gregoriou *et al.* (1990) observed that for IgG and IgA, the majority of antibody binding was located on the entire sperm with a minor amount bound to

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

the head and tail. For the IgM, the majority of the binding was detected on the tail tip, and tail binding was observed only in a minority of cases. Bronson *et al.* (1992) observed that immunoglobulins of IgG class directed against antigen on the sperm head and tail were the most prominent in men with cystic fibrosis with sperm antibodies.

Carson *et al.* (1988) studied antibody binding patterns in infertile males and females and also showed that IgG bound mostly to the head and tail, IgA to the head and tail and IgM to tail tip only. This is in conformity with the studies conducted by Clarke *et al.* (1985a) and Shulman *et al.* (1985). Shulman et al (1985) also showed that IgG bound predominantly to the head and tail, IgA predominantly to the tail and IgM was not described.

2.4.5 Sex differences:

El-Roeiy *et al.* (1988) observed that levels of sperm antibody titres were significantly lower in women than in men. Both men and women with anti-sperm antibodies demonstrated elevated total IgG levels compared with those of normal control subjects. Only women showed elevated levels of total IgM (El-Roeiy *et al.*, 1988). However, Crister *et al.* (1989) found no difference in percentage immunobead binding between males and females in the fertile population. Sperm-bound IgG and IgA both occurred at a significantly higher frequency ($p<0.05$) in partners of women with serum antisperm antibodies. Increased incidence of both IgG and IgM ($p<0.005$) circulating antisperm antibodies in females were observed when the male partners had antibody-bound sperm. Carson *et al.* (1988) indicated that there are differences in the characteristics of

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

the antibodies (IgA, IgG, IgM) found in the male versus female sera. While these three classes of immunoglobulins are distributed equally in the females, males have a higher percentage of IgG and IgA. In addition, the regional binding of these immunoglobulins differs. IgG and IgA from male sera bind heavily to almost the entire sperm-head, tail and tail tip. However, these antibodies from female sera bind mostly to the sperm head. IgM binds similarly in both male and female (Carson *et al.*, 1988).

2.4.6 Sperm antibodies and semen quality

Investigators have failed to demonstrate an association between sperm associated immunoglobulin and a specific alteration in semen parameters (Haas, 1987; Turek and Lipshultz, 1994). Takahashi *et al.* (1992) observed no significant relationship between results of semen analysis, hormones (LH, FSH, PRL) levels and the rates of binding. Similar observation was made by Upadhyaya *et al.* (1984). Sperm antibodies were not related to sperm concentration, morphology, ejaculate volume, seminal plasma zinc concentration acid phosphatase or fructose. However, they found significant reduction in sperm motility and viability in the presence of sperm antibodies and the overall reduction in sperm motility was maintained over an extended period of time. Broderick *et al.* (1989) also observed an inverse relationship between the proportion of antibody bound sperm and the percentage of motile sperm in the ejaculate of vas reversal patients. The percentage motility was significantly lower among patients with greater quantities of sperm surface antibodies.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

2.4.7 Sperm antibody positivity in relation to infertility

Reduced penetration can result if more than 50% of sperm are antibody bound. This inhibition also correlates with higher antibody concentration. It has been shown that if less than 50% of sperm are antibody bound, then postcoital tests approximate fertile controls (Turek and Lipshultz, 1994). Intra uterine insemination fails in the treatment of male immunological infertility when all spermatozoa have sperm antibodies regardless of semen quality (Francavilla *et al.*, 1992). Takahashi *et al.*, (1992) observed that in all of the cases with proven fertility, the binding rate was less than 60% of IgG sperm antibody, and less than 40% of IgA antibodies. Antibodies attached to the sperm head with binding rate of less than 40% were not considered to be a causative factor in male infertility.

2.5 Aetiology of sperm antibodies and their clinical relevance in the male

In the normal course of events, tolerance to most self constituents is thought to occur as a result of the elimination of self-reactive clones either during foetal development or shortly after birth. As spermatogenesis does not commence until long after this tolerance-susceptible phase, a number of alternative mechanisms are necessary to ensure that the adult male does not become sensitized to differentiation and other potential autoantigens associated with his own sperm (James and Hargreave, 1984).

During meiosis, differentiation molecules are expressed by germ cells in the testis. Testis-specific autoantigens appear on late pachytene spermatocytes and persist through spermiogenesis in the mouse. Additional antigens appear on the plasma membrane after the mid spermatid stage of spermatogenesis and on the

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

sperm surface during epididymal transit. Similar germ cell and sperm-specific antigens, revealed by mapping studies with monoclonal antibodies, develop during human spermatogenesis. Various sperm and germ cell antigens can stimulate the production of sperm antibodies and/or experimental orchitis. However, under normal conditions, humoral responses to testicular germ cell or sperm antigens are usually not detected (Alexander and Anderson, 1987).

During puberty new antigens make their appearance on the sperm surface at the time of spermatogenesis. As spermatozoa in the later stages of meiosis and subsequent spermiation are isolated within the lumen of the seminiferous tubule by the blood-testis barrier, which has its anatomic correlate in the tight junctions between the sertoli cells, these sperm antigens, to which the adult organism is not tolerant, are sequestered from the immune system (Bronson *et al.*, 1984). Additional evidence for the sequestration theory is provided by the deficiency of immunoglobulins, macrophages and other leukocytes in the seminiferous tubules.

Another theory to explain the lack of an immunologic response to sperm antigens is the immune suppression theory. According to this theory, limited amounts of spermatozoal antigens constantly leak from the male reproductive tract. A small amount of antigenic stimulus results in the activation of suppressor T-lymphocytes, which inhibit immune responses to that antigen (Haas, 1987). Such tolerogenic doses of antigen would preserve an unresponsive immunologic state (Turek and Lipshultz, 1994).

Along with the blood-testis barrier and alterations in cell-mediated immunity, cytokines and other humoral mediators of the immune response may contribute to tolerance within the testis. Interferon-γ, soluble Fe receptor and

UNIVERSITY OF IBADAN LIBRARY

IBADAN UNIVERSITY LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

transforming growth factor-β have immunosuppressive properties attributed to them and may indeed be active in the testicular environmem. Clinical evidence of an inverse of serum levels of antisperm antibodies and testosterone suggests that testosterone may act to suppress the immune response through T-suppressor cell induction (Turek and Lipshuhz, 1994).

Other extratesticular mechanisms may contribute to the safe production and delivery of sperm. Immune modulators are known to exist in seminal plasma (James and Hargreave, 1984) and may be important in the induction of a tolerant state once sperm are delivered to a second foreign environment, the female reproductive tract (Turek and Lipshuhz, 1994). Occasionally the mechanisms that prevent generation of adverse reactions to testicular germ cell or sperm antigens fail with the resultant formation of sperm antibodies (Barlow, 1988, Speroff *et al*, 1989).

Several theories exist to explain the formation of sperm antibodies. Breaches in the blood-testis barrier, overwhelming inoculations with sperm antigens, or a defect in active immunosuppression may all account for pathologic antibody production.

2.5.1 Conditions associated with sperm antibodies

The blood-testis barrier may be damaged by conditions associated with sperm antibodies such as physical injury, obstruction, infection, thermal and genetic factors with the resultant production of sperm antibodies.

Obstruction: Vasectomy, vasectomy reversal, vas or seminal vessicle

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

agenesis and testicular obstruction

Infection: Orchitis, genital infection and prostatitis

Thermal: Varicocoele and cryptorchidism

Physical Injury: Trauma, torsion, biopsy and coitus

Genetic factors and testicular cancer (Haas, 1987, Turck and Lipshultz 1994).

In 1959, Rumke and Hellinger initially reported an association between acquired obstruction of a portion of the ductal system of the male reproductive tract and autoantibodies to spermatozoa. They speculated that extravasation of spermatozoa into the interstitial tissue might stimulate antibody production. Subsequently, several investigators have documented an association between autoimmunity to spermatozoa and ductal obstruction, whether acquired secondary to infection or sterilization by vasectomy.

Exposure of the male immune system to sperm antigens frequently results from vasectomy - the most carefully studied of all the conditions associated with sperm antibody. The factors that influence the formation of antisperm antibodies following vasectomy, include leakage of sperm at the time of surgery, sperm granulomas, the amount of antigenic inoculum, and genetic influences (Haas, 1987). Spermatozoa also continue to be produced after vasectomy, degenerate in the male reproductive tract and leak soluble products through distended rete testis, epididymis, and efferent duct into interstitial spaces which are accessible to immunological mediators. The millions of sperm that continue to be produced daily after vasectomy often stimulate high titres of antisperm antibodies that persist for years (Alexander and Andersen, 1979).

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

It is estimated that 50% to 80% of men have measurable serum levels of sperm antibodies after vasectomy. These titres tend to peak at 6 to 12 months after the procedure and decrease to 30% several years later. The formation of sperm antibodies is unrelated to whether the ligation of the vas deferens occurs prior to or after puberty. Despite time related change in antisperm antibody activity, antisperm antibodies can be found as early as 2 months or as late as 20 years after vasectomy. It however appears that sperm agglutinating antibodies can only rarely be detected in the seminal plasma of vasectomized men who have circulating antibodies (Broderick *et al*., 1989, Haas, 1987; Turek and Lipshultz, 1994). Witkin *et al*. (1982) found that sperm antigens associated with circulating immune complex can be detected in vasectomized men in the first few months following vasectomy. However, Naz (1990) observed that none of the sera tested from immunoinfertile patients was found to contain immune complexes indicating that antibodies were present in free form. This was supported by observation by Oyeyinka *et al*. (1987) in the activation of complement in the azoospermic and oligospermic men studied.

The number of vasectomies performed annually in the United State is more than a half million. Unfortunately, more than one percent of the patients regret the procedure and eventually undergo vasovasostomy (Lee *et al*., 1980). Reported pregnancy rates following technically successful vasectomy reversal vary from 12 to 60 percent (Requeda *et al*., 1983; Amelar and Dublin, 1979). An anatomically successful vasovasostomy does not necessarily lead to recovery of male infertility. Recognition of the role of immune subfertility after anatomically

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

successful vas reversal is growing. For men who remain infertile after vasovasostomy, the possibility exists that antisperm antibodies were generated as a consequence of vasectomy and/or reversal. However, vasovasostomy itself is not sufficient immunological stimulus to convert a negative indirect immunobead antisperm antibody test during a 6 month latency (Broderick *et al*, 1989). The fact that 100% of vasectomized men do not develop detectable antisperm antibodies may be due to genetic variables or an inhibition of the immune response by the male's high levels of testosterone (Haas, 1987). On a more systemic level, animal models exhibiting spontaneous orchitis and sperm antibody production as well as clinical work in humans suggest that tolerance to sperm may be HLA related, in that genetic links may predispose an individual to auto immune reactions to sperm (Turek and Lipshultz, 1994). Non responder individuals could be genetically predisposed to low immunological response to sperm antigens (Alexander and Andersen, 1979, Haas, 1987, Turek and Lipshultz, 1994). Moreover, many men develop antisperm antibody with any of these factors. In support of the possibility of immunogenetic influences on sperm antibody responses in men, Law *et al* (1979) and Haas (1987) reported, an association between sperm antibodies in vasectomized men and the HLA phenotypes -A-28 and B22. Marsh and associates failed to demonstrate this relationship. However, the variability could also be due to as yet undefined surgical and other physical factors (Alexander and Anderson, 1987).

Tung and associates (1976) documented the presence of sperm antibodies in sera of men and women of all ages when tested against methanol fixed, permeabilized spermatozoa. Evidence was provided that these naturally occurring

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

antibodies were directed against sub- surface antigens of spermatozoa that cross-reacted with epitopes present on bacteria, suggesting the possibility that these antibodies are not directed primarily against sperm. Bronson *et al.* (1992), in contrast, provides evidence that antibodies directed against epitopes expressed on the sperm surface are not commonly seen in children, suggesting that pubertal development may play a role in the evolution of these antibodies. Isahakia (1988) suggested that in men with congenital obstructive azoospermia secondary to cystic fibrosis (CF), the immune system may become exposed to developmental antigens expressed on spermatocytes and spermatids to which it is not tolerant after activation of the pituitary-testicular axis and the initiation of spermatogenesis. Alternatively, T-dependent, epididymally derived antigens might also be secreted at puberty and be involved in the aetiology of autoimmunity to sperm (Bronson *et al*., 1992).

An association of congenital bilateral absence of the vas deferens and autoimmuntiy to spermatozoa was initially reported by Amelar and associates in 1975. Patrizio *et al.* (1989) demonstrated that infertile men with congenital absence of the vas deferens (CAVD) have low incidence of serum antisperm antibodies and that human vasa efferentia sperm like ejaculated sperm have full surface antigenic competence (Patrizo *et al*., 1992). However, the frequency of those men demonstrating antisperm antibodies varied widely, ranging from 11% to 62% (Patrizo *et al* 1989). In assessing the effect of repeated microsurgical epididymal sperm aspiration (a condition in which exposure of sperm to blood is inevitable) on the development of sperm antibodies in serum and epididymal sperm of men with CAVD, Patrizo *et al.* (1992b) observed that the procedure can

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

safely be repeated in patients with CAVD without the risk of developing new sperm autoimmunity. Their findings support the concept that there is a population of individuals that despite multiple exposure to sperm antigens are genetically predisposed to be non-immunological responders. Recently, D'Cruz *et al.* (1991) showed that some men with cystic fibrosis (CF) with congenital blockage or absence of the vas deferens develop low-titred serum antisperm antibody while Bronson *et al.* (1992) suggested that at puberty, and presumably, active spermatogenesis is requirement for development of autoimmunity to sperm in men with cystic fibrosis.

Although genital trauma or injury has been found to be associated with an increased risk of sperm antibodies, other studies have failed to confirm this association. Testicular torsion is a form of trauma to the male genital tract that has been intensely studied (Haas, 1987). The presence of primary testicular lesions in the twisted testes suggest that these lesions might be involved in the development of sperm antibodies.

Testicular biopsy has also been implicated in the initiation of sperm antibody formation, since this procedure obviously could injure the protective mechanism of the blood/testis barrier (Haas, 1987).

Varicocoele is another controversial issue that has been associated with infertility. Some investigators have considered that this condition might be associated with antisperm antibody formation (Haas, 1987).

Cryptorchidism is another potential cause of damage to the testicle that could increase the risk of antisperm antibodies. The interest regarding the sequelae of cryptorchidism stems from the fact that 10% of men with unilateral

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

safely be repeated in patients with CAVD without the risk of developing new sperm autoimmunity. Their findings support the concept that there is a population of individuals that despite multiple exposure to sperm antigens are genetically predisposed to be non-immunological responders. Recently, D'Cruz *et al.* (1991) showed that some men with cystic fibrosis (CF) with congenital blockage or absence of the vas deferens develop low-titred serum antisperm antibody while Bronson *et al.* (1992) suggested that at puberty, and presumably, active spermatogenesis is requirement for development of autoimmunity to sperm in men with cystic fibrosis.

Although genital trauma or injury has been found to be associated with an increased risk of sperm antibodies, other studies have failed to confirm this association. Testicular torsion is a form of trauma to the male genital tract that has been intensely studied (Haas, 1987). The presence of primary testicular lesions in the twisted testes suggest that these lesions might be involved in the development of sperm antibodies.

Testicular biopsy has also been implicated in the initiation of sperm antibody formation, since this procedure obviously could injure the protective mechanism of the blood/testis barrier (Haas, 1987).

Varicocoele is another controversial issue that has been associated with infertility. Some investigators have considered that this condition might be associated with antisperm antibody formation (Haas, 1987).

Cryptorchidism is another potential cause of damage to the testicle that could increase the risk of antisperm antibodies. The interest regarding the sequelae of cryptorchidism stems from the fact that 10% of men with unilateral

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

disease are infertile when treatment for the abnormal testicular orchidism occurs late in childhood 70 to 80 percent are infertile if the disease is bilateral (Haas, 1987).

Non-immunological factors such as mumps, virus, bacteria and certain mycoplasma strains can also cause agglutination of spermatozoa (Peleg and Ianconescu, 1966, Taylor-Robinson and Manchee, 1967) but it is not known to what extent spermatozoa are coated with antibodies which are not provoked by sperm antigen (Upadyaya *et al*., 1984).

Infectious aetiology are well documented as causes of epididymitis and subsequent spread of various pathogens from the urethra or prostate can cause epididymitis and subsequent scarring and obstruction (Pelouze 1941). Several workers found an association between genitourinary infection and antisperm antibodies suggesting that infection may play a role in cases of immunologic infertility not associated with bilateral obstruction (Shahamenesh *et al*., 1986; Witkin and Toth, 1983; Ingerslev *et al*., 1986, Haas, 1987). Hypothetical mechanisms that would explain a correlation between past or present infection and sperm antibodies include vasoepididymitis with unilateral obstruction, and exposure of sperm to immunologically competent cells present in the urethra during acute urethritis. Subclinical epididymitis could result in unilateral epididymal obstruction, subsequent phagocytosis of trapped spermatozoa, and systemic exposure to sperm antigens, resulting in the development of sperm antibodies (Close *et al*., 1987). Witkin and Zelikovsky (1986) suggested that

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

decreased cellular immunity and enhanced humoral reactivity to sperm are common in men with chronic prostatitis. Both may contribute to an increased rate of prostatic infection in these men. Patients with genital infection were observed to exhibit positive sperm antibodies (Mazzolli and Barrera, 1989). Further supportive evidence that both genital infection and autoantibodies are not only interrelated but are also associated with infertility in man were provided by several investigators (Quesada *et al*., 1968, Witkin and Toth, 1983, Sofer *et al*., 1990). They therefore suggested that sperm antibodies must be considered in the clinical management of this problem. In 1995, Ekwere in Nigeria demonstrated high incidence of sperm antibody in infertile subjects and related this to the prevalence of STDs.

Male homosexuals who participate in recipient anal intercourse have an increased incidence of sperm antibodies. It is reported that the formation of sperm antibodies in this group of men could play a role in the development of the acquired immune deficiency syndrome (AIDS) (Haas, 1987).

2.6 Mechanism of Action

It is well established that sperm antibodies are actiologically implicated in fertility. However, the mechanism by which it causes infertility has not been established. Several hypotheses had been put forward. Bronson *et al*. (1984a) in his review discussed the interference of antibodies with fertilization by affecting sperm transport and gamete interaction.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

2.6.1 Sperm transport

a) Binding of immunoglobulins to the surface of spermatozoa within semen impairs their ability to penetrate cervical mucus. Immunoglobulin binding also leads to altered motion of sperm within cervical mucus-shaking, vibration or incomplete immobilization.

b) The presence of sperm-reactive antibodies within cervical mucus subjects spermatozoa to complement mediated cell membrane damage. Tail directed antibodies (IgM and IgG but not IgA) promote loss of motility. Head directed antibodies may not impair sperm motility.

c) Opsonization of antibody coated spermatozoa through the binding of complement component C_3 could in theory lead to phagocytosis of sperm by macrophages within the female reproductive tract.

2.6.2 Gamete Interaction

a) Antibodies directed against sperm head may occlude binding sites of the zona pellucida, preventing sperm attachment.

b) Complement-fixing antibodies (IgM and IgG) directed against the sperm head may impair the ability of spermatozoa to penetrate eggs without loss of motility by promoting damage of the acrosomal and plasma membranes.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

2.7 Tests for detection of auto and iso antibodies

(Rose *et al*., 1976, Jones, 1980, Bronson *et al*., 1984a) Several methods have been used to detect and quantify sperm specific antibodies. Donor sperm had been used as an antigen source in antisperm antibody testing. This is because there is no epidemiologic or serologic evidence that the HLA or ABO systems are involved in immunologic infertility. The reactions, therefore are tissue rather than individual specific (Jones, 1980, Rose *et al* 1976; Broderick *et al*., 1989).

The methods used include sperm agglutination, sperm immobilization, indrect immunofluorescnce, enzyme-linked immunosorbent assay, radiolabelled antiglobulin assay and mixed agglutination reaction (MAR). These have been critisized for their lack of specificity (Broderick *et al*., 1989, Bronson *et al*., 1984a).

The Immunobead antisperm antibody test (IBT) is a new immunoglobulin specific assay that avoids many of the problems of older methods. It is currently, the most widely used assay system for the detection of sperm antibody. (Turek and Lipshultz, 1994). This test assays sperm antibodies on only live motile spermatozoa and it does not create false positive results by altering sperm membranes or exposing internal antigens. Introduced by Bronson and associates (Bronson *et al*., 1982a), the test is specific for class of immunoglobulin (IgA, IgG and IgM) and identifies the regions of sperm binding and proportion of sperm antibody can be accurately assessed. The immunobead antisperm antibody test uses commercially available micro-sized polyacrylamide spheres or beads (Immunobead, BioRad) that are bound covalently to rabbit antihuman immunoglobulins IgG, IgA and IgM. Mixing these beads with washed sperm (to

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

eliminate free antibody) results in direct binding of the bead to the sperm through this bead-attached antihuman antibody. The immunobead anusperm antibody test can be applied to detect antibodies already bound to sperm in semen directly (direct immunobead antisperm antibody test) or detect circulating sperm antibodies indirectly in fluid (indirect immunobead antisperm antibody test) (Carson *et al*., 1988; Bronson *et al*., 1984; Broderick *et al*., 1989; Turek and Lipshultz, 1994).

The indirect IBT has been applied to serum for some years and had been found useful (Shulman *et al*., 1992). Franco *et al* (1989) disclosed that IBT has been shown to be immunloglobulin and location-specific, and reproducible. It compares well with other indicators of autoimmunity to sperm, including reduced conception rate (Ayvaliotis *et al*., 1984), in vitro binding to the zona pellucida by spermatozoa (Bronson *et al*., 1982b) and correlates well with abnormal results on post coital testing (Bronson *et al*., 1984b). Jennings *et al* (1985) have found the indirect IBT to have a 99% correlation with the standard tray agglutination test and sperm immobilization test suggesting that IBT is a good index of specific antisperm antibodies (Khoo *et al*., 1991).

The indirect IBT has been used to study the presence of immunoglobulin classes of sperm antibodies in serum (Clarke *et al*., 1985a), cervical mucus (Clarke *et al*., 1984a) and follicular fluid (Clarke *et al*., 1984b) while the direct IBT has been applied to semen (Clarke *et al*., 1985b). The use of IgG and IgA IBTs has consequently become routine in many laboratories, both for direct testing of spermatozoa and for indirect testing (Pattinson and Mortimer, 1987).

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Broderick *et al* (1989) described IBT as rapid, inexpensive and clinically relevant assay.

2 8 Treatment of immunologic infertility

Therapy for antisperm antibodies in the male includes the use of condoms, corticosteroid immunosuppression, intrauterine insemination of husband's sperm, *in vitro* fertilization and donor insemination (Haas, 1987)

Candidates for treatment of immunologic infertility include those men with sperm antibodies without anatomic obstruction whose female partners have been fully investigated. Only patients with greater than 50% of sperm bound antibodies should be treated. In addition it is thought that only head-directed or midpiece-directed sperm antibodies are clinically relevant in immobilization or penetration assays; tail-directed sperm antibodies at least theoretically, need not to be treated. In selecting a treatment regimen, the physician should take into account the severity of the observed deficit, the overall health of the patient and the side effects.

Condoms were advocated in the past as a means by which to decrease the female exposure to repeated doses of sperm antigens. Although it is the oldest and least complicated form of therapy, its efficacy is at best uncertain.

Sperm wash has been reported to remove antibodies from the seminal fluid or from the sperm surface, but the results have met with variable success. Rapid dilutional washes of fresh ejacualtes may remove free antibody in the seminal plasma but are unsuccessful at disloding tightly bound, high affinity

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

sperm antibodies from the sperm surface. Potentially such sperm processing techniques, although unable to definitively remove sperm antibodies, may be effectively used in conjunction with assisted reproductive procedures to overcome immunologic infertility.

The most common form of treatment for immunologic infertility is immunosuppressive therapy with corticosteroids. Although the mechanism is not completely understood, it is known that steroids can impede the chemotaxis of inflammatory cells, prevent the release of cytokines or lymphocyte growth factors, decrease antibody production, and weaken antibody-antigen association. In instances in which sperm/ovum interaction is inhibited, only corticosteroids immunosuppression or donor insemination may be successful. However, the risk of corticosteroids cannot be under estimated. Effects such aseptic necrosis of the hip have occurred (Turek and Lipshultz, 1994).

Among the postulated pathologic effects of sperm antibodies in immunologic infertility, the inhibition of sperm migration and penetration of cervical mucus is well described. It is thought that one way to overcome the subfertility induced by this effect is to combine sperm wash techniques with intrauterine insemination (IUI) of sperm, and in essence, by-pass the cervical factor. Also, this form of therapy has potential in female derived sperm antibody, a condition in which sperm antibody may reside in the cervical mucus. However, Francavilla *et al.* (1992) suggested that overcoming the cervical mucus barrier through intrauterine inseminations in the treatment of male immunological infertility is imputable to antisperm antibodies when they involve all spermatozoa, regardless of semen quality. IUI is less risky than immunosuppression and is

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

commonly used in male factor infertility for a variety of indication - oligospermia, asthenospermia, poor post coital tests, low semen volume, and anatomic abnormalities (Turek and Lipshultz, 1994).

In vitro fertilization (IVF) has also been applied to overcome male factor infertility because of the relatively low numbers of sperm required for successful IVF in cases of female factor infertility. Assisted reproductive technology can generate definite, although variable, success rates for certain couples with male factor immunologic infertility. The promise it offers, however, is at great financial and emotional expense.

2.9 Protective ability of sperm antibodies

Sperm antibodies are associated with decreased fertilization in vitro and in vivo. Reports have suggested that capacitation and acrosome reaction (AR), physiologic prerequisites for mammalian fertilization, may quantitatively increase sperm antigenicity (Silverberg *et al*., 1990). However, sperm antibodies can also enhance the ability of human spermatozoa to bind to and penetrate the zona free hamster oocyte (Edward, 1990a). Silverberg *et al* in their study suggested that sperm antibody binding may facilitate the acrosome reaction in human spermatozoa in vitro (Silverberg *et al*., 1990). Sperm antibodies prevent fertilization by agglutinating or being toxic to spermatozoa, so reducing the number available for penetration, but their action in improving fertilization has been a matter of speculation. It seems to depend on the actions of antibodies in enhancing the proportion of spermatozoa undergoing the acrosome reaction,

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

which might explain why the number of spermatozoa binding to the oolema of the hamster oocyte is raised

Such evidence implies that the presence of a specific sperm antibody enhances sperm-egg binding, perhaps through the presence of shared epitopes between spermatozoan and oocyte e g oncofetal antigens Another possibility is that the antibodies against spermatozoa bind to Fc receptors on the oocyte membrane, so bringing spermatozoa and oocyte to close proximity and enhancing their fusion. This possibility has now been examined in a study involving a search for Fc receptors on zona-free hamster oocytes and the membrane of mouse oocytes together with an analysis of the effect of Fc receptors on the binding of antibody-treated human spermatozoa to zona-free hamster oocytes (Edward, 1990b).

Various methods were used to assess the binding of antibodies to zona-free hamster oocytes. These included the use of IgG Fab and IgG Fc specific antibodies in tests using immunoflourescence or immunobeads. Initially, human IgG aggregates were found to become attached to the oolema of zona-free hamster oocytes, and so did human IgFc fragments (Edward, 1990a).

These forms of binding were blocked if the oocytes were first exposed to specific antibodies against Fe Antibodies to Fab also bound to many mouse oocytes, whereas myeloma proteins used as control did not. These studies thus indicated that Fc receptors were present on mouse and hamster oocytes (Edward, 1990b).

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

The effect on fertilization *in vitro* of antibodies against the human sperm-head was then tested. Exposure of human spermatozoa to these antibodies resulted in their increased binding to zona-free hamster oocytes. Binding was depressed when eggs were exposed to IgG Fc before insemination, implying that the blockage of Fc receptors on oocytes impaired the ability of spermatozoa coated with antibody to bind to the oolemma. In essence, the Fc receptors had enhanced the binding of antibody-coated human spermatozoa to the oolemma (Edward, 1990b).

Fc receptors must be present on the membranes of mammalian oocytes. They have a role in enhancing sperm attachment in the presence of antibodies against the sperm head, but they could also be involved in normal sperm/egg attachment. There are various indications that Fc receptors are involved in transmembrane signalling in other types of cells, and act (as receptor ionophores) causing Na^+/K^+ ion fluxes in membranes (Edward, 1990b).

2.10 Hormones and Infertility

Successful and complete male germ cell development is dependent on the balanced, endocrine interplay of the hypothalamus, the pituitary and the testis. The hypothalamus secretes gonadotrophin - releasing hormone in a pulsatile manner which in turn, elicits the pulsatile release of the gonadotrophins LH and FSH from the pituitary. Luteinizing hormone stimulates spermatogenesis indirectly via testosterone, where as FSH acts directly on the seminiferous tubules. The synthesis and release of gonadotrophic hormones is under the

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

feedback control of testosterone. The involvement of testicular peptides such inhibin and activin is not clear. Luteinizing hormone/testosterone and FSH are the prime regulators of germ cell development. On the spermatogenic process. Receptors for androgens and FSH are confined to the somatic cells of the testis, hence, the trophic effects of these hormones on germ cells must be indirect (Weinbauer and Nieschlag, 1995).

The role of prolactin in males has not been satisfactorily explained. It has been proved that hyperprolactinemia has a negative effect both on spermiogenesis and spermatogenesis (Rogoza *et al*., 1994).

Saeed *et al*. (1994) observed normal levels of serum FSH in infertile patients with mild to moderate oligospermia while an increase in serum FSH was observed in patients with severe oligospermia. In another study, Merino *et al*. (1997) observed significantly higher ($p<0.001$) levels of prolactin in the men with asthenozoospermia, oligozoospermia and azoospermia compared with controls. However, normal levels of prolactin were observed in 66.9% while 33.1% of cases (167) had hyperprolactinemia. Serum FSH and LH concentrations in azoospermic men were significantly higher ($p<0.0001$) which indicates some disturbance of the spermatogenic process. No significant differences were found in serum testosterone. Takahashi *et al*. (1992) observed no significant relationship between results of traditional semen analysis, LH, FSH and prolactin and the rates of antibody binding.

IBADAN UNIVERSITY LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

CHAPTER THREE

MATERIALS AND METHODS

3.1 Subjects

A total of 182 male subjects aged 18-56 were recruited under three groups – fertile subjects, subjects with STDs and infertile subjects.

Fertile subjects(Group A) These were 85 randomly selected, apparently, 'normal' male individuals(group A) with no evidence of STDs who also served as controls. Evidence of fertility was based on a satisfactory semen profile and achievement of at least one pregnancy.

Infertile subjects(Group B) These were 50 patients(group B) without evidence of STDs who were married and had problems with fertility for at least one year. They were recruited from urology clinic and husbands of spouses from Obstetrics and Gynaecology clinic-both of the UCH, and from some private hospitals in Ibadan. 37 of these males were dyspermic (BA) while the other 13 males were normospermic (BN).

Subjects with STDs(Group C) These were 47 acutely infected patients(group C) recruited from the STDs clinics of the UCH. They were

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

otherwise 'healthy' adult male patients with incontrovertible evidence of STDs Patients were investigated before treatment

3 2 Study design

All subjects in the study gave informed consent and the following investigations were carried out

a Ages and past history of STD were obtained from subjects

b Anthropometric measurements Weight and height of subjects were obtained after which body mass index (BMI) was calculated as weight (kg) /height^2 (m^2)

c Biophysical and biochemical examination of semen Semen was analysed biophysically in accordance with World Health Organisation (WHO) guidelines (WHO,1987) Semen was examined macroscopically for appearance, liquefaction, consistency and volume, and microscopically for morphology, concentration, motility, white blood cells (WBC) and pus cells at x40 magnification Biochemical analysis of zinc was estimated in seminal plasma using atomic absorption spectrophotometry (Parker *et al* , 1967, WHO , 1987)

d Immunological analysis (in blood plasma and semen) IgG, IgA and IgM antisperm antibodies were detected in blood plasma and seminal plasma using the indirect immunobead binding technique while sperm antibodies on spermatozoa were detected using the direct immunobead binding technique (WHO 1987, Carson *et al* , 1988, Broderick *et al* ,1989)

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

e Chlamydia antigen and antibody detection: Enzyme linked immunosorbent assay (ELISA) technique was used in detecting both chlamydial antigen and antibody using commercially available kits (Orthodiagnostics System Inc and Orgenics Ltd respectively).

f Microbiological analysis: *N. gonorrhoea* was examined by direct microscopy using gram stain in semen and urethral swab. Incubation of samples was done on thayermatin medium to specifically grow *N. gonorrhoea* if present. Chocolate medium was also used to see if other microbial organisms were present. Urine was examined by direct mucroscopy (Arya *et al*., 1988).

g Venereal disease research laboratory test (VDRL): This was a serological test used to detect antibodies against syphilis (Arya *et al*., 1988).

h. Endocrinological Analysis: Folicle stimulating hormone (FSH), luteinising hormone (LH) and prolactin (PRL) were estimated using Immunoradiometric assay method (IRMA) while testosterone (T) was estimated using radioimmunoassay technique (RIA) (ICN Biomedicals Inc.).

3.3 Sample collection

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Blood 10-20mls of venous blood were collected in lithium heparin tubes, centrifuged for 5 minutes at 3000 rpm. Plasma was collected and stored at - 20°C for analysis.

Semen Semen was collected by masturbation after abstinence from sexual relations for 3 days, put in a graduated sterile universal bottle and allowed to liquefy within one hour before biophysical tests were performed. Part was used for microbiological tests and direct immunobead test for antibodies on spermatozoa. The remaining semen was centrifuged at 3000 rpm for 30 mins and seminal plasma obtained was stored at - 20°C for analysis of zinc and sperm antibodies.

Urethral swab 2 urethral swabs were collected from urethra of each male subjects by rotating swab approximately for 5 seconds after inserting 2 to 4 cm into the urethra. One swab was used for microbiological analysis while the other was used for ELISA technique for the detection of chlamydial antigen after storing in transport medium and stored at - 20 °C for a maximum of seven days for the determination of chlamydial antigen.

3.4 Validation of Assay

Specificity is defined as the extent of freedom from interferences by substances other than the one intended to be measured.

i.e $\frac{TN}{TN+FP} \times 100$

TN = True Negative FP = False Positive

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Sensitivity of the assay is defined as the smallest detectable amount of substance that can be distinguished from zero

ie $\frac{TP}{TP + FN} \times 100$

TP = True Positive, FN = False Negative

Accurncy of assay was assessed by dctennining the recovery of known amounts of substance

Precision of an assay represents the variation of a given set of estimations of the same sample from the mean of tbat set. It usually expressed the standard deviation as the percentage of the mean value ie $SD/x \times 100$ = Coefficicnt of variation (CV%)

SD – Standard deviation

x- the mean of the set of estimations

Precision was assessed by detennining the intra-assay (within batch) coefficient of variation and inter-assay (between batch) coefficient of variation of same sample after a series of measurements

% Error is the percentage of deviation of sample value from its real value

3.5 Biophysical analysis of semen

The following biophysical analyses of semen were carried out by examinining semen both macroscopically and microscopically according to WHO methods (WHO, 1987)

3.5.1 Macroscopic Examination

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

The semen sample was evaluated by simple inspection at room temperature for appearance, liquefaction, consistency (viscosity) and volume. A semen sample with grey-opalescent appearance and is homogenous, liquefies within 1 hour (60mins) and is able to produce small discrete drops of not more than 2 cm in length was regarded as normal

3.5.2 Microscopic Investigation:

10µl of semen was delivered with a sterile dropping pipette on a clean glass slide with a coverslip 20mm x 20mm. The weight of the coverslip spread the sample for optimal viewing. The freshly made, wet preparation was left to stabilize for approximately one minute. Examination was carried out at room temperature using a light microscope at x.10 magnification. Estimates of motility, concentration, morphology, white blood cells and pus cells were observed. The microscopic field was scanned systematically and motility of each spermatozoa was classified under:

a. rapid and linear progressive motility ('excellent' or 'good' progression) (RPL)

b. slow or sluggish linear or non-linear movement ('weak or 'moderate' progression) (SLP)

c. non-progressive motility. (NPM)

d. immotility (MI)

Percentage of each motility category per one hundred successive spermatozoa was recorded. Concentration of spermatozoa per ml was made from mean

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

number of spermatozoa in several microscopic fields and multiplied by 10^6 Morphologically normal sperm cells were also counted and scored as percentage while white blood cells (WBCs) and pus cells /field were scored as

1 = 1- 4 cells

2 = 5-9 cells

3 = 10 and above

4 = Numerous

3 5 3 Classification of semen characteristics

1) Normospermia	Appearance = Normal
	Liquefaction = Normal
	Consistency = Normal
	Volume = 2-5 mls
	Concentration > 20 million/ml
	Motility = RPL ≥ 25%
	or RPL + SLP ≥ 50%
	Morphology = ≥ 50%
2) Azoospermia	Concentration = 0 million/ml
3) Oligospermia	0 < Concentration < 20 million/ml
4) Asthenozoospermia	< 50% spermatozoa with rapid linear + slow linear progression
5) Teratozoospermia	Morphology < 50%
6) Oligoasthenozoospermia	Combination of 3 and 4
7) Oligoasthenteratozoospermia	Combination of 3, 4 and 5

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

8) Asthenoteratozoospermia	Combination of 4, 5
9) Aspermia	No ejaculate
10) Necrozoospermia	Immotility = 100%
11) Hypospermia	Volume<2ml
12) Hyperspermia	Volume >5ml
13) Dyspermia	Pathologic Semen

3.6 Biochemical estimation of Zinc

Zinc in seminal plasma was estimated with an atomic absorption spectrophotometer (Perkin-Elmer model 403) (Parker *et al*, 1967, WHO, 1987)

Principle: In the ground (unexcited) state, zinc atoms absorb light of the same wave length as emitted by the element in the excited state. The amount of light absorbed is directly proportional to the concentration of zinc

Reagents

Glycerol solutions, 5% and 10%

Zinc powder

Concentrated Hydrochloric acid, HCl, diluted (1 + 1) with deionized water

Standard solution: Zinc stock solution, 50, 000 µg% Zn (500 µg/ml/Zn)- 0 500 g of zinc powder was dissolved in a minimum amount of (1 +1) HCL. This was diluted to 1 litre with deionized water

Zinc standard solutions: Zinc stock solution 500 µg/ml was diluted with 5% (v/v) glycerol solution in 1/2, 1/5, 1/10, 1/20, 0 corresponding to 250, 100, 50, 25, 0, µg/ml concentrations

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Sample Preparation: Seminal plasma was diluted to 1/200 with deionized water (e.g. 10 µl of seminal plasma added to 1.99ml of distilled water. The trichloroacetic acid (TCA) precipitation step for proteins is unnecessary because of the high dilution of seminal plasma)

Calculations: Zinc (µg%) = (µg% in diluted solution). All glass ware and tube used in collecting or storing samples of zinc were cleaned by being kept at least 24 hr in HCL diluted with an equal volume of water thoroughly washed with doubly distilled water/deionized water, and dried (Lampugnani and Maccheroni, 1984)

3.6.1 Validation of Zinc assay.

Intra-assay variation of zinc are shown on Table 1

Table 1

Intra-assay variation of Zinc assay.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Table I

Intra-assay variation of Zinc assay.

	Zinc (µg/ml) A	Zinc(µg/ml) B	Mean Zinc (µg/ml)	S D	C V%
Deionised water	4.0	4 0	4 0	0	0%
P Sample I	50	48	49	1 4	2 9
P Sample 2	72	70	71	1 4	2 0

S.D = Standard deviation, C.V = Coefficient of variation, P Sample 1 and 2 = Seminal plasma samples from different subjects ; A & B = replicate samples

3 7 Immunobead binding technique for the detection of sperm antibodies on spermatozoa, in blood plasma and seminal plasma

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Sperm antibodies were detected on sperm cells using the direct immunobead test and in seminal and blood plasma using the indirect immunobead test (Broderick *et al*, 1989, WHO, 1987, Carson *et al*, 1988).

Reagents:

a Immunobeads Rabbit anti human IgG, IgA and IgM were obtained from Bio Rad Laboratories, Richmond CA. 10mg of immunobeads were reconstituted in 2ml of tyrode's buffer and stored at 4 ^{o}C. These were kept for one month.

b Buffer: Tyrode's Solution - 0.2g $CaCl_2$, 0.2g KCl, 0.05g NaH_2PO_4, 1.0g $NaHCO_3$, 1.0g glucose, 0.2g $MgCl_2 6H_2O$, 8.0g NaCl. These were dissolved in little distilled water and made up to one litre. The solution was passed through a millipore (22µm) filter before use.

c Bovine serum albumin (BSA) buffer (0.4% w/v): 0.4g of BSA was weighed out and made up to 100ml with Tyrode's solution (T-BSA). This was prepared just before analysis so as to prevent bacterial growth that could affect the assay.

3.6.1 Direct immunobead test:

Principle Rabbit anti-human IgG, IgA and IgM were directly bound to polyacrylamide beads and sperm cells are washed in order to eliminate free antibody. Mixing the immunobeads with washed sperm resulted in direct binding of the bead to sperm antibodies on the sperm cell.

Procedure: 0.2 ml (1mg) reconstituted immunobeads - anti-IgG, anti-IgA and anti-IgM beads were put into separate centrifuge tubes, washed once by making it up to 10ml with T-BSA and centrifuged at 3000rpm for five minutes. The

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

supernatant was decanted and immunobeads were resuspended in 500µls of T-BSA buffer (i.e. 2mg/ml). The amount of semen required for the assay was determined by sperm count and motility according to Table 2. The required amount of semen was made up to 10ml with T-BSA washed twice by centrifuging and decanting. The resultant semen pellet was resuspended in 0.2ml of T-BSA.

5µl of beads were added to 5 µl of the semen suspension. These were mixed well with the coverslip on each of the mixtures. Observation was done after leaving the slide for 15 minutes in a moist chamber at 400x magnification under a phase-contrast microscope. Percentage of motile spermatozoa attached to the immunobeads in the IgG, IgA, IgM-containing mixtures were scored separately. 100 motile spermatozoa were scored for the presence and location of bead binding. Binding was considered positive when one or more beads adhered to the surface of a motile sperm cell. Location was demarcated as head only (H), tail only, head and tail (H/T), and tail-tip (t). The test was regarded as positive if 10% or more of the motile spermatozoa were attached to the beads (WHO, 1987).

3.7.2 Indirect immunobead test

Principle: Antisperm antibodies present in plasma were absorbed onto antibody-free donor sperm using a passive antibody transfer technique. Rabbit anti-human IgG, IgA and IgM were directly bound to polyacrylamide beads and sperm cells are washed in order to eliminate free antibody. Mixing the immunobeads with washed sperm resulted in direct binding of the bead to sperm antibodies on the sperm cell.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Procedure Following liquefaction, semen samples were diluted in 3 volumes of (T-BSA), and washed twice by centrifugation at 3,000 rpm for 10 mins. The resulting pellet was gently loosened and 0.5ml T-BSA was carefully layered onto the top. The tube was placed upright in a 37° C incubator for 30 minutes to allow the highly motile sperm fraction to swim up into the medium. The top 0.4ml was then transfered to a new tube and the sperm count and motility were determined and adjusted to 50 x 10^6 motile sperm/ml. Heat inactivated plasma (at 56°C) was thawed and diluted 1:4 in T-BSA. Known human antibody-positive seminal plasma and antibody- negative plasma were run as controls in every assay. Sperm suspensions (40µl) were mixed with 400µl diluted plasma (4.5 x 10^6 motile sperm/ml final concentration) and allowed to incubate at 37°C for 30 minutes. Sperm cells were then washed free of plasma by centrifugation for 5 minutes at 3000 rpm, resuspended in 0.5ml T-BSA and centrifugation was repeated. The final pellet was resuspended in 100µl T-BSA (10 x 10^6 motile sperm/ml) and these sperm cells were used in the immunobead assay.

Ten microliters of the sperm suspension and 20µl of the bead suspension were mixed on a glass slide, covered with a coverslip, and allowed to react for 10 minutes at room temperature. Using a phase-contrast microscope (400x), 100 motile spermatozoa were scored for the presence and location of bead binding. Binding was considered positive when one or more beads adhered to the surface of a motile sperm cell. Location was demarcated as head only (H), tail only, head and tail (H/T), and tail-tip (t). A specimen was considered IBT-positive

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

when ≥ 10% of the motile sperm showed positive bead binding for IgG, IgA, and/or IgM(WHO, 1987)

UNIVERSITY OF IBADAN LIBRARY

IBADAN
UNIVERSITY LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Table 2

Required amount of semen for analysis

Concentration (10^6/ml)	Motility (%)	Semen required (ml)
>50		0.2
20 – 50	>40	0.4
20 – 50	<40	0.8
<20	>40	1.0
<20	<40	2.0

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

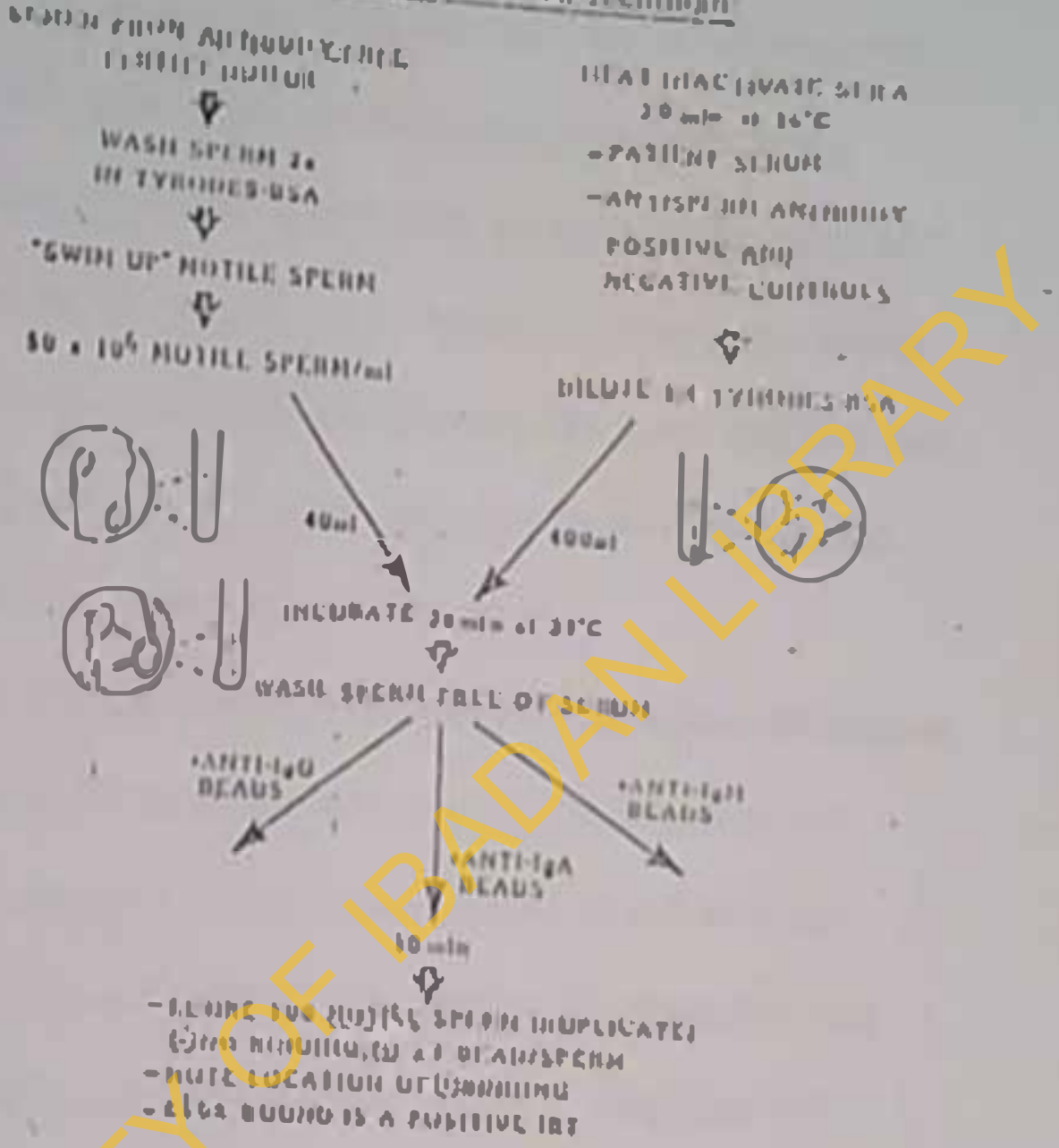


Fig 1. Schematic diagram depicting the principles used in the IBT test

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

3 7 3 Validation of Sperm antibody assay

(1) Semen from 5 sperm antibody free donors were used for the indirect sperm antibody tests. These were healthy' normospermic adult males. Semen analysis was performed on each semen sample donated after abstinence for 3 days to ensure satisfactory semen profile. In order to be admitted as donors, direct immunobead sperm antibody test was done after sperm cells have been washed free of seminal plasma. Percentages of immuoobead bnding were recorded for each of the donors as shown on Table 3.

(2) Each semen brought by each antibody free donor was tested for spermatozoal sperm antibodies by the direct immunobead method in every assay run.

(3) Known positive and negative controls were included in each assay run.

(4) Negative control plasma was obtained from plasma of subjects that were found to be antibody negative by IBT indirect tests. Assay runs with negative controls of ≤5% immunobead binding was accepted as valid.

(5) Controls were lyophilised seminal plasma samples which were reconstituted with 2mls of deionised water before use. These were obtained from Professor W. R. Jones, Flinders Medical Centre, Bedford, Australia. Assay runs with

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

≥ 15% immunobead binding for positive in any of the antibody isotypes

were valid by IBT. Percentage binding of positive controls are shown on Table 4.

(6) The sensitivity of IBT is 98%

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Table 3

Percentage Immunobead binding of sperm donors for indirect assay.

Donors	IgG	IgA	IgM
1	0	1 (H)	0
2	0	0	0
3	0	0	1 (tt)
4	0	0	1 (T)
5	0	0	0

H = binding is on the head region, T = binding is on the tail region, tt = binding is on tail tip

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Table 4

Inter-assay runs of positive control samples in the immunobead binding test.

	A	S	S	A	Y		R	U	N	S			
Ig	A	B	C	D	E	F	G	H	I	J	K	L	M
G	19	1	66	2	31	16	5	47	43	40	1	4	19
A	35	18	85	3	35	0	24	54	24	62	38	40	37
M	2	16	97	30	19	54	0	43	3	0	1	11	8

Ig = Sperm antibody isotype; values are in percentages showing % binding of sperm antibodies; alphabets represent assay runs

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

3 8 Detection of chlamydial antigen

Ortho® Chlamydia antigen ELISA test was used for the detection of Chlamydia antigen (Ortho Diagnostic Systems Inc. Raritan)

Principle Ortho Chlamydia antigen ELISA test utilised the microwell format. The male urethra swab was extracted by adding a buffer directly to the transport tube. An aliquot of the specimen was subsequently transfered to a treated microwell where chlamydia antigen present in the sample bound to the surface of the microwell. The bound antigen was detected using a two-step procedure. First, detector antibody (rabbit anti-Chlamydia) was added which specifically bound to the immobilized antigen. Next, conjugate antibody (horse radish peroxidase -conjugated goat anti-rabbit (IgG) was added which bound to the detector antibody-antigen complex. This was visualized by the addition of the chromogemic substrate 0-phenylene diamine dihydrochloride (OPD) which was converted by the peroxidase into a coloured end product. The intensity of the colour produced was dependent on the amount of chlamydia antigen present and was read on a spectrophotometer.

Reagents

Treated microwells	-	8 x 12 well treated microwell strips
Extraction buffer	-	1 x 50 ml 0.05M phosphate buferred saline (PBS) containing 0.85% extraction reagents and 0.1% Sodium Azide (NaN_3)

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Antigen standard	-	1 x 2.0 ml Chlamydia antigen LGV2 strain in 0.05M PBS containing 1% carrier protein and 0.1% NaN_3
Detector antibody	-	1 x 11 ml anti-Chlamydia (rabbit) in 0.05M PBS containing 40% carrier protein, 0.01% Teen Tm and 0.1% NaN_3
Conjugate antibody	-	2 vials lyophized peroxidase-conjugated anti-rabbit IgG (goat) in 0.5 M PBS containing 10% carrier protein and 3.5% preservatives and stabilizers
Conjugate Reconstitution buffer	-	1 x 13ml 0.05M PBS containing 10% carrier protein and 0.4% preservatives and stabilizers
Substrate reagent	-	1 x 50 ml 0.01% thimerosal in water
Substrate tablets	-	1 x 10 tablets containing O-phenylene diamine dihydrochloride (OPD) (10 ng per tablet)
Hydrogen peroxide	-	1 x 1.5 ml 3% H_2O_2 in water
Wash buffer Concentrate	-	1 x 50 ml 20 x concentrate of 0.05M PBS containing 0.05% Tween Tm and 0.005% preservative

Procedure: Assay controls were arranged in microwells as follows-A_1-substrate blank, Wells A_2 and A_3 - Negative control, Well A_4-Antigen standard. Well A_5 and above contained patients samples. 0.5ml extraction buffer was added to all patient specimens and antigen standard and mixed. Samples were incubated at room temperature for 15 minutes and then vortex extracted for 15 seconds. 100µl

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

of controls, antigen standard and patient specimens were pipetted into appropriate microwells and incubated for 60 mins at room temperature. The samples were washed five times manually, incubating exactly one minute each time. 100µl of Detector antibody was added to all the wells excluding the blank (Well A1) - Samples were then incubated for 30 mins at room temperature. After incubation, samples were washed again for five times manually incubating exactly one minute each time. 100µl of conjugate antibody were added to all wells (excluding well A_1). The samples were again incubated for 30 mins at room temperature. Washing was repeated for five times incubating exactly one minute each time. Fresh substrate solution was prepared (one substrate tablet per 5 ml of substrate reagent) after adding a full drop of Hydrogen peroxide. 100µl of substrate were added to all wells within 15 mins of preparing substrate reagent. Incubation was repeated for 30 minutes at room temperature. 100µl of sulphuric acid were added to all the wells. The samples were then transferred to ELISA plates and absorbance was read at a wavelength of 492 nm within 30 minutes.

Calculation of results

1. The negative control mean was calculated by adding the two negative control values and dividing by two. This was rounded off to the nearest hundredth.
2. The cut off value was calculated as the negative control mean + (plus) 0.15
3. Any specimen yielding a value greater than the cut off value (negative control mean + 0.15) was positive for chlamydia antigen.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

3.8.1 **Validation of Chlamydia antigen Assay**

In order for the assay results to be considered valid the following criteria were met

1. Maximum negative Control value- Both negative control values were less than or equal to 0.10 absorbance units
2. Negative control variation: The difference between the two negative control values were less than or equal to 0.06 absorbance units
3. Minimum antigen standard value - The antigen standard value was greater than two times the cut off value (negative control mean + 0.15)
4. Interassay and intra assay variation was determined using negative, low positive and high positive sample. Each sample was run in replicates of five or four different runs. The results are presented in Table 5.
5. The sensitivity of ortho chlamydia ELISA antigen test for males is 97% (31/32).
6. The specificity of orthochlamydia ELISA antigen test for males is 96% (96/100).

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Table 5

Inter assay and Intra assay varintion of chlamydia antigen

Sample	Mean Absorbance	Inter assay C V	Intra assay C V
Negative	0.03	38.7%	21.0%
Low Positive	0.51	6.1%	3.5%
High Positive	1.21	6.6%	3.1%

C V = Coefficient of variation

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

3 9 Detection of chlmydial antibodies by serology

Imniunocomb (R) *Chlamydia Trachomatis* IgG (Orgenics Ltd) was used in detecting IgG antibodies to Chlamydia Trachomatis in human plasma

Principle

The Imniunocomb *Chlamydia Trachomatis* IgG kit was based on the principle of the enzyme-linked Immunosorbent assay. Results were obtained in less than an hour

The plastic Immunocomb card acted as solid phase and was sensitized with inactivated antigens of *C. Trachomatis* on each of 12 pointed tips (teeth). A second spot containing goat antibodies to human immunoglobulin was also present on each of the teeth and served as internal control, enabling the operator to verify that the kit reagents functioned properly and to confirm that the specimens were added to the test wells

Specimens were deposited into the wells of the developing plate and brought in contact with the antigens by insertion of the Immunocomb card After a wash step, the card was moved to wells containing enzyme labelled antibodies to human IgG which bind with the antibody complex on the card teeth The card underwent two successive wash steps and was then moved to wells where a chromogenic reaction took place, resulting in the appearance of an insoluble dye at the site of the antibody-antigen complex. The chromogemc reaction was

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

stopped by returning the immunocomb card to the wash solution. The card was dried in air, and the results were read visually.

Composition of Kit: Inimunocomb card was composed of antigens of *C. Trachomatis* as a lower spot, 2-4mm from the tip, and goat antibodies to human immunoglobulin as an upper spot, 6-8 mm from the tip. The teeth of the immunocomb card were numbered 1-12. Each of the teeth was also identified by the number "10" above the tooth number inorder to identify each portion as belonging to the Chlamydia trachomatis IgG kit, if the card is broken into sections.

Developing plate: Each developing plate contained reagent solutions (Compartments A-F) ready for use in all steps of the test. Each compartment was divided into 12 wells. 3 developing plates per kit were provided. The contents of the developing plate compartments were as follows.

A: Specimen diluent

B: Washing solution

C: Diluted goat anti-human IgG antibodies conjugated to alkaline phosphatase

D: Washing solution

E: Washing solution

F: Chromogenic substrate solution containing nitro blue tetrazolium (NBT) and 5 bromo-4 - chloro-3-indolyl phosphate (BCIP).

Positive Control 1 vial, 150µl. Human plasma diluted to an anti-chlamydial IgG titre of 1:32 as determined by microimmunofluorescent assay; non-reactive for hepatitis B surface antigen (HBsAg) and for antibodies to HIV.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Negative Control 1 vial, 150µl Reconstituted serum, non-reactive for hepatitis B surface antigen (HBs Ag) and for antibodies to HIV

Comb key card A printed colour scale for translating the colour density of the reaction spots to microimmuno-fluorescent titre levels

Procedure: The developing plate was brought to room-temperature

Antigen-Antibody Reaction 10µl of each specimen including negative and positive control were added into their respective wells

The immunocomb card was inserted and incubated for 10 mins in compartment A after which there was antibody antigen reaction During insertion, the card was withdrawn and reinserted rapidly several times to remove air bubbles

Wash The immunocomb card was washed by inserting card into the wells of compartment B and incubated for 2 minutes at room temperature during which the card was agitated periodically to ensure proper washing The card was withdrawn and the pointed edge was cleaned with clean absorbent paper in order to remove liquid adhering to the teeth

Conjugate The immunocomb was inserted in compartment C with the usual withdrawing and reinserting of card rapidly several times to remove air bubbles It was then incubated for 20 mins at room temperature At the end of the incubation, card was withdrawn and teeth cleaned with clean absorbent paper

Wash The card was inserted into the wells of compartment D and washed It was incubated for 2 mins and cleaned

Wash Washing was repeated in compartment E with agitation of card to ensure proper washing and cleaned

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Chromogen The immunocomb card was inserted into the wells of compartment F and incubated for 10 minutes for colour reaction after which the reaction was stopped by removing the card into the well of compartment E and incubating for 1 minute The card was withdrawn and air dried The results were read visually

Visual rending with combkey card:

The level of anti-chlamydial IgG antibodies was determined by comparing the colour intensity of the spot produced by each specimen with combkey colour scale.

This was performed in three steps

1. Selection of the purple-grey scale as indicated by Scale 2 (BCIP-NBT) in the upper portion of the combkey card
2. Calibration of the Combkey in relation to the lower C.T. spot on the tooth developed with the positive control (having a titre of 1:32 by immunofluorescence assay)
3. The colour wheel was turned until the colour density in the window labelled "1/32" and "C" matched that on the lower spot on the positive control tooth.

3.10 Chlamydia IgG antibody Validation

In order for the assay results to be considered valid, the following conditionswere fulfilled

(1) Positive control must produce 2 spots on the immunocomb card tooth

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

(2) The negative control must produce an upper spot 6-8mm from the tooth tip, indicating the proper functioning of the kit reagents.

3.11 Detection of *Neisseria Gonorrhoea*

Smears: Smears were prepared by rolling the urethral swab on a slide or by gentle spreading of semen with a platinum loop, making sure that the smear was thin and evenly spread and avoiding rubbing on the slide which distort the morphology of the organism. The smear was then dried and fixed by passing it through a bunsen burner flame without overheating. The slide was stained by Gram's method.

Gram's staining method: Smear was stained with 1% cystal violet for 60 seconds and rinsed with tap water. Thereafter gram's iodine was poured on slide and left for 60 seconds. It was then rinsed with tap water and decolourized with acetone or alcohol for a few seconds until fluid flowing out was colourless. It was washed with tap water and finally counterstained with safranine for 60 seconds. It was then blot dried and examined under microscope using oil immersion lens. Gonococci appeared as gram-negative bean-shaped, intracellular and extracellular diplococci.

Culture media: Culture media used were (a) Chocolate agar which is an enriched non-selective medium and (b) Modified Thayer-Martin medium which was both enriched and selective medium incorporating vancomycin, colistin, nystatin and trimethoprim which inhibit other bacteria and yeast.

Technique: Specimen was inoculated directly onto culture plates. The urethral swab or platinum loop (in the case of semen) was rolled on to the each medium

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

in a large 'Z' pattern to provide adequate transfer of material from all surfaces of the swab to the plate. The plate was then cross-streaked and incubated at 37 ^{0}C for 48 hours in an atmosphere of 3% CO_2 by using a candle jar. A moistened pad was kept in the jar which ensured the maintenance of moisture which was another essential requirement for growth of gonococci (Arya *et al*, 1988).

3.12 Venereal disease laboratory test (VDRL)

This is a flocculation test which utilized a purified cardiolipin antigen, which when mixed with small amounts of syphilitic serum caused flocculation which was observed microscopically or with the naked eye. VDRL test was performed in a well slide. Patient's inactivated serum was mixed with antigen suspension and rotated mechanically for four minutes on a ringed slide. The flocculation was then detected microscopically using a low power objective (x 10) (Arya *et al*, 1988).

3.13 Endocrinological Assays

Follicle stimulating Hormone (FSH), luteinising hormone (LH) and prolactin (Prl) were estimated using Immuchem ^{TM} FSH, LH and Prl-CT ^{125}I IRMA kits respectively (ICN Biomedicals, Inc) which employed an immunoradiometric assay technique to quantitate human FSH, LH and Prl in plasma sample.

Principle: Two antibodies were generated against different portions of the same antigen (FSH/LH/Prl). One antibody was bound to a solid phase support (the tube) while the other antibody was labelled with ^{125}I. Thus, antigen present, simultaneously bound both antibodies in a "bridge" or "sandwich" fashion. This

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

entire complex remained bound to the tube, while the unbound radioisotope remained in solution and was later decamed. The bound radioactivity was then quantitated using a computerised packard gamma counter. Standard curves were constructed from which unknown concentrations were calculated.

3.13.1 Follicle stimulating Hormone (FSH)

Reagents

1. Anti-FSH coated tubes
2. FSH standards - 0,2.5, 5, 10, 25, 100 containing NaN_3 and gentamicin and 200µl/ml in horse serum based matrix.
3. Wash diluent - 5x solution of phosphate buffered saline containing tween It was diluted 1 in 5.
4. Assay diluent - phosphate buffered albumin containing NaN_3 as a preservative.
5. Anti-FSH ^{125}I - It was provided in phosphate albumin diluent containing NaN_3 as preservative.

Procedure: 100µl of the standards, controls and samples were pipetted into appropriately labeled coated tubes. 200µl of assay diluent was pipetted into each tube, vortexed and incubated for 45 minutes at 37°C. The contents of the tube were decanted. The tubes were washed by pipetting 1.0ml of diluted wash solution into each tube, shaken vigorously for approximately 5 seconds and decanted. 300µl of the anti FSH ^{125}I was pipetted into each tube, vortexed and incubated for 45 minutes at 37°C. Contents of the tubes were decanted, washed

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

twice as indicated above and emptied tubes were counted in a computerised gamma counter calibrated for ^{125}I (Packard Instrument, Indianapolis).

Calculations:

1. Counts of duplicates were averaged.
2. A standard curve was constructed by plotting the average cpm on (y-axis) versus the concentration of the FSH standards (x-axis) (Fig 4)
3. FSH concentrations were determined for each patient sample using the standard curve.

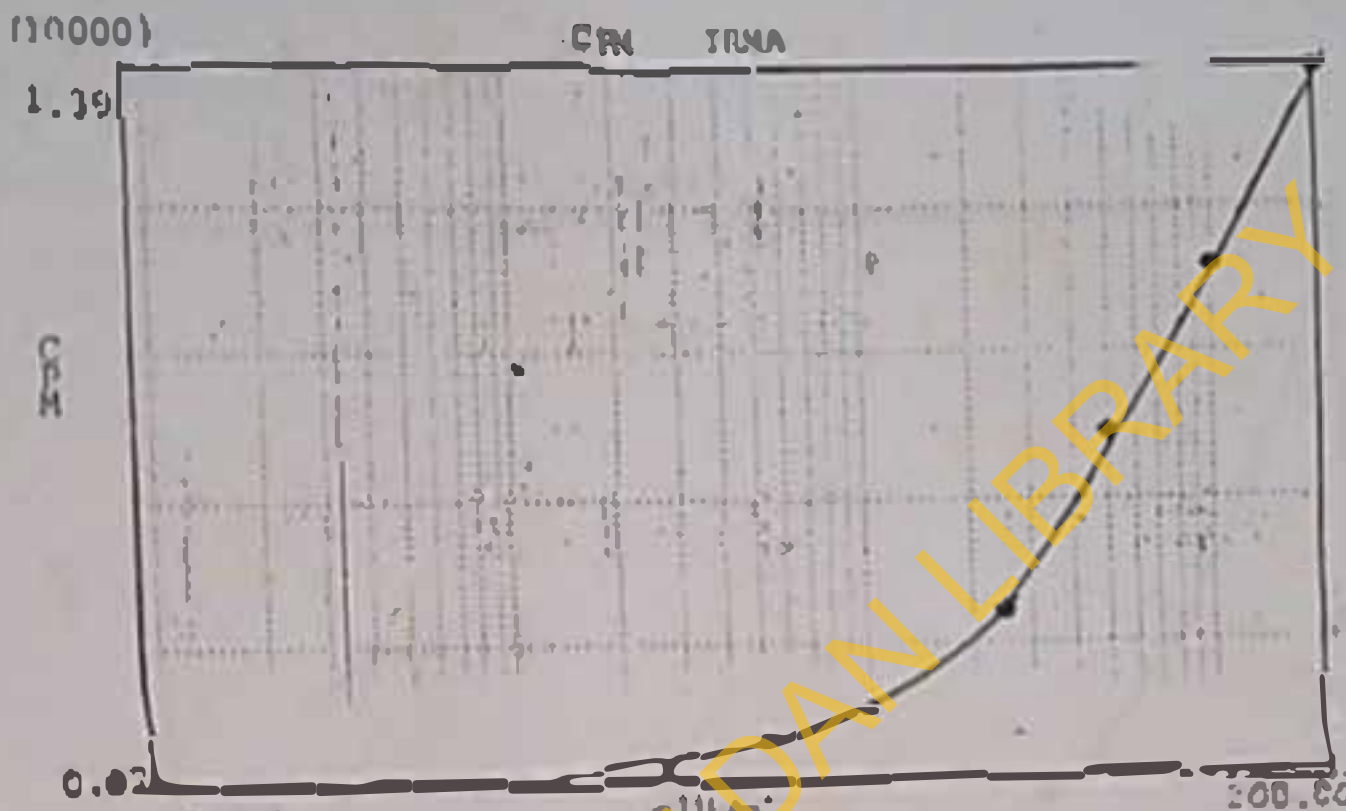
3.13.2 Luteinizing hormone (LH)

Reagents, procedure and calculations are same as for FSH. Fig 5 shows standard curve of LH assay.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

ASSAY TYPE: RIA



STD #	CPM	DEFINED DOSE	CALC. DOSE
1	DET LIM	0.08824	0.08824
2	419	2.50000	2.49734
3	979	5.00000	5.59512
4	1618	10.00000	10.03673
5	3591	25.00000	24.41446
6	6874	50.00000	54.10021
7	10035	100.00000	100.49693
8	13917	200.00000	206.59542

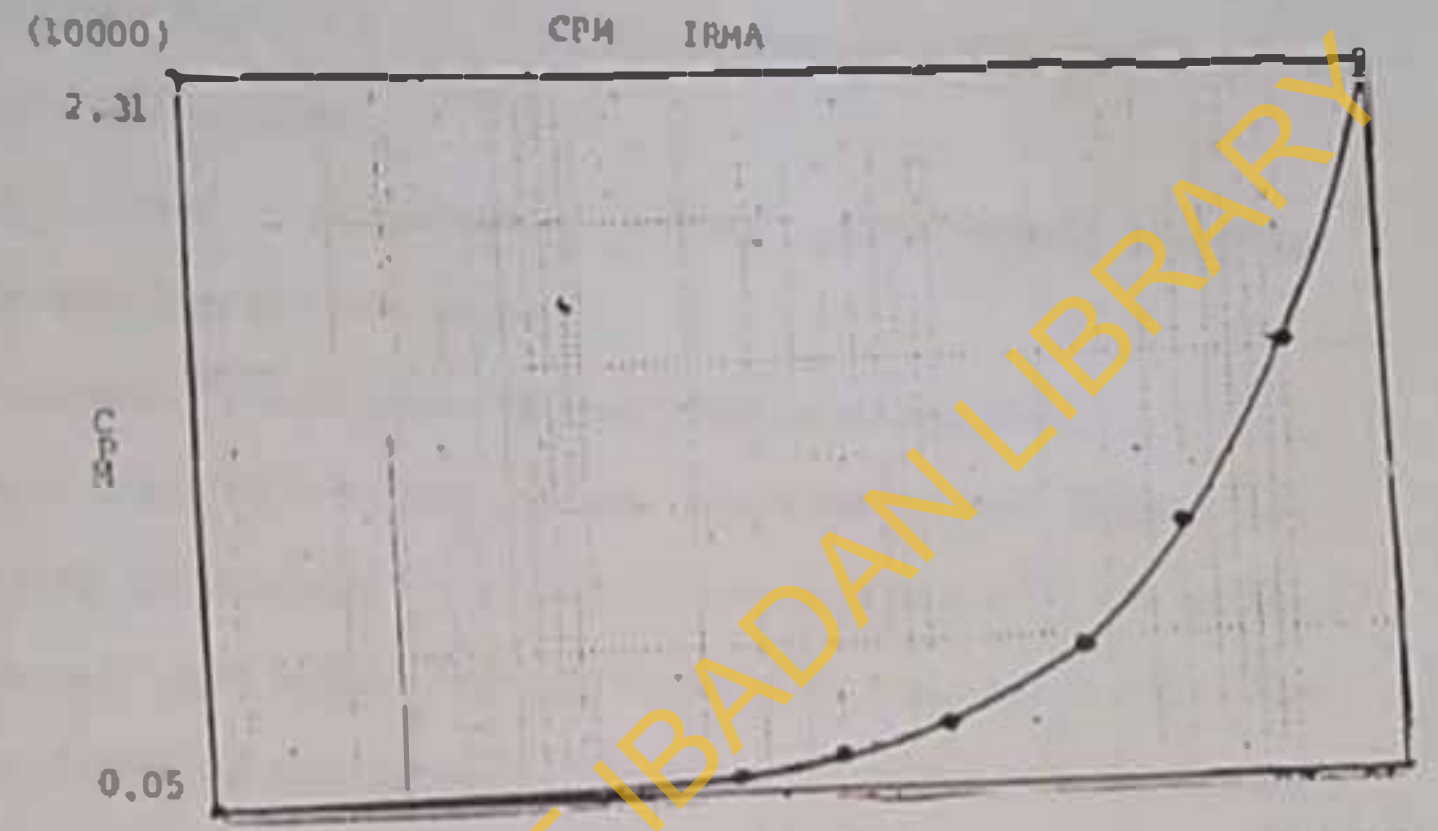
Fig 4: Standard curve of FSH assay

UNIVERSITY OF IBADAN LIBRARY

IBADAN UNIVERSITY LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

ASSAY TYPE:RIA



SID #	CPM	DETERMINED DOSE	CALC. DOSE
1	DET LIM	0.08146	0.08146
2	950	2.50000	2.49986
3	1602	5.00000	5.00077
4	2468	10.00000	10.00000
5	4767	25.00000	24.79875
6	8568	50.00000	50.71302
7	14321	100.00000	90.40372
8	23105	200.00000	200.34189

Fig 5: Standard curve of LH assay.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

3.13.3 Prolactin (PRL)

Reagents

1. Anti-human prolactin coated tubes
2. Prolactin standards - 0, 2.5, 5, 10, 25, 50 and 100ng/ml in horse serum based matrix containing NaN_3
3. Wash diluent - A phosphate buffered saline diluent containing polyoxyethylene sorbitan monolaurate
4. Anti-prolatin ^{125}I in phosphate - albumin diluent containing NaN_3

Procedure 25µl of standards, controls and samples were pipetted into appropriately labeled coated tubes 200µl of the anti-prolactin ^{125}I was put into each tube and vortex mixed This was incubated for two hours at 37°C The contents of the tubes were decanted The tubes were washed twice by pipetting 1.0ml of wash diluent into each tube The test tube rack was shaken vigorously for approximately 5 seconds Wash solution was emptied from tubes by decanting The emptied tubes were counted in a gamma counter

Calculation - same as for FSH Fig 6 shows standard curve of prolactin assay

3.14 Testosterone (T)

Testosterone was estimated using ^{125}I RIA kit with Immu Chem™ double antibody technique (ICN Biochemicals, Inc)

Principle Radioimmunoassay depends on the ability of an antibody to bind its antigen - testosterone To quantitate testosterone, the radioactive and non-radioactive form of the antigen compete for binding sites on a specific

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

antibody -anti testosterone As more non radioactive testosterone was added, less radioactive antigen remained bound, until equilibrium between the free and antibody bound antigen occurs.

In the assay, a limited amount of specific antibody-antitestosterone (anti-T) was reacted with the corresponding hormone (*T) labeled with ^{125}I Upon addition of an increasing amount of the hormone (T), a correspondingly decreasing fraction of *T added was bound to the antibody After separation of the bound from the free T by centrifuging and decanting, the amount of radioactivity was evaluated and used to construct a standard curve against which the unknown samples were measured.

Reagents:

a) Anti-testosterone - 19-carboxylmethylether-BSA was used as the antigen to generate antiserum in rabbits

b) Teststerone standards - 0.1, 0.25, 0.5, 1.0 2.5, 5, 10ng/ml were prepared in human serum matrix and contain NaN_3 and gentamycin sulphate

c) Testosterone ^{125}I

d) Steroid diluent - Phosphosaline gelating buffer (pH 7.0± 0.1) containing rabbit gamma globulin

e) Sex hormone binding globulin inhibitor (SBGI) solution

f) Precipitating antiserum (2nd antibody)

Procedure 500µl of diluent buffer was added to tube numbers 1 and 2. 50µl of the 0.0ng/ml standard (testosterone free serum) was added to the non-specific binding tube (blank) NSB and total binding tube (Bo) 50µl of each testosterone standard (0.1ng/ml - 10) was added to their respective tubes and patients plasma

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

antibody -anti testosterone. As more non radioactive testosterone was added, less radioactive antigen remained bound, until equilibrium between the free and antibody bound antigen occurs.

In the assay, a limited amount of specific antibody-antitestosterone (anti-T) was reacted with the corresponding hormone (*T) labeled with ^{125}I. Upon addition of an increasing amount of the hormone (T), a correspondingly decreasing fraction of *T added was bound to the antibody. After separation of the bound from the free T by centrifuging and decanting, the amount of radioactivity was evaluated and used to construct a standard curve against which the unknown samples were measured

Reagents:

a) Anti-testosterone - 19-carboxylmethylether-BSA was used as the antigen to generate antiserum in rabbits.

b) Testisterone standards - 0.1, 0.25, 0.5, 1.0 2.5, 5, 10ng/ml were prepared in human serum matrix and contain NaN_3 and gentamycin sulphate

c) Testosterone ^{125}I

d) Steroid diluent - Phosphosaline gelating buffer (pH 7.0± 0.1) containing rabbit gamma globulin

e) Sex hormone binding globulin inhibitor (SBGI) solution

f) Precipitating antiserum (2nd antibody)

Procedure 500µl of diluent buffer was added to tube numbers 1 and 2. 50µl of the 0.0ng/ml standard (testosterone free serum) was added to the non-specific binding tube (blank) NSB and total binding tube (Bo). 50µl of each testosterone standard (0.1ng/ml - 10) was added to their respective tubes and patients plasma

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

were added to their respective labelled tubes. 100µl of SBGI solution was added to all the assay tubes and mixed by shaking the test tube rack for 10 seconds. 500µl of testosterone ^{125}I was added to all the tube (caution was taken to add the tracer before the antiserum). 500µl of anti-testosterone was added to all the tubes with the exception of NSB and Bo tubes. All tubes were vortex mixed and incubated at 37°C for 120 minutes. After 120 minute incubation, 100µl of second antibody was added to all the tubes. They were then, vortex mixed and incubated at 37°C for 60 minutes. After 60 minutes incubation, all assay tubes were centrifuged at 2500 rpm for 15 minutes. The supernatant was decanted (bloting the rim of the test tubes on absorbent paper before turning right side up. The precipitate was counted in a gamma counter.

Calculations

a) The counts of all duplicates were averaged. Average NSB counts were subtracted from averages obtained above. This yielded the corrected counts. The corrected counts were divided by the corrected Bo count to obtain the percent bound (B/Bo).

b) **Formula**

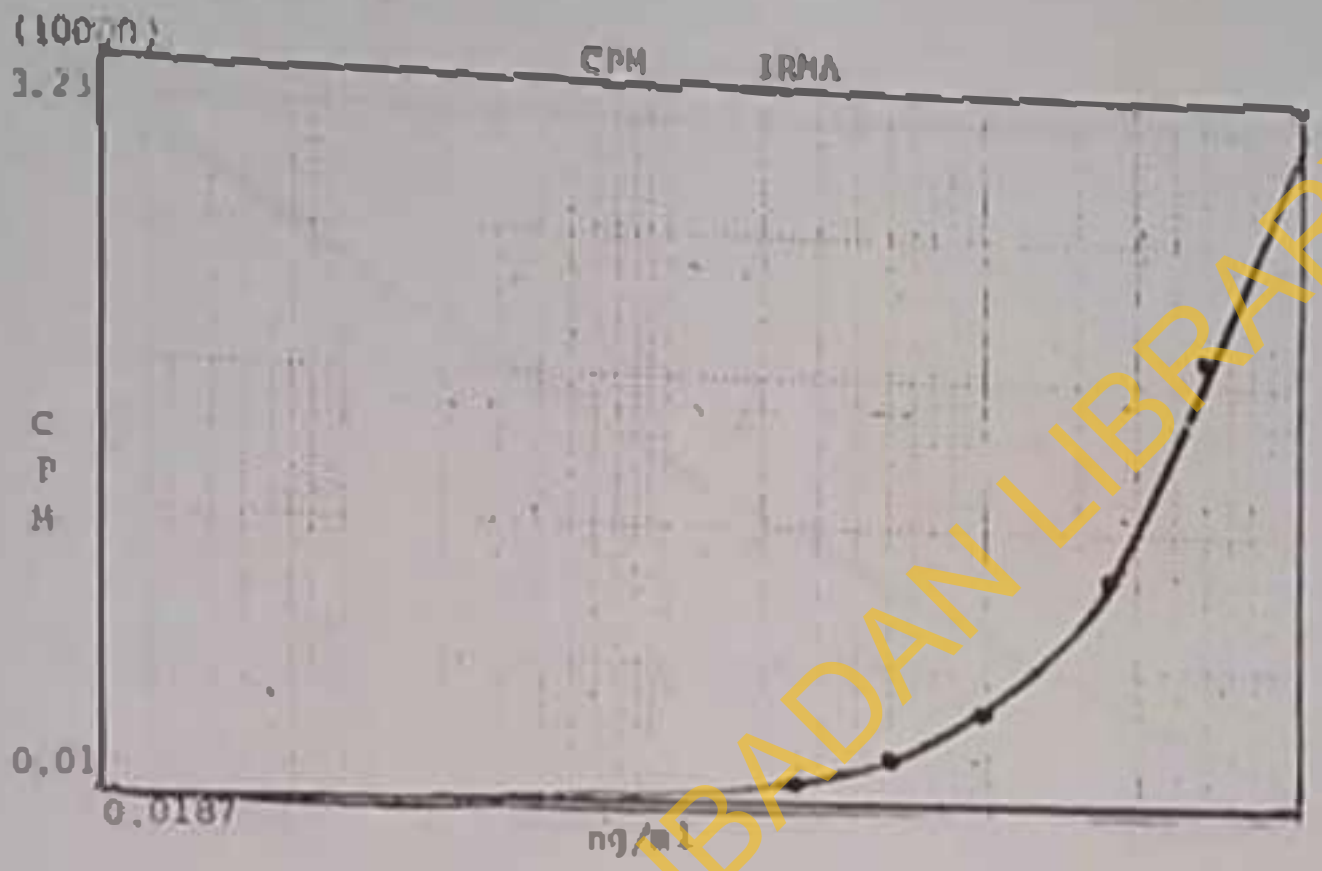
$$\%B/Bo = \frac{\overline{CPM}(sample) - \overline{CPM}(NSB)}{\overline{CPM}(0ng/ml) - \overline{CPM}(NSB)} \times 100$$

A standard curve was constructed with %B/Bo along the Y-axis and testosterone concentration (ng/ml) along the X-axis as shown on Fig. 7. Sample readings were extrapolated from it in packard computerised counter.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

ASSAY TYPE: RIA



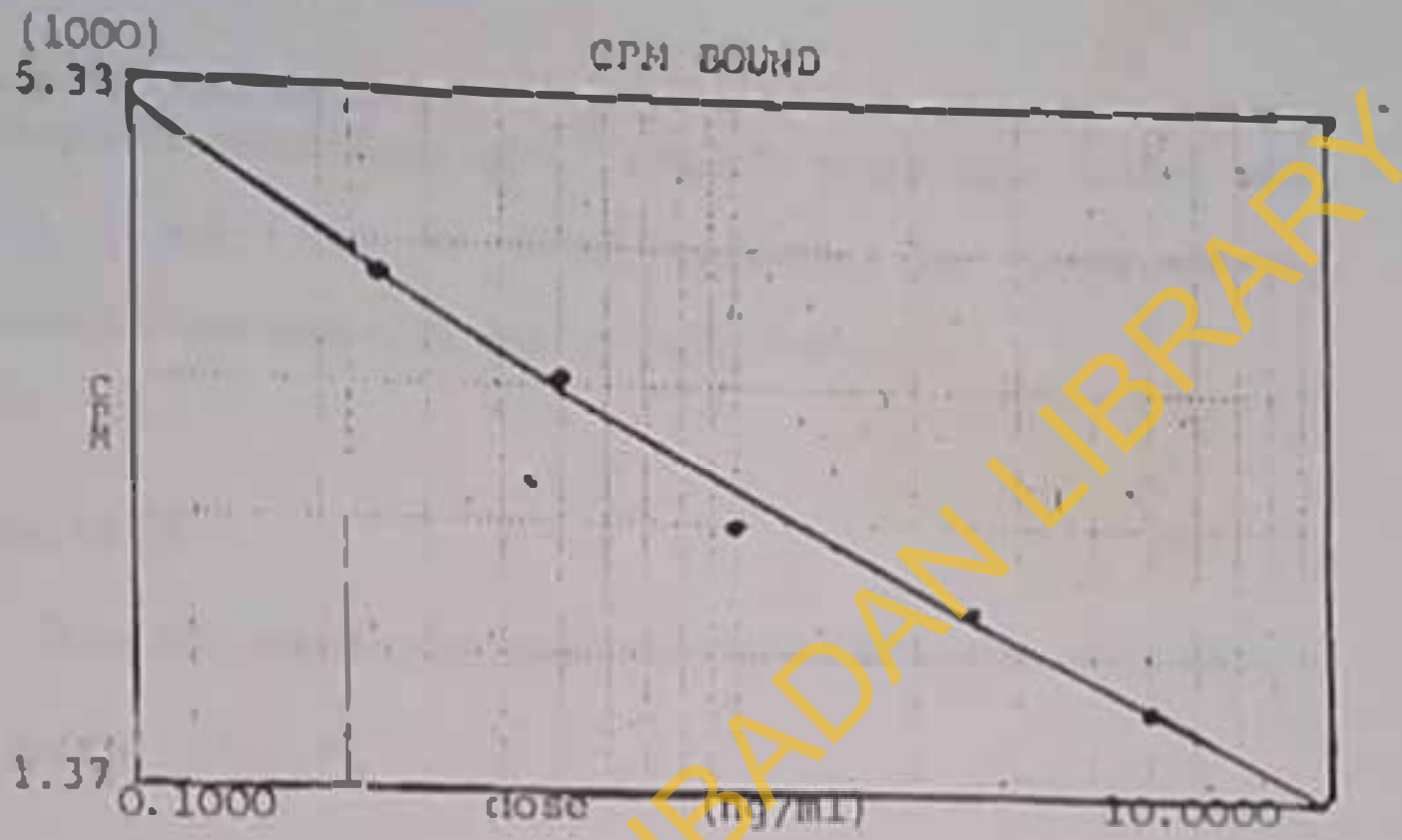
SID #	CPM	DEFINED DOSE	CALC. DOSE
1	DET LIM	0.01871	0.01871
2	1234	2.50000	2.50019
3	2399	5.00000	5.00016
4	4512	10.00000	10.01166
5	10930	25.00000	24.01539
6	20706	50.00000	52.16534
7	32290	100.00000	115.90227

Fig. 6 Standard curve of Prolactin assay

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

ASSAY TYPE:RIA



STD #	CPM	DEFINED DOSE	CALC. DOSE
1	6331	0.10000	0.09007
2	5137	0.25000	0.24708
3	4414	0.50000	0.46252
4	3342	1.00000	1.24353
5	2730	2.50000	2.29285
6	1984	5.00000	5.00211
7	1373	10.00000	9.60486

Fig 7 Standard curve of Testosterone assay

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

3.13.5 Hormonal Assay Validation

1. Intra-assay and Inter-assay variations for samples with different concentrations A,B,C are tabulated for FSH, LH, Prl and T on Table 6

3.13.6 Quality Control (QC)

Commercial control-Lyphochek ® (BioRad) immunoassay control serum levels 1, 2 and 3 (lot No 40040) representing low, normal and high concentrations were used in the assay as quality controls

% Error of QC

Three different QCs were used for the various hormonal assays – QC 1, QC 2 and QC 3 (Table 7).

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Table 6

Intra-assay and inter-assay variations of Hormonal Assays

Hormone		Samples	Mean	S E	%C V
FSH (mIU/ml)	Intra-assay n = 10	A	13.1	0.1	2.6
		B	35.0	0.4	4.0
		C	68.6	1.2	5.4
	Inter-assay n = 10	A	13.7	0.3	8.0
		B	37.1	0.7	5.9
		C	72.4	1.0	4.6
LH (mIU/ml)	Intra-assay n = 10	A	4.1	0.2	11.7
		B	28.8	0.2	2.4
		C	69.6	0.8	3.6
	Inter-assay n = 10	A	8.4	0.2	8.6
		B	26.2	0.6	7.3
		C	75.2	1.9	7.8
Prl (ng/ml)	Intra-assay n = 12	A	11.4	0.2	7.0
		B	16.8	0.2	4.8
		C	27.1	0.3	1.8
	Inter-assay n = 36	A	12.3	0.2	8.9
		B	17.1	0.2	8.2
		C	27.0	0.4	7.8
T (ng/ml)	Intra-assay n = 10	A	0.348	0.0	6.0
		B	2.83	0.0	4.6
		C	8.22	0.2	9.1
	Inter-assay n = 20	A	0.425	0.0	12.7
		B	3.85	0.1	7.5

FSH = Follicle stimulating, LH = Luteinising hormone, Prl = Prolactin, T = Testosterone, A, B, C = low, medium and high concentrations (respectively) of quality control samples, S E = Standard error, CV = Coefficient of variation

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Table 7

% Error of Quality control sera – Lyphotech 1, 2, 3

	QC sample	Assay Value	MA Value	% Error
FSH	1	17.7	17.5	1.14
	2	24.9	25.7	3.1
	3	69.8	78	10.5
LH	1	4.95	4.9	1.02
	2	32.98	31	6.4
	3	95.8	81	10.3
Prl	1	18.33	16.0	14.6
	2	28.93	27	7.15
T	1	0.71	0.76	6.6
	2	6.12	5.9	3.7
	3	79	7.4	6.8

FSH = Follicle stimulating; LH = Luteinising hormone; Prl = Prolactin; T = Testosterone; QC = Quality control sample. MA value= Manufacturer's assigned value

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

3.14. Diagnosis of STDs

All STDs diagnosis were made first on clinical grounds by a consultant microbiologist at the STD clinic, UCH, Ibadan and confirmed by laboratory tests except for lymphogranuloma venereum, genital wart, herpes genitalis, genital ulcer and tinea cruris which were diagnosed on clinical grounds only. Gonococcal urethritis was diagnosed if gram-negative intra-cellular diplococci were present in gram-stained urethral swab and/or culture. Non-specific urethritis was diagnosed if gram-stained urethral swab showed > 5 WBC or urine sediment showed > 15 WBC and gram-negative diplococci wee absent by microscopy and/or culture (Aryn *et al.*, 1988; Ekweozor *et al.*, 1995).

3.15. Statistical Analysis:

Statistical analysis was carried out by means of computer statistical software – Epi-info 6.02 and Stat-Pac 5.0. Student's t-test (pair and unpaired) and analysis of variance (anova) – one way were used for comparison of means. Chi squared test (X^2) for contingency tables was used to test association between two qualitative variables.

Student's t-test was used for statistically comparing means of

1) BMI between normospermic and dyspermic infertile males

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

2) Seminal characteristics between normospermic and dyspermic infertile males

3) Seminal characteristics between infertile men and fertile controls

4) Seminal Characteristics between STD subjects and fertile controls

5) Mean seminal plasma zinc levels in normospermic and dyspermic infertile men

6) FSH, LH, PRL and T between normospermic and dyspermic infertile males

7) Mean percentage binding of spermatozoal (X) sperm antibodies between normospermic and dyspermic infertile men

8) Mean percentage binding of seminal plasma (Y) sperm antibodies between normospermic and dyspermic infertile men

9) Mean percentage binding of blood plasma (Z) sperm antibodies between normospermic and dyspermic infertile men

Analysis of variance (anova) – one way was used for statistical comparison of means of

1) BMI of fertile, infertile and STD subjects

2) Semen characteristics between fertile, infertile and STD subjects

3) Seminal plasma zinc levels between fertile, infertile and STD subjects

4) FSH, LH, PRL, T between fertile, infertile and STD subjects

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

5) Mean percentage binding of spermatozoal (X) sperm antibodies between fertile , infertile and STD subjects

6) Mean percentage binding of seminal plasma (Y) sperm antibodies between fertile infertile and STD subjects

7) Mean percentage binding of blood plasma (Z) sperm antibodies between fertile, infertile and STD subjects

Chi-square test (X^2) for contingency tables was used to test statistical association of

1) Chlamydia IgG antibody and past history of STD between fertile, infertile and STD subjects

2) Chlamydia IgG antibody and past history of STD within the infertile group

3) Subjects with past STD and subjects with Chlamydia IgG antibodies in fertile, infertile and STD groups

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

CHAPTER FOUR

RESULTS

The mean (S E) ages of fertile men, infertile men and men with STDs were 35 3 (0 7,) 33 2 (0.6) and 28 .4 (0.7) yrs respectively.

4 1 Anthropometric Measurements.

The mean (S E) weights of subjects in fertile, infertile and STDs groups are 61 3 (1 0) kg, 68.0(1.7)kg and 65 1(2 0) 0kg respectively while their mean (S E) heights were 1 7 (0.01)m,1 7(0.02)m and 1 7 (0.02)m respectively. The mean (S E) BMI of the fertile group was 20.9 (0.3) kg/m^2, infertile group was 22 7 (0.5) kg/m^2 while men with STDs had mean (S E) BMI of 22 3 (0.7)kg/m^2 (Table 8). Men in the infertile group had significantly higher BMI compared to fertile controls ((t = 3 128, p = 0 03). Comparison of normospermic infertile men with dyspermic infertile men showed significantly higher BMI in the later than former (t = 2 754, p = 0 009). Similarly, men with STDs had higher BMI than fertile controls that was significant (t = 1 917, p = 0 05)

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Table 8

Mean (±S.E) anthropometric measurement in fertile, infertile and STDs subjects

	Groups		
	A	B	C
n	82	44	16
Weight (kg)	61.3 ± 1.0	68.0 ± 1.68	65.1 ± 2.0
Height (m)	1.7 ± 0.01	1.7 ± 0.02	1.7 ± 0.15
Body Mass Index (kg/m)	20.9 ± 0.03	22.7 ± 0.5	22.3 ± 0.7

A = fertile men ; B = infertile men, C = STDs subjects, values in mean ± standard error (S.E); n = number of subjects.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

4.2 Biophysical and Biochemical Analysis of Semen

Sperm count of men in the various groups - fertile, infertile and the STDs groups are shown on Table 9. All subjects in the fertile group were normospermic. In the infertile group, about a third (30%) of subjects were oligospermic, 26% were normospermic, 14% were hypospermic 20% were azoospemic while the last 10% had varying other forms of dyspermia. 73.4% of oligospermic infertile men had problems with sperm motility and /or morphology (Table 10).

35.4% of STDs subjects were hypospermic and only 23.6% were normospermic. The remaining 41% had various other forms of dyspermia (Table 9).

Oligospermia was most frequently observed in infertile subjects this study while hypospermia was observed most frequently among STDs subjects. More than two thirds of STDs subjects (76.4%) were dyspermic.

Seminal biophysical indices of men in fertile, infertile and STDs groups are tabulated on Table 11 while similar indices in normospermic and dyspermic infertile men are shown on Table 16. No significant differences ($p > 0.05$) were observed in seminal volume, white blood cells and pus cells between infertile men and fertile controls (Table 12). Similar observations were made between normospermic and dyspermic infertile men ($p > 0.05$) (Table 17). Seminal volume,

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

white blood cells and pus cells were also not significantly different between STDs subjects and their fertile controls ($p>0.05$) (Table12). However, sperm count, percentage with normal morphology and percentage with normal motility were significantly lower in infertile men than fertile controls ($p = 0.000$) (Table 13). Significant differences in the seminal indices were also observed between normospermic and dyspermic infertile men. Normospermic infertile men had significantly higher sperm count, percentage with normal motility and percentage with normal morphology than infertile subjects with dyspermia ($p<0.001$) (Table 17). Contrary to observations in seminal indices between fertile and infertile men, percentage with normal motility was the only index that differed significantly between men with STDs and fertile controls – the later being higher than the former ($p = 0.005$) (Table 14).

There were no significant differences between the mean values of zinc in fertile, infertile and STDs males ($p>0.05$) (Table 22). Within the group of infertile men, there

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Table 9

Classification of fertile, infertile and STDs subjects based on their seminal characteristics

Sperm Count	Groups		
	A	B	C
N	85	50	17
Normospermia	85 (100)	13 (26)	4(23.6)
Azoospermia		10 (20)	1 (5.9)
Oligospermia		15 (30)	1 (5.9)
Asthenozoopermia		1 (2)	1 (5.9)
Asthenoteratozoospermia		1 (2)	
Incomplete liquefaction		2 (4)	1 (5.9)
Hypospermia		7 (14)	6 (35.4)
Hyperspermia		1 (2)	1 (5.9)
Abnormal appearance			1 (5.9)
Aspermia			1 (5.9)

A = fertile men, B = infertile men, C = STDs subjects, values are in proportion, n = number of subjects, percentages in parentheses

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Table 10

Sub-classification of oligospermic subjects in infertile men, based on their seminal characteristics

Sperm Count N	B group 15
Hypospermia (H)	3 (20)
Asthenozoopermia (S)	1 (6.7)
Asthenoteratozoospermia (ST)	4 (26.7)
Teratozoopermia (T)	1 (6.7)
H and T	1 (6.7)
H and ST	4 (26.7)
Hyperspermia	1 (6.7)

B = infertile men, values are in proportions with percentages in parentheses; n = number of subjects.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Table 11

Biophysical analysis of semen in fertile, infertile and STDs groups

	Groups		
	A	B	C
N	85	50	16
Appearance			
Normal	85 (100)	46 (92.0)	13 (81.3)
Abnormal	0 (0)	4 (8.0)	3 (18.8)
Liquefaction			
Normal	85 (100)	42 (84.0)	14 (87.5)
Abnormal	0 (0)	8 (16.0)	2 (12.5)
Consistency			
Normal	85 (100)	42 (84.0)	14 (87.5)
Abnormal	0 (0)	8 (16.0)	2 (12.5)
Volume (mls)	3.0 ± 0.1*	2.6 ± 0.3*	2.4 ± 0.5*
% Normal Morphology	78.3 ± 1.11*	48.4 ± 5.0*	75.3 ± 5.6*
Sperm count (10^6 /ml)	67.9 ± 21*	37.7 ± 58*	76.5 ± 13.4*
% Normal Motility	79.3 ± 1.4*	41.5 ± 47*	67.2 ± 6.6*
White blood cells /field			
0	39 (45.9)	26 (52)	5 (31.3)
1	16 (18.8)	13 (26)	3 (18.8)
2	29 (34.1)	11 (22)	6 (37.5)
3	1 (1.2)	0 (0)	0 (0)
4	0 (0)	0 (0)	2 (12.5)
Pus cells /field			
0	40 (47.1)	21 (42)	7 (43.8)
1	15 (17.6)	15 (30)	3 (18.8)
2	28 (32.9)	9 (18)	2 (12.5)
3	2 (2.4)	2 (4)	4 (25.0)
4	0 (0)	1 (2)	0 (0)

A = fertile, B infertile, C = STDs, values are in proportions with percentages in parentheses, n = number of subjects, * = mean ± standard error

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Table 12

Statistical comparison of characteristics (using ANOVA) between fertile, infertile and STDs groups.

	A n = 85	B n = 50	C n = 16	F	p
Volume (mls)	3.0(0.1)	2.6(0.3)	2.4(0.5)	1.848	0.160
Sperm count(10^6/ml)	67.9(2.1)	37.7(5.8)	76.5(13.4)	16.108	0.0001+
% Normal Morphology	78.3(1.11)	48.4(5.0)	75.3(5.6)	26.799	0.0001+
% Normal Motility	79.3(1.4)	41.5(4.7)	67.2(6.6)	48.340	0.0000+
WBC				4.609	0.1
0	39(45.9)*	26(52)*	5(31.3)*		
1	16(18.8)*	13(26)*	3(18.8)*		
2	29(34.1)*	11(22)*	6(37.5)*		
3	1(1.2)*	0(0)*	0(0)*		
4	0(0)*	0(0)*	2(12.5)*		
Pus Cell				0.602	0.74
0	40(47.1)*	21(42)*	7(43.8)*		
1	15(17.6)*	15(30)*	3(18.8)*		
2	28(32.9)*	9(18)*	2(12.5)*		
3	2(2.4)*	2(4)*	4(25.0)*		
4	0(0)*	1(2)*	0(0)*		

A= fertile group, B=infertile group, C=STDs group, values are in mean with standard error in parentheses, n = number of subjects, * = proportions in percentages parentheses, + = Significant, p = probability

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Table 13

Statistical comparison of seminal characteristics between fertile and infertile groups (using student's t-test).

	A n = 85	B n = 50	t	p
Sperm count (10^6/ml)	67.9(2.1)	37.7(5.8)	5.775	0.000+
% Normal Morphology	78.3(1.11)	48.4(5.0)	7.173	0.000001+
% Normal Motility	79.3(1.4)	41.5(4.7)	10.060	0.00000+

A = fertile group, B = infertile group, values in mean with standard error in parentheses, n = number of subjects, + = Significant, p = probability

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Table 14

Statistical comparison of seminal characteristics between fertile controls and STDs subjects using (student's t-test).

	A n = 85	C n = 16	t	p
Sperm Count (10^6/ml)	67.9(2.1)	76.5(13.4)	1.1637	0.246
% Normal Morphology	78.3(1.11)	75.3(5.6)	0.839	0.59
% Normal Motility	79.3(1.4)	67.2(6.6)	2.888	0.005+

A = fertile group, C = STDs group, values in mean with standard error in parentheses, n = number of subjects, + = significant, p = probability

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Table 15

Statistical comparison of mean seminal plasma zinc levels in fertile, infertile and STD subjects using ANOVA

	Groups				
	A	B	C	F	p
Zinc(μ g/ml) N	25	45	15		
	128.3 ±15.0	133.9 ± 11.0	134.0 ± 18.0	0.046	0.97

A= fertile men, B = infertile men, C = men with STD, values given as mean ± standard error, n = number of subjects, p = probability

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Table 16

Biophysical analysis of semen in infertile subjects

	BN	BA
N	13	37
Appearance		
Normal	13 (100)	33 (89.2)
Abnormal	0 (0)	4 (10.8)
Liquefaction		
Normal	13 (100)	29 (78.4)
Abnormal	0 (0)	8 (21.6)
Consistency		
Normal	13 (100)	29 (78.4)
Abnormal	0 (0)	8 (21.6)
Volume (mls)	3.3 ± 0.25	2.3 ± 0.3*
% Normal Morphology	81.2 ± 3.0*	36.9 ± 5.5*
Sperm count(10^6/ml)	66.7 ± 5.32*	27.5 ± 6.8*
% Normal Motility	71.9 ± 6.1*	30.8 ± 4.8*
White blood cells/field		
0	7 (53.8)	19 (51.4)
1	4 (30.8)	9 (24.3)
2	2 (15.4)	9 (24.3)
Pus cells/ field		
0	7 (53.8)	14 (40.0)
1	5 (38.5)	10 (28.6)
2	1 (7.7)	8 (22.9)
3	0 (0)	2 (5.7)
4	0 (0)	1 (2.9)

BN = Normospermic males, BA = dyspermic males, values are in proportions with percentages in parentheses, n = number of subjects, * = mean ± standard error

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Table 17

Statistical comparison of seminal characteristics between normospermic and dyspermic infertile males (using student's t-test)

	BN	BA	t	p
Volume (mls)	3.3(0.25)	2.3(0.3)	1.641	0.10
Sperm count (10^6/ml)	66.7(5.32)	27.5(6.8)	3.176	0.002+
% Normal Morphology	81.2(3.0)	36.9(5.5)	4.554	0.001+
% Normal Motility	71.9(6.1)	30.8(4.8)	4.312	0.0002+
WBC			0.132	0.72
0	7(53.8)*	19(51.4)*		
1	4(30.8)*	9(24.3)*		
2	2(15.4)*	9(24.3)*		
Pus Cell			1.885	0.170
0	7(53.8)*	14(40.0)*		
1	5(38.5)*	10(28.6)*		
2	1(7.7)*	8(22.9)*		
3	0(0)*	2(5.7)*		
4	0(0)*	1(2.9)*		

BN = normospermic males, BA = dyspermic males, values are in mean with standard error in parentheses, * = proportions with percentages in parentheses, n = number of subjects, + = significant, p = probability

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Table 18

Statistical comparison of mean seminal plasma zinc levels in normospermic and dyspermic infertile men (using students t-test.)

	Group B			
Zinc (μ g/ml)	BN	BA	t	p
n	12	23		
	135.2 ± 20.0	133.4 ± 16.0	0.070	0.94

BN = normospermic infertile men, BA = dyspermic infertile men, values are in mean ± standard error, n = number of subjects, p = probability

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

was no significant difference in mean zinc values between normospermic and dyspermic infertile men ($p>0.05$) (Table 18)

4.3. Sexually Transmitted diseases.

Men in the STDs group had various sexually transmitted diseases namely, gonococcal urethritis, non-specific urethritis, herpes genitalis, genital ulcer, genital wart, candidal urethritis and tinea cruris (Table 19). Gonococcal and non-specific urethritis were the most common findings representing about two-thirds of total diagnosis made. STDs were not detected in men in fertile and infertile groups.

Chlamydia antigen was not detected in any of the individuals of the fertile, infertile and STDs groups showing absence of current chlamydia infection. However, 80% of fertile men, 13.3% of infertile men and 33.3% of men with STDs tested positive for chlamydia IgG antibody. Significant differences were observed in these groups ($p = 0.02$) (Table 20). The presence of chlamydia IgG antibody was significantly higher in fertile than infertile males ($x^2 = 5.324$, $p = 0.02$). Similarly, chlamydia IgG antibody was significantly higher in fertile controls than in men with STDs ($x^2 = 6.97$, $p=0.008$).

The incidence of past history of STDs infection in fertile subjects was 35.8%, infertile subjects was 26.7% while the incidence in the STDs group was

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

63 0% (Table 20) Past history of STDs was significantly lower in fertile men than in men with STDs ($p = 0.05$). Such a difference was not observed between infertile and fertile men ($p = 0.3$). Similarly, no significant differences were observed between normospermic and dyspermic infertile men in chlamydia IgG antibody and past history of STDs ($p>0.005$) Table 21 An association of past history of STDs with chlamydia IgG antibody was observed in subjects within each of the groups tested ($p>0.05$) (Table 22)

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Table 19

Actiolological classification of men with STDs

	CS (sub-group)	C group
A	n = 17	n = 47
Gonococcal urethritis	6(35.3)	18(38.2)
Non specific urethritis	7(41.2)	13 (27.7)
Herpes genitalis	2 (11.8)	4(8.5)
Genital ulcer		4(8.5)
Genital wart	1(5.9)	5(10.6)
Candidal urethritis		1(2.1)
Tinea cruris	1(5.9)	2(4.3)

C = total men in STDs group, CS = men in STDs group from whom semen was obtained for analysis, values are in proportions with percentages in parentheses, n = number of subjects

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Table 20

Statistical association of Chlamydia IgG antibody with past history of STDs in fertile , infertile and STDs subjects (using chisquare test X^2)

	Groups				
	A	B	C	X^2	P
Chlamydia IgG antibody	n=15	n= 30	n = 18		
Positive	12 (80.0)	13 (43.3)	6 (33.3)	7.794	0.02+
Negative	3(20.0)	17(56.1)	12 (66.7)		
Past History of STDs	n = 81	n = 45	n = 46	13.911	0.0009+
Yes	29(35.8)	12(26.7)	29 (63.0)		
Nil	52 (64.2)	33 (73.3)	17 (37.0)		

A = fertile men, B = infertile men, C = men with STDs, values are in proportions with percentages in parentheses, + = significant, p = probability, n = number of subjects

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Table 21

Statistical association of Chlamydia IgG antibody with past history of STDs between normospermic and dyspermic infertile groups using (Chisquare test X^2).

	Group B			
	BN	BA	X^2	p
Chlamydia IgG antibody	n = 6	n = 24	0.295	0.58
Positive	2(33.3)	11(45.8)		
Negative	4 (66.7)	13 (54.2)		
	n = 20	n = 25	0.154	0.70
Past History of STDs	6(30.0)	6(24.0)		
Yes	14(70.0)	19(76.0)		
Nil				

Values are in proportions with percentages in parentheses. p = probability. n = number of men. BN = normospermic infertile men. BA = dyspermic infertile men

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Table 22

Statistical association of subjects with past STDs and subjects with Chlamydia antibodies in fertile, infertile and STDs groups (using Chi-squared test X^2)

Groups	Past history STDs		Chlamydia IgG antibody		X^2	p
	Pos	Neg	Pos	Neg		
A	29(35.8)	52(64.2)	12(80.0)	3(20.0)	0.250	0.62
B	12(26.7)	33(73.3)	13(43.3)	17(56.1)	0.602	0.44
C	29(63.0)	17(37.9)	6(33.3)	12(66.7)	0.5	0.47

A = fertile men, B = infertile men, C = men with STDs, p = probability, values are in proportions with percentages in parentheses, number of subjects with past history of STDs in A, B, C = 81, 45 and 46 respectively; number of subjects with chlamydia IgG antibody in A, B, C = 15, 30 and 18 respectively, Pos = positive, Neg = negative

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

4.4 Endocrinological Assays

The mean concentrations of follicle stimulating hormone (FSH), luteinising hormone (LH), prolactin (Prl), and testosterone (T) in fertile, infertile and STDs groups are shown on Table 23. There were no significant differences in the comparison of means of FSH, LH and T between fertile, infertile and STDs groups ($p>0.05$)

There was significantly lower difference in prolactin in men with STDs compared with fertile controls ($p = 0.03$). No significant difference was observed between fertile and infertile men in prolactin ($p = 0.69$). Comparisons of hormonal levels (FSH, LH, Prl and T) between infertile men with normospermia and dyspermia did not reveal any significant differences in all the hormones tested ($p>0.05$) (Table 24).

4.5 Sperm antibodies

4.5.1 Location, class and region of binding

Sperm antibodies were detected in three different locations in the human male in all groups tested – fertile, infertile and STDs groups. These locations are

(a) On the sperm cell – Spermatozoal sperm antibodies (X)

(b) In seminal plasma – Seminal plasma sperm antibodies (Y)

(c) In blood – Plasma sperm antibodies (Z)

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

IgG, IgA and IgM sperm antibodies were detected on spermatozoa, in seminal blood plasma and in plasma in all groups tested –fertile, infertile and STDs groups Comparison of binding percentage of sperm antibodies between the different classes of X in fertile, infertile and STDs groups did not reveal any difference that was significant ($p>0.05$)

Similar observations were made between IgG, IgA and IgM of Y and Z ($p>0.05$) in fertile, infertile and STDs subjects

IgG sperm antibodies were not significantly different between the three body locations –spermatozoa, seminal plasma and blood plasma ($p>0.05$) Neither were there significant differences in IgA and IgM sperm antibodies between spermatozoa, seminal plasma and blood plasma ($p>0.05$)

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Table 23

Statistical comparison of Follicle stimulating hormone, Luteinizing hormone, Prolactin and Testosterone between fertile, infertile and STDs subjects (using ANOVA).

	Groups				
	A	B	C	F	P
FSH(mIU/ml)	n=25 18.0 ± 2.3	n = 44 19.9 ±2.9	n = 42 12.1 ± 2.9	2.190	0.11
LH(MiU/ml)	n = 25 6.6 ± 0.7	n = 44 8.6 ± 0.8	n = 42 7.2 ± 0.6	1.358	0.26
Prl(ng/ml)	n = 24 20.1 ± 2.1	n = 42 21.4 ± 2.1	n = 42 15.0 ± 1.2	3.851	0.02+
T(ng/ml)	n = 25 7.3 ± 0.3	n = 39 7.0 ± 0.3	n = 30 7.2 ±0.3	0.328	0.73

A = fertile men, B = infertile men, C = men with STD, FSH = follicle stimulating hormone, LH = luteinising hormone, Prl = prolactin, T = testosterone, values are in mean ± standard error, + = significant, n = number of subjects, p = probability

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Table 24

Statistical comparison of Follicle stimulating hormone, Luteinising hormone, Prolactin and Testosterone between normospermic and dyspermic infertile males (using student's t-test).

	Group B			
	BN	BA	T	p
FSH (mIU/ml)	n = 12 14.7 ± 3.7	n = 32 21.9 ± 3.9	1.07	0.29
LH (mIU/ml)	n = 12 6.7 ± 0.9	n = 32 9.4 ± 1.0	1.561	0.12
Pri (ng/ml)	n = 12 22.3 + 3.0	n = 32 21.1 ± 2.7	0.267	0.79
T (ng/ml)	n = 10 7.1 ± 0.4	n = 29 6.9 ± 0.4	0.619	0.55

BN = normospermic infertile men, BA = dyspermic infertile men, FSH = follicle stimulating hormone, LH = luteinising hormone, Pri = prolactin, T = testosterone, values are in mean ± standard error, n = number of subjects, p = probability

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Differences in region of binding of sperm antibodies were observed in this study Head, tail and tail tip binding of X, Y and Z were observed in all groups – fertile, infertile and STDs groups However, differences exist in their preference for regions on the sperm cell X preferentially bound to tail, Y to head and tail while Z preferentially bound to tail and tail tip regions

4.5.2 Spermatozoal Sperm Antibodies (X)

Mean percentage binding of IgG, IgA and IgM X were low ranging from 2 1% -4 1% for IgG, 1 1% - 2 4% for IgA and 1 2% - 3 0% for IgM in all groups tested (Table 25)

Comparison of mean percentage binding of X-IgG, IgA and IgM are shown on Table 25 No significant differences were observed in IgG X between fertile, infertile and STDs groups ($p > 0.05$) Similar observations were made in comparisons between fertile, infertile and STDs subjects in X- IgA and IgM

Comparisons between normospermic and dyspermic infertile males did not reveal significant differences in X – IgG, IgA and IgM ($p > 0.05$)

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

The incidences of men with X vary between the different groups studied. Incidence of fertile men with IgG was 6.3%, infertile men was 5.6% while the incidence of STDs subjects with IgG was 14.3%.

The incidence of men with IgA X was 4.4% in fertile. 5.9% in infertile and 0% in STDs groups. The incidences of men with IgM X were 6.1% in fertile and 0% in infertile and 0% in STDs groups.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Table 25

Percentage binding of sperm antibodies – IgG, IgA and IgM to spermatozoa in fertile, infertile and STDs groups.

	Groups			
	A	BN	BA	CS
IgG	n = 48 2.1 ± 0.7	n = 8 3.0 ± 1.1	n = 10 2.6 ± 1.0	n = 7 4.1 ± 3.5
IgA	n = 45 1.5 ± 0.5	n = 8 1.1 ± 0.6	n = 9 1.6 ± 1.3	n = 5 2.4 ± 1.7
IgM	n = 49 1.4 ± 0.5	n = 8 2.4 ± 0.7	n = 8 3.0 ± 1.0	n = 5 1.2 ± 0.7

A = fertile men, BN = normospermic infertile men,
BA = dyspermic infertile men, CS = men with STDs in whom seminal analysis was done, values in mean ± standard error, n = number of subjects

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Table 26

Incidence of men with spermatozoal sperm antibodies – IgG, IgA and IgM in fertile, infertile and STDs groups.

	Groups			
	A	BN	BA	CS
IgG	n = 18 3(6.3)	n = 8 0 (0)	n = 10 1(10)	n = 7 1(14.3)
IgA	n = 45 2(4.4)	n = 8 0 (0)	n = 9 1 (11.1)	n = 5 0(0)
IgM	n = 49 3(6.1)	n = 8 0 (0)	n = 8 0 (0)	n = 5 0 (0)

A = fertile men; BN = normospermic infertile men, BA = dyspermic infertile men; CS = men with STDs in whom seminal analysis was performed; values are in proportions with percentages in parentheses.
n = number of subjects

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Incidences of men with spermatozoal sperm antibodies – IgG, IgA and IgM were each 0% in normospermic infertile group. Contrarily, 10% of dyspermic infertile men had IgG X, 11.1% had IgA X while the incidence of men with IgM X was zero (Table 26).

Tail binding was observed in all isotypes of X – IgG, IgA and IgM in all groups tested – fertile, infertile and STDs subjects.

4.5.3 Seminal Plasma Sperm Antibodies (Y)

The mean percentage binding of IgG, IgA and IgM were also low ranging from 1.2% to 1.4% for IgG, 1.0% -2.1% for IgA, 0.8% - 1.8% for IgM in all groups tested (Table 27).

Comparison of mean percentage binding of Y- IgG, IgA and IgM are shown on Table 27. No significant differences were observed in IgG Y between fertile, infertile and STDs groups ($p > 0.05$). Similar observations in comparisons were made between fertile, infertile and STDs subjects in Y- IgA and IgM. Comparisons between normospermic and dyspermic infertile males did not reveal significant differences in Y- IgG, IgA and IgM ($p > 0.05$).

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

The incidences of men with Y vary between the different groups studied Incidence in fertile men with IgG was 3.8%, in infertile men was 5.1% while the incidence in STDs subjects with IgG was 0%. Similar variances in incidence were observed for Y- IgA and IgM. The incidence of men with IgA Y was 3.8% in fertile, 2.6% in infertile and 0% in STDs groups. The incidences of men with IgM Y were 3.8% in fertile, 2.6% in infertile and 0% in STDs groups

Incidences of men with Y- IgG, IgA and IgM were 10%, 0% and 0% respectively in normospermic infertile group. 3.4% of infertile men with dyspermia had positive IgG Y 3.4% also had positive IgA Y while the incidence of men with IgM Y was 3.4% (Table 28)

Unlike X, Y bound to different regions of sperm. However, tail and head binding by Y were more commonly observed in all groups

4.5.1 Blood Plasma Sperm Antibodies (Z)

Similar to X and Y, the mean percentage binding of IgG, IgA and IgM Z were low ranging from 1.3 % - 2.1% for IgG, 1.2% - 2.1% for IgA and 0.9% - 1.7% for IgM in all groups tested (Table 29)

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Comparison of mean percentage binding of Z - IgG, IgA and IgM are shown on Table 29. No significant differences were observed in IgG Z between fertile, infertile and STDs groups ($p > 0.05$). Similar observations were made in comparisons between fertile, infertile and STDs subjects in Z- IgA and IgM.

Comparisons between normospermic and dyspermic infertile males did not reveal significant differences in Z – IgG, IgA and IgM ($p > 0.05$).

The incidences of men with Z vary between the different groups studied. Incidence of fertile men with IgG was 3.8%, infertile men was 4.4% while the incidence of STDs subjects with IgG Z was 0%. The incidence of men with IgA positive was 0% in fertile, 0% in infertile and 5.9% in STDs groups. The incidence of men with IgM Z was 0% in fertile, 4.4% in infertile and 0% in STDs groups.

Incidences of men with IgG, IgA and IgM were 7.7%, 0% and 0% respectively in normospermic infertile group. 3.1% of infertile men with dyspermia had positive IgG Z. None had positive Z IgA while the incidence of men with IgM Z was 6.2% (Table 30).

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Binding of Z-IgG, IgA and IgM were observed on the different regions of the sperm cell – head, tail and tail tip. These antibodies bound to tail and tail tip more commonly in all groups tested - fertile, infertile and STD groups.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Table 27

Percentage binding of sperm antibodies – IgG, IgA and IgM in seminal plasma of men in fertile, infertile and STDs groups.

	Groups			
	A	BN	BA	CS
IgG	n = 26 1.2 ± 0.4	n = 10 1.4 ± 1.2	n = 29 1.4 ± 1.0	n = 14 1.1 ± 0.4
IgA	n = 26 2.1 ± 1.1	n = 10 1.0 ± 0.61	n = 29 1.8 ± 0.4	n = 14 1.0 ± 0.3
IgM	n = 26 1.2 ± 0.4	n = 10 0.8 ± 0.32	n = 29 1.8 ± 0.7	n = 14 1.1 ± 0.5

A = fertile men, BN = normospermic infertile men, BA = dyspermic infertile men, CS = men with STDs in whom semen was analysed, values are in mean ± standard error, n = number of subjects

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Table 28

Incidence of seminal plasma sperm antibodies – IgG, IgA and IgM in the different Groups of men studied.

	Groups			
	A	BN	BA	CS
IgG	n = 26 1 (3.8)	n = 10 1 (10)	n = 29 1 (3.4)	n = 14 0 (0)
IgA	n = 26 1 (3.8)	n = 10 0 (0)	n = 29 1 (3.4)	n = 14 0 (0)
IgM	n = 26 1 (3.8)	n = 10 0 (0)	n = 29 1 (3.4)	n = 14 0 (0)

A = fertile men; BN = normospermic infertile men; BA = dyspermic infertile men; CS = men with STDs in whom semen was analysed; values are in proportions with percentages in parentheses; n = number of subjects

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Table 29

Percentage binding of sperm antibodies – IgG, IgA and IgM in blood plasma in men studied.

	Groups			
	A	BN	BA	C
IgG	n = 26 1.7 ± 0.5	n = 13 1.9 ± 1.0	n = 32 2.1 ± 0.5	n = 43 1.3±0.3
IgA	n = 26 1.4 ± 0.3	n = 13 1.2 ± 0.3	n = 32 1.2 ± 0.3	n = 42 1.7 ±0.4
IgM	n = 26 0.9 ± 0.3	n = 13 1.5 ± 0.4	n = 32 1.7 ± 0.5	n = 42 1.3 ±0.3

A = fertile men; BN = normospermic infertile men, BA = dyspermic infertile men, C = men with STDs, values in mean ± standard error, n = number of subjects.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Table 30

Incidence of blood plasma-sperm antibodies – IgG, IgA and IgM in fertile, infertile and STDs groups

	Groups			
	A	BN	BA	C
IgG	n = 26 1 (3.8)	n = 13 1 (7.7)	n = 32 1 (3.1)	n = 43 0 (0)
IgA	n = 25 0 (0)	n = 13 0 (0)	n = 32 0 (0)	n = 42 2(4.8)
IgM	n = 26 0 (0)	n = 13 0 (0)	n = 32 2 (6.2)	n = 42 0 (0)

A = fertile men, BN = nonnospermic infertile men, BA = dyspermic infertile men, C = men with STDs, values in proportions with percentages in parenthesis, n = number of subjects

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

CHAPTER FIVE

DISCUSSION

Infertility is a medico-social problem that has gained pronúnence in the world over including sub-Saharan Africa (Leke *et al*., 1993). Although the high contribution of males to infertility is now widely recognised (Marinho, 1986, Ilesanmi *et al*., 1996), knowledge of male infertility is limited (Moskowitz and Mellinger, 1992). Proof of the ability of normospermic men to fertilise remains indirect (Bar-Charma and Lamb, 1994) and the choice of semen values as objectives indices of male infertility is in doubt (Adejuwon *et al*., 1996). Sperm antibodies have therefore, been implicated as a cause of infertility especially in normospermic infertile males or couples with unexplained infertility (Koksal *et al*., 1991, Skakkabaek *et al*., 1994, Hameed *et al*., 1995). Though a subject of current interest worldwide (Hameed *et al*., 1995), its role in male infertility remains controversial (Heidarreich *et al*., 1992). Ekwere in his study conducted in Calabar, Nigeria, reported a high incidence (44%) of sperm antibodies in association with STDs among infertile men in sub-Saharan Africa (Ekwere, 1995). Several investigations have earlier observed incidence of sperm antibodies in association with STDs and reduced fertility in men (Quesada *et al*, 1968, Witkin and Toth, 1983, Soffer *et al*, 1990).

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

IgG, IgA and IgM antibody isotypes have been observed as the most common sperm antibodies (Gonzales *et al.*, 1992) in serum, seminal plasma and bound to sperm (Koksal *et al.*, 1991; Turek and Lipshultz, 1994). Similarly, sperm antibodies – IgG, IgA and IgM were present on spermatozoa, in seminal plasma and blood in all groups studied-fertile, infertile and STDs groups.

There has been no consistency in immunoglobulin class of sperm antibodies. IgG was observed as the most predominant immunoglobulin class in sera and semen (Lehmann *et al.*, 1987; Broderick *et al.*, 1989). Comhaire (1993) observed that secretory IgA does occur in semen and not serum. Systemic inoculation of sperm antigen is thought to stimulate IgG production whereas local reproductive tract antigens are stimuli for IgA production, thus suggesting a primary, locally induced antibody of IgA type which may be more harmful than a secondary, systemically produced IgG antibody. Theoretically, the Fc or constant region of the IgA molecule binds receptors in the cervical mucus and impairs sperm motility. Penetration of cervical mucus by sperm becomes ineffective when sperm contacts cervical mucus resulting in the change of forward motility to a 'shaking' motion. Sperm directed antibodies may also cause sperm to autoagglutinate or clump, which equally inhibits cervical passage (Turek and Lipshultz, 1994). Other studies have observed IgA more commonly than IgM

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

(Gonzales *et al.*, 1992, Clarke *et al.*, 1985, Takahashi *et al.*, 1992) IgM sperm antibodies are large molecules and are uniformly confined to serum and only rarely found in organs or secretions of the male genital tract (Haas, 1987) Hence, IgM sperm antibody is not routinely measured in detection systems because their role in antibody mediated infertility is presumably limited (Turek and Lipshultz, 1994)

Comparison of X between the different classes – XIgG, XIgA and XIgM in this present study did not reveal any significant differences in fertile, infertile and STDs groups ($p > 0.05$)

Neither were there significant differences in the comparisons between YIgG, YIgA and YIgM nor between ZIgG, ZIgA and ZIgM ($p > 0.05$). These findings do not show a significantly higher level of binding of one antibody class over the other classes in all the body locations – spermatozoa, seminal plasma and plasma thus indicating the possibility of both systemic and local production of sperm antibodies – IgG, IgA and IgM. IgM may, like IgG and IgA have a role in responding to sperm antigenic challenge. A new approach in including the measurement of IgM to IgG and IgA in semen and serum may be worthwhile in resolving the existing conflicts of their role in infertility. Moreover either of the immunoglobulin classes can be measured in detection systems

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Many studies have detected sperm antibodies in serum probably because of the conveniences of assaying blood (Haas, 1987). However, circulating sperm antibodies were reportedly unable to properly reflect immunological causes of infertility (Ruijs *et al*., 1990). Similarly, Haas (1987) observed that sperm antibodies identified directly on a man's sperm's surface probably correlate best with the presence of antibody mediated infertility in males while seminal plasma antibodies may or may not be antibody bound (Turek and Lipshultz, 1994). These investigators argued that serum sperm antibodies cannot logistically bind to sperm unless they transudate into semen, hence they are considered clinically less important than sperm-bound antibodies. Sperm-bound antibodies are said to be the most appropriate to measure for sperm functional capacity because sperm alone reach the female reproductive tract and the sperm plasma membrane remains intact in sperm participating in in-vivo fertilization until after the acrosome reaction (Haas, 1987; Eggert-Kruse, 1990; Turek and Lipshultz, 1994). Koksal *et al*. (1991) found 70% positive sperm antibody in semen and 30% positive in blood. The higher incidence of semen sperm antibodies over serum sperm antibodies was explained in their study by the fact that sperm antibodies are derived from local production in the genital tract or from transudation from the blood stream. Moreover, only 1% of serum IgG content was found in male

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

genital secretions and sperm bound IgG can be measured in the absence of assayable serum IgG levels (Turek and Lipshultz, 1994)

Comparisons of IgG sperm antibodies between the different locations spermatozoa, seminal plasma and blood plasma did not show any significant differences in all groups ($p>0.05$) Similarly comparisons between X-IgA, Y-IgA and Z-IgA did not reveal any significant differences in all groups ($p>0.05$) Neither were there significant differences in the comparisons between X-IgM, Y-IgM and Z-IgM in all groups tested ($p>0.05$) Contrary to the observations of Koksal *et al.* (1991) of higher sperm antibodies in semen than serum, similar sperm antibody binding percentages were observed in semen and blood plasma This further confirms the local and systemic production of sperm antibodies but contrasts the more clinical relevance of semen sperm antibodies over serum sperm antibodies. A positive correlation between antibodies in blood plasma and seminal plasma has been observed (Upadyaya *et al.*, 1984) Moreover, 50% - 80% of vasectomized males (the most thoroughly studied association with sperm antibodies) have measureable levels of sperm antibodies in serum and rarely in seminal plasma (Broderick *et al.*, 1989, Turek and Lipshultz, 1994)

However, in the present study, the mean percentage binding of sperm antibodies on spermatozoa – X-IgG, X-IgA, X-IgM, in seminal plasma – Y-IgG, Y-IgA, Y-IgM and in plasma – Z-IgG, Z-IgA, Z-IgM were low with a maximum

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Many studies have detected sperm antibodies in serum probably because of the conveniences of assaying blood (Haas, 1987). However, circulating sperm antibodies were reportedly unable to properly reflect immunological causes of infertility (Ruijs *et al.*, 1990). Similarly, Haas (1987) observed that sperm antibodies identified directly on a man's sperm's surface probably correlate best with the presence of antibody mediated infertility in males while seminal plasma antibodies may or may not be antibody bound (Turek and Lipshultz, 1994). These investigators argued that serum sperm antibodies cannot logistically bind to sperm unless they transudate into semen, hence they are considered clinically less important than sperm-bound antibodies. Sperm-bound antibodies are said to be the most appropriate to measure for sperm functional capacity because sperm alone reach the female reproductive tract and the sperm plasma membrane remains intact in sperm participating in in-vivo fertilization until after the acrosome reaction (Haas, 1987, Eggert-Kruse, 1990, Turek and Lipshultz, 1994). Koksal *et al.* (1991) found 70% positive sperm antibody in semen and 30% positive in blood. The higher incidence of semen sperm antibodies over serum sperm antibodies was explained in their study by the fact that sperm antibodies are derived from local production in the genital tract or from transudation from the blood stream. Moreover, only 1% of serum IgG content was found in male

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

genital secretions and sperm bound IgG can be measured in the absence of assayable serum IgG levels (Turek and Lipshultz, 1994)

Comparisons of IgG sperm antibodies between the different locations – spermatozoa, seminal plasma and blood plasma did not show any significant differences in all groups ($p>0.05$). Similarly comparisons between X-IgA, Y-IgA and Z-IgA did not reveal any significant differences in all groups ($p>0.05$). Neither were there significant differences in the comparisons between X-IgM, Y-IgM and Z-IgM in all groups tested ($p>0.05$). Contrary to the observations of Koksal *et al.* (1991) of higher sperm antibodies in semen than serum, similar sperm antibody binding percentages were observed in semen and blood plasma. This further confirms the local and systemic production of sperm antibodies but contrasts the more clinical relevance of semen sperm antibodies over serum sperm antibodies. A positive correlation between antibodies in blood plasma and seminal plasma has been observed (Upadyaya *et al.*, 1984). Moreover, 50% - 80% of vasectomized males (the most thoroughly studied association with sperm antibodies) have measureable levels of sperm antibodies in serum and rarely in seminal plasma (Broderick *et al.*, 1989, Turek and Lipshultz, 1994).

However, in the present study, the mean percentage binding of sperm antibodies on spermatozoa – X-IgG, X-IgA, X-IgM, in seminal plasma – Y-IgG, Y-IgA, Y-IgM and in plasma – Z-IgG, Z-IgA, Z-IgM were low with a maximum

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

of 4 1% in fertile, infertile and STDs groups (Tables 24, 26, 28) Sperm antibody percentage binding of less than 60% for IgG and less than 40% for IgA were observed in all cases with proven fertility (Takahashi *et al*, 1992) Turek and Lipshultz (1994) reported that sperm bound antibody of less than 50% need not be treated since post-coital tests approximate fertile controls Francavilla *et al* (1992) also suggested that the failure of intrauterine insemination in the treatment of male immunological infertility is imputable to sperm antibodies when they involve all spermatozoa regardless of sperm quality Thus sperm antibody percentage binding of a maximum of 4 1% observed in this present study appears far too low to affect fertility in males The type of immunoglobulin present, their reactivity with different antigens and their concentrations within the reproductive tract will all determine whether immunity to sperm will alter reproduction (Bronson *et al*, 1984)

Differences exist in the region of binding of sperm antibodies Head, tail and tail tip binding of sperm antibodies have been observed (Carson *et al*, 1988, Gregoriou *et al*, 1990, Bronson *et al*, 1992, Turek and Lipshultz, 1994) Similar observation were observed in this present study Head, tail and tail tip binding of X, Y and Z were observed in all groups – fertile, infertile and STDs groups However X preferentially bound to tail, Y to head and tail while Z preferentially bound to tail and tail tip regions irrespective of immunoglobulin class and group

UNIVERSITY OF IBADAN LIBRARY

UNIVERSITY LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

These observations were contrary to findings in other studies where specific immunoglobulin classes have been associated with specific regions of binding (Shulman *et al.*, 1985; Clarke *et al.*, 1985a; Carson *et al.*, 1988; Gregoriou *et al.*, 1990). The effect of sperm antibodies on sperm function depends on the area to which the antibodies are directed (Kaufman and Nagler, 1987). Tail directed antibodies only weakly affect cervical mucus interactions (Barlow, 1988). Turek and Liphshultz (1994) suggested that tail directed sperm antibody need not be treated since it is thought that only head-directed or midpiece directed sperm antibodies are clinically relevant in immobilisation or penetration assays (Takahashi *et al.*, 1992) also observed that antibodies that are attached to the sperm head with binding rate of less than 40% were not considered to be causative factor of male infertility. Head directed sperm antibodies inversely correlated with zona adherence, are said to be more potent blocks of normal gamete interaction than are tail directed antibodies. Sperm-zona binding via specific receptors could be blocked by steric interference from attached head-directed antibodies thus affecting fertility at the level of gamete interaction (Bronson *et al.*, 1984). Antibodies to tail tip rarely have any effect on sperm function (Bronson *et al.*, 1984, Barlow, 1988). The finding of head-directed sperm antibodies in seminal plasma in this present study may be important but antibody binding rates are far two low to affect fertility. Moreover, this

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

observation was similar in all groups – fertile, infertile and STDs groups. These findings in addition to the observation of tail binding as the most prominent region of binding of sperm antibodies further support the fact that sperm antibodies may not affect fertility in males.

Clinically, antibodies to sperm are found in 3% to 12% of infertile men (Turek and Liphsultz, 1994; Skakkaback *et al.*, 1994; Jennings *et al.*, 1985). Similar variations were observed in this study. The incidences of men with X, Y and Z varied between the groups in all antibody classes with a range of 0% - 14.3% in X (Table 26), 0%-10% in Y (Table 28) and 0% - 7.7% in Z (Table 30). Comparison of the incidences of men between infertile and fertile groups or between STDs and fertile groups did not follow a definite pattern in all immunoglobulin classes on spermatozoa, in seminal plasma and plasma in this study. Similar discrepancies have been observed in other studies. Upadyaya *et al.* (1984) observed a higher incidence of sperm antibodies in infertile than fertile subjects. A significant number of patients with normal spermatogenesis was said to have antibodies that exhibited a strong binding to spermatozoa in serum and semen (Lehmann *et al.*, 1987; Koksal *et al.*, 1991). Patients with genital tract infection or sperm duct obstruction exhibited positive sperm antibodies (Mazzolli and Barrera, 1989). Contrarily, Bronson *et al.*, 1984a) reported a higher incidence

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

of sperm antibodies in fertile than infertile men. Similarly, genital tract infection was not related to sperm antibodies (Upadyaya *et al.*, 1984)

In order to elucidate this conflict, comparisons of sperm antibodies between test groups and controls were carried out in the present study. Comparison of X-IgG between infertile group and fertile controls did not show any significant difference ($p>0.05$). Neither was the difference between STDs group and fertile controls significant ($p>0.05$). Similar observations were made in X-IgA and X-IgM ($p>0.05$). Further comparisons within the infertile group – between normospermic infertile males and dyspermic infertile males also showed no significant differences in X-IgG, X-IgA and X-IgM ($p>0.05$). Similar comparison as X above in Y-IgG, Y-IgA and Y-IgM are significantly indifferent ($p>0.05$). Comparisons as X above in Z-IgG, Z-IgA and Z-IgM did not also show differences that were significant ($p>0.05$). Micil and associates (1990) confirmed the observations made in the study. Comparisons of seminal plasma sperm antibodies showed no significant difference between infertile and fertile men as well as between genitourinary tract infection and the formation of seminal sperm antibodies. No significant enhancement in serum levels of antibodies in serum was observed in the study of Meisel *et al.* (1994). Sperm concentration and viability are not associated with sperm antibody presence (Clarke *et al.*, 1985) as sperm killing by antibody-induced cytotoxicity is not important in semen

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

(Anderson and Hill, 1988). Although specific semen abnormalities may be associated with sperm antibody, no specific set of parameters can as yet fully predict antibody presence (Turek and Lipshultz, 1994). Serum and seminal plasma sperm antibodies were not related to concentration, morphology, ejaculate volume nor the seminal zinc, magnesium and fructose levels (Upadhyaya *et al*., 1984). Takahashi *et al*. (1992) also observed no significant relationship between the results of traditional semen analysis, LH, FSH, prolactin and rates of spermatozoal sperm antibody binding.

Sperm antibodies have been observed to have no relationship with STDs (Upadhyaya *et al*., 1984; Ruijs *et al*., 1990). However, Close *et al*. (1987) showed that past STDs especially the often asymptomatic chlamydial infection has been associated with a history of non-specific urethritis and with the presence of sperm antibodies in infertile men. Hypothetical mechanisms put forward to explain the correlation between past and present infection and sperm antibodies is that sub-clinical epididymitis caused by Chlamydia trachomatis could result in unilateral epididymal obstruction, subsequent phagocytosis of trapped spermatozoa and systemic exposure to sperm antigens resulting in the development of sperm antibodies. Contrary to the observations of Close *et al*. (1987), Chlamydial IgG antibody was significantly higher in fertile than infertile men ($p<0.02$) in this study (Table 27). Similarly, in the present study, chlamydial IgG antibody was

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

significantly higher in fertile men than men with STDs ($p=0.008$) suggesting that past Chlamydial infection is not significantly associated with male infertility. Neither does past Chlamydial infection relate to sperm antibody production because of the comparably low levels (≤4.1%) of sperm antibodies observed in fertile men and men with STDs. Soffer *et al.* (1990) also observed Chlamydial infection paradoxically more frequent in the apparently normal than dyspermic infertile males. Chlamydial infection was also found to have no relationship with assessory gland evaluation or sperm variables such as sperm count, motility and morphological characteristics (Ruijs *et al.*, 1990). Eggert-Kruse *et al.* (1990) also in agreement with the observations made in the present study did not reveal past Chlamydial infection as shown by Chlamydial IgG antibodies to adversely affect semen characteristics and sperm mucus interaction or to correlate with circulating or local sperm antibodies. Other studies have also not shown the relevance of Chlamydial antibody to the reduction of fertility potential (Hellstrom *et al.*, 1987, Gregoriou *et al.*, 1989).

Despite the significantly higher difference of Chlamydia IgG antibodies in fertile than infertile men, none of the subjects had current Chlamydia infection since no Chlamydia antigen was detected in any individual in fertile, infertile, and STDs groups. This suggests a lack of relationship between current and past Chlamydial infection. This view is similar to observations in other studies

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

(Eggert – Kruse *et al* 1990, Ruijs *et al.*, 1990) Although females are not included in the present study, is it thought that the main influence of C. trachomatis on male infertility is due to sexual transmission to the female and subsequent negative influence on tubal function but not on reduced sperm functional capability (Eggert-Kruse et al, 1990). A high prevalence of C. trachomatis colonisation of the tubal mucosa was observed in the groups of infertile patients studied thus demonstrating the possibility of silent Chlamydial colonisation of the tubal mucosa, in the absence of active pelvic infection which can lead to infertility because of progressive tubal damage (Marana *et al.*, 1990).

However, an association of Chlamydia IgG antibody and past history of STDs was observed in subjects in fertile, infertile and STDs groups in this study. Gregoriou *et al.*(1989) similarly observed a positive correlation between past genitourinary infection and the presence of antibodies to Chlamydia. Similarly, Close *et al.* (1987) observed an association of past Chlamydial infection and past history of non-specific urethritis. In addition, they found a relationship between STDs correlates such as high number of life time partners and Chlamydial seropositivity. These suggest that subjects with Chlamydial seropositivity might have been exposed to Chlamydial infection at an earlier date without knowing. As many as 20% of men with Chlamydial urethritis may be asymptomatic (Soffer *et al.*, 1990; Zenilman, 1992). Hence, many men with current chlamydial infection

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

may not present themselves for treatment. This is evident in the absence of Chlamydial antigen even in subjects with current STDs. This indicates that the contribution of current Chlamydial infection in patients with current STDs infection especially non-specific urethritis is minimal. Although current Chlamydial infection is absent (from the study) it appears that Chlamydial infection is prevalent in our society judging from the high incidence of Chlamydial antibodies in the subjects studied (Table 20).

Comparison of past history of STDs between infertile and fertile men in the present study did not show any significant difference ($p>0.05$, Table 20) probably indicating that past STDs without complications is not likely to cause infertility. However, it appears that past exposure to STDs is a risk factor in contacting STDs. These observations are important in informing policies and strategies aimed at STDs control and infertility management.

Sexually transmitted diseases are said to be epidemic throughout most of the world (Moskowitz and Mellinger, 1992). In Africa, STDs are highly prevalent and continue to constitute a major health problem (Ekweozor *et al.*, 1994, Ekwere, 1995). In this study, gonococcal and non-specific urethritis were the most common findings of the total diagnosis made (Table 19). Similar observations were made by other investigators in sub-Saharan African (Ekweozor *et al.*, 1993, Osoba *et al.*, 1975).

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

The significance of the role of a subclinical genital tract infection in infertility is controversial. In the majority of male infertility investigations, the patient is asymptomatic. However, STDs have been linked to infertility by clinical and epidemiological studies and studies of subjects with post infection are well documented (Moskowitz and Mellinger, 1992; Gilbaugh and Lipshultz, 1994). In sub-Saharan Africa, the role of STDs in male infertility has been demonstrated (Ekwere, 1995; Alemnji and Thomas, 1997). These workers postulate that STDs retrograde into the testicular accessory organs leading to post inflammatory obstruction possibly causing atrophy or may secrete toxins which alter the spermatozoal characteristics and this can cause infertility (Moskowitz and Mellinger, 1992; Alemnji and Thomas, 1997). Cates *et al.* (1985) observed that infection related infertility is common (35%) in Africa.

Men with STDs in the present study had poor sperm count. Only 23.6% were normospermic. Hypospermia was the most frequent cause of dyspermia affecting 35.4% of men with STDs (Table 8). The dyspermia observed in most subjects with STDs may possibly be as a result of genital tract infection. Fertility may then be affected through the impairment of semen quantity and quality. Alemnji and Thomas (1997) and Ekwere (1995) in related studies observed significant involvement of bacterial infection of the genital tract of infertile

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Nigerian subjects Reduction in semen volume can impair fertility by reducing the total sperm production of the testes Moreover, Overstreet and Katz (1987) reported that alterations in semen volume per se (independent of sperm numbers) below 1ml appear to affect fertility

Comparison between men with STDs and fertile subjects did not reveal any significant differences in semen volume, sperm count and sperm morphology ($p<0.05$). Soffer *et al.* (1990) demonstrated that infection was unrelated to accessory gland evaluation or semen quality. However, percentage motility was the only seminal index that differed significantly between men with STDs and fertile controls in this study (the later being higher than the former, $p=0.005$). It is likely that the involvement of infection of the genital tract in infertility may be through the impairment of sperm function i.e lowering the motility of sperm. Ladipo *et al.* (1978) observed that the sperm motility index is by far the most important parameter in determining semen quality and can be a strong compensating factor when sperm count is low (<20 million/ml)

Semen analysis begins the evaluation of the infertile couple and has been used routinely in infertility clinics in the world to assess the fertility of the males (Bar-Charma and Lamb, 1994). Sperm counts, the percentage of motile and normally formed sperm, and the quality of sperm motions are essential components of semen analysis (Ilesanmi *et al.*, 1996). Akande (1986) observed

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

that low concentration of sperm in semen could result in infertility while Obafunwa *et al* (1993) observed varying degrees of hypospermatogenesis in 40% of testicular biopsis of infertile men. Similarly, in the present study, oligospermia was most frequently demonstrated in infertile males representing 30% of men studied in this group. 74.3% Oligospermic infertile men in this study had oligoasthenozoospermia and/or oligoteratozoospermia.

Similar findings were made by Charvania *et al.* (1995). Idiopathic oligoasthenozoopermia was demonstrated as the highest cause of infertility in their study.

Sperm count per se is a relatively insensitive indicator of infertility. Men with low sperm counts (10 x 10^6/ml) may be fertile. Thus relatively low numbers of functional sperm cells (<5 x 10^6/ml) are adequate for fertility. However, the chances of initiating pregnancy declines as the sperm count decreases from 20 to <5 million/ml (Overstreet and Katz, 1987, Bar-Charma and Lamb, 1994).

Only motile sperm are able to penetrate through cervical mucus, migrate through the reproductive tract, penetrate the zona of the ova, and achieve fertilization. Morphology on the other hand is an important factor in semen analysis because it is a reflection of spermatogenic development (Bar-Charma and Lamb, 1994). Semen volume is important in assessing the total sperm production by the testes (Overstreet and Katz, 1987). Sperm count, percentage morphology

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

and percentage motility were significantly lower in infertile than fertile men ($p< 0.000$, Table 13) in the present study. Similarly, within the infertile group, normospermic infertile men had significantly higher sperm count, percentage motility and percentage morphology than infertile subjects with dyspermia ($p<0.001$, Table 17). These findings accord those reported by Ladipo *et al.* (1978) thus suggesting that these three parameters are predictive of male infertility. Hall and Krause (1992) showed that progressive motility and morphology alone were sufficiem to discriminate between normal and pathologic semen quality. Ilesanmi *et al.* (1996) demonstrated a positive correlation between sperm density and percentage of motile sperm. Katz *et al.* (1982) indicated that sperm with abnormal morphology are more likely to be immotile and if motile, to swim slower than normal sperm. Conversely, in the present study, comparisons in seminal volume between infertile and fertile controls did not show any significant difference ($p>0.05$, Table 12). Neither was there any significant difference between normospermic and dyspermic infertile men ($p>0.05$, Table 17). Similar observations were made by other investigators in the same geographical sub-region (Ladipo *et al.*, 1978, Adejuwon *et al.*, 1996).

Seminal zinc may be associated with seminal and prostatic function. However, its role in genital tract infection and infertility is controversial. Male accessory gland inflammation may interfere with the reproductive potential and

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

can result in the glands secretory dysfunction (Ladipo *et al.*, 1978; Bumazian *et al.*, 1992). Soffer *et al.* (1990) demonstrated significantly lower zinc levels in men with prostatovesiculitis with positive mycoplasma and/or Chlamydia cultures compared with non-infected case indicating a decreased prostatic function. Low levels of zinc were also reported in oligospermic and azoospermic patients (Stankovic and Mikac – Devic, 1978). In the present study, no significant difference in mean zinc levels were demonstrated in the fertile, infertile and STDs groups ($p>0.05$, Table 22). Within the group of infertile men, no significant difference was also observed in mean zinc levels between normospermic and dyspermic infertile men ($p>0.05$, Table 25). This suggests that the contribution of zinc to either infection of infertility is minimal. These findings are in agreement with that of Ladipo *et al.* (1978), who reported no significant correlation between the semen and blood zinc concentration and the fertility potential in the three groups of infertile men studied – normospermic group, cases with doubtful pathologic findings and cases with severe pathologic findings. Similarly, Adejuwon *et al.* (1996) did not find any significant difference in zinc levels between normospermic, oligospermic and azoospermic infertile men. Chia *et al.* (1994) also did not also find any relationship in zinc levels between idiopathic infertile men and normals. Neither did Upadhyaya *et al.*, (1984) find any relationship between zinc and infection of the genital tract.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Traditionally, the diagnosis of genital tract inflammation has been made through the evaluation of leukocytes in the seminal fluid (Moskowitz and Mellinger, 1992). The prevalence of leukocytospermia among infertile patients is approximately 10% to 20%. Controversy exists in the significance of WBC in semen. WBC numbers were found higher in infertile patients than fertile men and has been observed in association with decreased sperm numbers and impaired motility (Wolff, 1995). In this study, the incidence of men with leucocytospermia was high. 47.1%, 42% and 43% of men in fertile, infertile and STDs groups respectively have WBCs in their semen (Table 11). Similarly high incidences of WBCs were observed in normospermic and dyspermic infertile men (Table 12). Comparison between fertile, groups infertile and STDs groups in WBCs did not show a significant difference ($p>0.05$, Table 12). Further comparison between normospermic and dyspermic infertile men similarly showed no significant difference ($p>0.05$, Table 17). It appears that WBCs in semen is not an indication of current STDs or infertility. Moskowitz and Mellinger (1992) reported that an increased number of seminal leukocytes is specific for neither infertility nor infection.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Tomilson *et al.* (1993) had similar observations and suggested that measurement of seminal leukocytes in routine semen analysis appears to be of little prognostic value with regard to male fertilizing potential. Wolff (1995) observed that approximately 80% of leukocytospermic semen samples are microbiologically negative. C. trachomatis was presumed to trigger a persistent inflammatory reaction leading to leukocytospermia (Wolff, 1995).

The quantitative production of spermatozoa generally requires the presence of both LH/testosterone and FSH (Weinbauer and Nieschlag, 1995). Identifiable endocrine abnormalities have been reported in 10% of infertile men in developed countries where hormonal evaluations of infertile males are routinely undertaken (Kuku, 1995). Kuku (1995) indicated that hormonal disorders may be responsible for some case of oligospermia and azoospermia found in male partners of infertile marriages. He suggested that these abnormalities are not casually related to defective spermatogenesis but may be secondary to the resultant infertility in some of these patients. The actiological role of endocrine disorder is hardly understood and data regarding the frequency of these abnormalities are lacking in African countries (Kuku, 1995).

Normal levels of FSH in infertile patients with mild to moderate oligospermia and an increase in serum FSH in patient with severe oligospermia were observed by Saeed *et al.* (1994). Significantly higher FSH and LH

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

concentrations were observed in azoospermic men than controls indicating some disturbance of the spermatogenic process. However, no significant difference was observed in serum testosterone between azoospermic men and controls (Merino *et al.*, 1997). A third of infertile subjects with dyspermia were observed with hyperprolactinemia. In Nigeria, 52.9% of men with hormonal disorders with hyperprolactinemia were observed which was the most common form of hormonal abnormality (Kuku, 1995).

In the present study, comparison of the mean concentrations of FSH, LH, Prl and T between fertile and infertile subjects were not significantly different ($p > 0.05$, Table 23). Neither were these hormones significantly different between normospermic and dyspermic infertile males ($p > 0.05$, Table 24). These observations indicate that FSH, LH, Prl and T may not be responsible for infertility and the observed defect in semen quality in infertile men may be as a result of some other factors. Similar observations were made in other studies (Nkposong *et al.*, 1982, McClure, 1987, Takahashi *et al.*, 1992). Similarly, LH, FSH and T were not significantly different between men with STDs and fertile controls ($p > 0.05$, Table 23) in the present study. However, Prl was the only hormone that showed a significantly lower difference in men with STDs than fertile controls ($p = 0.03$). Low testosterone and prolactinemia were observed in association with high bacterial and non-bacterial infection (Ekwere, 1995). The

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

reason for this difference is not known and requires further study. The role of prolactin in male infertility is not clear. However, hyperprolactinemia has been proved to have a negative effect on spermiogenesis and spermatogenesis (Rogoza *et al.*, 1994). The finding also of abnormal prolactin levels as the most common observation in Kuku's study (Kuku, 1995) makes it perhaps necessary to elucidate the relationship between STDs, prolactin and male infertility. Kuku (1995) suggested that direct hormonal evaluation and identification of patients with abnormal Prl should be an important part of the workup of infertile males and females in this region, an uncommon practice at present.

The finding of no significant difference in plasma T levels ($p>0.05$) in the present study between infertile and fertile controls discussed earlier but significantly higher difference in BMI in infertile men than fertile controls ($p=0.03$) suggest a defect in spermatogenesis with resultant poor utilization of testosterone in infertile men in the study. Similar findings of significantly higher BMI in dyspermic than normospermic infertile males ($p=0.009$) in addition to the observed significant reduction in sperm count percentage morphology and percentage motility ($p>0.05$) in infertile than fertile and dypermic than normospermic infertile men, gives credence to this opinion. The relationship between obesity and infertility is well known. Testosterone is an anabolic steroid that initiates general body growth. There is thus, increase in muscle mass and

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

protein synthesis. loss subcutaneous fat and increased skeletal maturation and mineralisation (Verdaramc, 1986). Low levels of testosterone or failure in its utilization may affect all these functions resulting in impotence, obesity and muscular wasting. Most men in the STDs group (76.4%) exhibited various degrees of dyspermia. Alemnji and Thomas (1997) observed that infection of the genital tract could cause defective spermatogenesis. This probably explains the observed significant increase of BMI in men with STDs over fertile controls ($p<0.05$).

It is probable that sperm antibodies, though present, do not have a role in male infertility judging from the observations made in this study. Similarly, the production of sperm antibodies may not be influenced by STDs including 'Silent' STDs like *C. trachomatis*. Thus, the diagnosis of immunological infertility still remains one of exclusion as reported by Turek and Lipshultz (1994). The presence of immunoglobulin isotypes – IgG, IgA and IgM sperm antibodies in blood and semen and the similarity of incidences of men with these antibodies between this study and others (Turek and Lipshultz, 1994) is suggestive of their possible role in male infertility. However, such a role is less obvious due to the finding of low ($\leq$ 1%) percentage binding of these antibodies. In addition, tail binding - the most commonly observed region of sperm antibody binding in blood and semen, and the lack of significant differences of sperm antibody binding rates

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

between infertile men, men with STDs and fertile controls ($P>0.05$) make it unlikely that sperm antibodies are responsible for male infertility even at significant binding rates. Furthermore, past Chlamydial infection implicated as a causative factor of antibody mediated infertility was significantly higher in fertile men than infertile men and men with STDs ($p>0.05$), even when sperm antibody percentage binding was low in all groups, showing a lack of relationship between chlamydia IgG antibody and sperm antibodies, male infertility and genital tract infection.

Sperm antibodies appear to be produced both systematically and locally in the reproductive tract since similarities were demonstrated in circulating sperm antibodies and semen sperm antibodies ($p>0.05$). Thus semen sperm antibodies do not show more clinical relevance than serum sperm antibodies. This indicates that sperm antibodies detected in either serum, seminal plasma or on spermatozoa represent the sperm antibody status of the individual. The absence of significant differences between immunoglobulin classes in blood and semen ($p>0.05$) and in IgG, IgA and IgM (tested separately) between blood and semen ($p>0.05$) also show the lack of importance of one immunoglobulin class over the other. Thus, sperm antibody IgM can be included in detection systems and either of the immunoglobulin isotypes could represent the other classes. This would minimise the cost of testing for these antibodies. However, the low binding rates of sperm

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

antibodies make these observations difficult to establish and requires further studies.

The lack of relationship between sperm antibodies, STDs and male infertility ($p>0.05$) demonstrated in this study is contrary to the observations of Ekwere (1995) and others (Quesada *et al.*, 1968; Witkin and Toth, 1983; Soffer *et al.*, 1990). Perhaps, the high incidence (44%) of agglutination observed by Ekwere (1995) in infertile men in Nigeria might be due to the high incidence of bacterial and non-bacterial (17%) infection rather than sperm antibodies. Non immunological factors such as mumps virus, bacteria and certain mycoplasma stains can also cause agglutination and it is not known to what extent spermatozoa is coated with antibodies which are not provoked by sperm antigen. In addition, the frequency of detecting sperm antibodies depends on methodology apart from patient selection and interpretation of positive test used (Upadhyaya *et al.*, 1984). In this study a method – the immunobead sperm antibody test was used. The advantages of this method far out weigh the agglutination method used by Ekwere (1995). It is immunoglobulin specific, location specific, sperm region specific and is currently, the most widely used assay system (Broderick *et al.*, 1989; Turek and Lipshultz, 1994). Hence, sperm antibodies were detected not only in blood (as was the case in Ekwere's study, but also in the 'clinically relevant' semen in order to resolve the existing conflict on its contribution to infertility in males. It is also

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

likely that the low testosterone and prolactinemia observed in Ekwere's study might be primarily and clinically responsible for their infertility. Pregnancies observed after steroid therapy may be attributed to improvement in hormonal levels rather than sperm antibodies. In this study, no significant differences was observed in testosterone values between fertile, infertile and STDs ($p>0.05$). Sperm antibodies were not related to LH, FSH, prolactin and traditional semen analysis (Takahashi *et al.*, 1992). On the other hand, STDs may cause a breach in the blood-testis barrier resulting in the overwhelming inoculations of sperm antigens thus compromising the immune status of the individual. Deficiency of testosterone and cytokines may reduce the recruitment of T suppressor/cytotoxic cells to the 'leaky areas' or alter the state of normal non recognition of sperm antigens (Turek and Lipshultz, 1994). Decrease in T supressor/cytotoxic cells have been observed to create conditions permissible for the formation of autoantibodies for sperm specific antigens (Witkin, 1988, 1989). Thus primary deficiency of testosterone may result in decrease of T supressor/cytotoxic cells in conjunction with a defect in active immunosuppresion could lead to sperm antibody formation. Low testosterone and cross-reactivity of bacteria and/or non-immunological factors with spermatozoa in agglutination tests (and not sperm antibodies) may be responsible for the observed infertility in their study.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

A study carried out in Benin City, Nigeria, a similar geographical sub-region as this study and Ekwere's (Ogbimi *et al*., 1988), amongst infertile women did not relate infertility to the presence of sperm antibodies. This is similar to the findings in this study although this study was on males. Law *et al*. (1979) reported immunogenetic influences in men and as reported by Alexander and Anderson (1987) it is possible that infertile men in this geographical sub-region may belong to the population of men that despite multiple exposure to sperm antigens are genetically predisposed to be non-immunological responders.

Sperm antibodies, seminal indices – seminal volume, zinc and leucocytospermia, chlamydia IgG antibody, past STDs, and hormones – FSH, LH, Prl and T appear to have no affect fertility in this study. However, testicular failure appears to be the major and primary cause of male infertility in this study. Similar observations were made by other investigators. Testicular failure is said to be the most commonly investigated testicular disorder (McClure, 1987; Awojobi *et al*., 1983). Seminal characteristics – sperm count, percentage motility and morphology appear to be factors that influence the fertility of males and discriminate between pathologic and normal semen. It does show from these findings that the cause of infertility may be the impairment of the spermatogenic process. STDs also appear to contribute to male infertility in this study. Infection

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

related infertility is common in Africa (Alemnji and Thomas, 1997, Cates *et al.*, 1985). Gonorrhoea and non-specific urethritis were most commonly found STDs in this study similar to findings of Obafunwa *et al.* 1993). The explanation that STDs may retrograde into the testicular accessory organs leading to post inflammatory obstruction may account for the observed reduction in seminal volume. The observed impairment of sperm percentage motility may be as a result of alteration of spermatozoal characteristics by toxins secreted by STDs causing organisms. The involvement of prolactin in this process in unknown and requires further investigation.

The cause of infertility in the Nigerian male may be at the level of the testes through impairment of the spermatogenic process while the contributions of STDs to male infertility may be post-testicular through the impairment of sperm function.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

CHAPTER SIX

SUMMARY AND CONCLUSIONS

Infertility is common in Africa and the male role is now well recognised. However, knowledge of male infertility is limited. In an attempt at elucidating this subject, this present study has comprehensively evaluated the role of sperm antibodies and determined the involvement of STDs and/or other factors in the pathogenesis of male infertility in Nigeria. This was done through the analysis of hormones, chlamydia, semen and performance of sperm antibody tests in infertile men (normospermic and dyspermic) and men with proven STDs, and compared with fertile controls using appropriate statistical tests. Currently the most widely used assay system for the detection of sperm antibodies-immunobead binding technique was used. By this technique, sperm bound antibody isotype, location, region of binding and proportion were accurately assessed. Thus, IgG, IgA and IgM sperm antibodies were observed on either head, tail, tail tip or on the entire sperm on spermatozoa, in seminal plasma and blood plasma.

Thus, the contributions of this present study to knowledge in the area of male infertility are -

1) Sperm antibody isotypes-IgG, IgA and IgM and sperm antibody locations-sperm cell, seminal plasma and blood plasma are all relevant when screening for sperm antibodies. IgG, IgA and IgM sperm antibodies were demonstrated in blood plasma and semen in fertile, infertile and STDs groups. Binding proportions of IgG were similar to those of IgA and IgM in blood plasma,

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

seminal plasma and on spermatozoa. IgG binding proportion in blood plasma were similar to those of seminal plasma and spermatozoa. Observations made on IgA and IgM were not different from those of IgG.

2) Sperm antibodies may not contribute to infertility in males. Tail binding was observed as the most prominent region of binding of sperm antibodies in test groups and controls is said to only weakly affect sperm-cervical mucus interactions. In addition, the maximum of sperm antibody percentage binding of 4.1% observed in all antibody isotypes in blood plasma and semen in fertile, infertile and STDs groups is far too low to affect fertility in males. Furthermore, comparisons of blood plasma sperm antibodies showed no significant differences between infertile men, men with STDs and fertile men ($p>0.05$). Similar findings were obtained in seminal plasma and on spermatozoa.

3) Chlamydial infection is prevalent in society but may be absent in men presently attending STDs clinics due to its 'silent' nature. Chlamydia antigen was observed absent in all groups and the percentage of men with chlamydial seropositivity was high (80%, 43.3%, 33.3% in fertile, infertile and STDs groups respectively).

4) Past chlamydial infection may not be related to male infertility or sperm antibodies. Chlamydial IgG antibody indicating past chlamydial infection was found higher in fertile than infertile or STDs groups. Moreover, low binding rates of sperm antibodies were observed in all groups.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

5) Past and successfully treated STDs without complications are probably not likely to cause male infertility but appears to be a risk factor in contacting STDs. Comparisons of past history of STDs between infertile and fertile men did not show any significant difference ($p>0.05$). However, proportion of men with past history of STDs was significantly lower in fertile than STDs groups ($p<0.05$).

6) Gonorrhoea and NSU are still prevalent in our society and were the major infections in men attending the STDs clinic in this study. STDs appear to affect fertility by impairing seminal volume and percentage motility.

7) Seminal analysis may be a more objective method of assessing male infertility. Sperm count, percentage motility and percentage morphology appear to discriminate between pathologic and normal semen. Thus, the cause of infertility appear to be testicular through the impairment of spermatogenesis while the contribution of STDs to infertility may be post testicular through the impairment of sperm function.

8) Leukocytospermia and seminal zinc are not related to infection or infertility since they did not show any significant differences in comparisons between infertile men, men with STDs and fertile controls ($p>0.05$).

9) Hormonal abnormalities may not be a major contributor to male infertility as no significant differences ($p>0.05$) were observed in FSH, LH, Prl and T in all groups tested. Similar findings were demonstrated in all these hormones ($p>0.05$) except in Prl ($p<0.05$) in comparisons between men with STDs and fertile controls ($p>0.05$). The reason for this is not clear. Hence, further studies are required in this respect.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

10) Obesity appears related to male infertility since BMI in infertile and STDs groups were significantly higher than controls ($p<0.05$) and STDs group showed significant dyspermia (76.4%).

In conclusion, sperm antibodies are present but may not be a factor of infertility in Nigerian males. Sperm-count, percentage motility and percentage morphology still appear to be objective indices that discriminate between normal and pathological semen. STDs may affect male infertility by interfering with seminal volume and motility.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

REFERENCES

Adeghe, A. J. H.(1987) Effect of washing of spenn surface auto antibodies
Br. J. Urol 60. 360-363

Adegoke, A. K.(1986) Infertility-Who is to blame?
Dokita 16. 32.

Adekunle, L.V. (1986) The Social aspect of infertility. Dokita 16. 44-45.

Adejuwon, C.A., A.O. Ilesanmi, E.O. Ode and K.S. Akinlade (1996) Biophysical and Biochemical analysis of semen in infertile Nigerian males.
Afr. J. Med. Sci 25: 217-219.

Akande, E.O.(1986) Problems of infertility in sub-Saharan Africa.
Dokita 16. 23-27.

Alausa, O. and O.A. Osoba(1978) The role of sexually transmitted diseases in male infertility in tropical Africa. Nig. Med. J. 8. 225-229.

Alemnji, G.A. and K.D. Thomas (1997). Sociological Status of Nigerian males with primary and secondary infertility. East. Afr. Med. J. 74(8) 519-522.

Alexander, N.J.(1987) Immunology of semen. Fertil. Steril. 47. 192-205.

Alexander, N.J. and D.J. Anderson(1979) Vasectomy consequences of autoimmunity to sperm antigens. Fertil. Steril. 32. 253.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Amelar, R D and L Dublin(1979)Vasectomy reversal J Urol 121: 547-450

Amelar, R D, L Dublin and C Schoenfeld(1975) Circulating sperm agglutinating antibodies in azoospermic men with congenital bilateral absence of the vasa deferentia Fertil Steril, 26: 228-231

Anate, M and O Akercdolu (1995) Surgical management of female infertility in Ilorin, Nigeria East African Medical Journal 72: 411-4

Anderson D.J. and J.A. Hill (1988) Cell-mediated immunity in infertility. Am. J. Reprod. Immunol. Microbiol 17,22.

Arya, O.P., A O Osoba and F J Benett(1988) Tropical Venereology 2nd Ed. Edinburgh, Churchil Livingstone Inc 370

Awojobi, O A, E O Nkposong and J Lawani(1983) Actiological factors of male infertility in Ibadan, Nigeria Afr J Med Med Sci 12 91-94

Ayvaliotis, B, R Bronson, D Rosenfeld, and G Cooper (1995) Conception rates in couples where autoimmunity to sperm is detected Fertil Steril 43:739-41

Bar-Chama, N E and D J Lamb(1994) Evaluation of sperm function-What is available in the modern Andrology Laboratory Urol Clin North Am 21: 433-446

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Barlow, D H (1988) Antisperm antibodies in infertility. British Med J 296: 310-311.

Beastall, G H (1993) Role of endocrine biochemistry laboratories in the investigation of infertility. Role of endocrine biochemistry laboratories in the investigation of infertility. J Clin Pathol 46: 790-794.

Bello, C S S and L H Lombin (1990) Ureaplasma Urealyticum and Mycoplasma Hominis in the genital tract of patients with gonorrhoea attending the veneral disease clinic in Zaria, Nigeria. Nig Med J 20: 109-111.

Bomman, M S., G W. Schulenburg G W., D Boomker, T R Chauke and G Reif (1994) Observations in infertile African males at an andrology clinic in South Africa. Archives of Androl 33: 101-4.

Broderick G A., A Tom and R D Mcclure(1989) Immunological status of patients before and after vasovasostomy as determined by the immunobead antisperm antibody test. J Urol 142: 752-755.

Bronson, R A., G Cooper and D L Rosenfeld(1982a) Correlation between regional specificity of antisperm antibodies to the spermatozoan surface and complement-mediated sperm immobilization. Am J

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Reprod Immunol 2: 222- 224 198

Bronson, R A (1982b) Sperm-specific isoantibodies and auto-antibodies inhibit the binding of human sperm to the human zona pellucida Fertil Steril 38: 724-730

Bronson, R A (1984a) Sperm antibodies: their role in infertility Fertil Steril 42: 171-183

Bronson, R A (1984b) Autoimmunity to spermatozoa: effect on sperm penetration of cervical mucus as reflected by post coital testing Fertil Steril 41: 609-614

Bronson, R A, J O Walter, T A Wilson, S K Bronson, F I Chasalow and K Droesch(1992) Correlation between puberty and the development of autoimmunity to spermatozoa in men with cystic fibrosis Fertil Steril 58: 1199-1204

Brunham, R C, M Cheang, J Mc Master, G Garnett and R Anderson (1993) Chlamydia trachomatis, infertility, and population growth in Sub-Saharan Africa Sexually Transmitted Diseases, 20: 168-73

Burnazian, R A and M N Oganesian (1992) The biochemical indices of the sperm in chronic inflammatory diseases of the accessory genital glands Urol Netrol Mosk 4-6: 36-8

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Carlsen, E., A. Giwercman, N Keiding and N E Skakkebaek (1992) Evidence of decreasing quality of semen during the past 50 years. B M J. *305* (6854): 609-13.

Carson, S A., J. Reiher, A. Scommegna and G.S. Prins(1988) Antibody binding patterns in infertile males and females as detected by Immunobead test, gel-agglutination test and sperm immobilization test. Fertil. Steril. *49*: 487-492.

Castle, P E., K J. Whaley, T E. Hoen, T R. Moench, and R A Cone (1997). Contraceptive effect of spermagglutinating monoclonal antibodies in Rabbits. Biol. Reprod. *56*: 153-9.

Cates, W., T M. Farley and P J. Rowe(1985) Worldwide patterns of infertility: Is Africa different? The Lancet 2: 596-598.

Chavarria, E., A. Reves, A. Carrera, G. Aquilera, A Rosado, I.D. Velchuis (1995) Kinetic study of pulsatility of gonadotropin and testosterone secretion in normospermic fertile and infertile men with idiopathic oligo-asthenozoospermia. Ginecogia obstetricia de Mexico *63*: 74-89.

Chia, S E., C.N. Ong, S T. Lee and F H. Tsakok (1994) Study of the effects of occupation and industry on sperm quality. Annals Acad. Med. Singapore. *23*: 645-9.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Clarke, G N , A Stojanoff, M N Cauchi, J C McBain, A L Speirs and W I H Johnston(1984a) Detection of antispermatozoal antibodies of IgA class in Cervical mucus Am J Reprod Immunol 5 61-5

Clarke, G N , C Hsieh, S H Koh and M N Cauchi(1984b) Sperm antibodies, immunoglobulins, and complement in Human Follicular Fluid Am J Reprod Immunol 5 179-81

Clarke, G N , A Stojanoff, M N Cauchi and W I H Johnston (1985) The immunologlobulin class of antispermatozoal antibodies in serum Am. J Reprod Immunol Microbiol 7 1-13

Clarke, G N , P J Elliot and C Smaila(1985) Detection of Sperm Antibodies in semen using the Immunobead test a survey of 813 consecutive patients Am J Reprod Immunol 7 118-123

Close, C E , S P Wang, P L Roberts and R E Berger(1987) The relationship of infection with Chlamydia Trachomatis to the parameters of Male infertility and sperm autoimmunity Fertil Steril 47 880-883

Comhaire, F H (1992) An approach to the management of male infertility Clin Endocrinol Metab 6 435-50

Comhaire, F H (1993) Sperm Antibodies-"Gold Standard" Fertil Steril 59 242,

Comhaire, F H L Vermeulen and L Monsieur(1989) Determination of

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Adenosine Triphospliate in human semen to estimate the fertilizing potential and to quantify sperm antibodies. J. Bioluminescence and Chemiluminescence 4: 399-405

Critser, J.K., P.M. Villines, G.B. Coulam and E.S. Critser (1989) Evaluation of circulating antisperm antibodies in fertile and patient populations. Am. J. Reprod. Immunol. 21: 137-42

D'Cruz, O.J., G.G. Haas, R. dela Rocha and H. Lambert (1991) Occurrence of Serum antisperm antibodies in patients with cystic fibrosis Fertil. Steril. 56: 519-27.

D'Cruz, O.J., G.G Haas and H. Lambert(1993) Heterogeneity of human sperm surface antigens identified by indirect immunoprecipitation of antisperm antibody bound to biotinylated sperm. J. Immunol 151: 1062-74

De Muylder, X. (1995) Role of hysterosalpingography in the evaluation of infertility in Black Africa. Review. Medicine Tropicale 55: 160-4.

Diekman, A.B. and J.C. Herr (1997) Sperm antigens and their use in the development of an immunocontraceptive (Review) Am. J. Reprod. Immunol. 37: 1117.

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

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

219-21.

Edwards R G. (1990a) Use of androgens as a male contraception. Research in Reproduction 22, 1

Edwards R G. (1990b) Fc fragments and sperm-egg binding. Research in Reproduction 22, 2.

Eggert-Kruse, W., M. Christmann, I. Gerhard, S.Pohl, K. Klinga and B. Runnebaum(1989). Circulating antisperm antibodies and fertility prognosis: A prospective study. Reprod. J. 513-20.

Eggert-Kruse I. Gerhard, H. Naher,W. Tilgen and Runnebaum. (1990). Chlamydial Infection: A male and/or female infertility factor? Fertil. Steril. 53: 1037-43.

Ekwcozor C C., O D. Olaleye, O. Tomori,, I. Saliu, E M. Essien (1994) Sexually Transmitted Diseases in Ibadan in the 1990's, HIV infection and additional dimension. Afr. J. Med. Sci. 33: 363-367.

Ekwcozor C C., O D. Olaleye, O. Tomori., I. Saliu, E M. Essien, R.A. Bakare, A. A. Oni, O. O. Oyewo, A. O. Okesola, T. N. Onyemenem, J. O. Ajayi (1995) Clinico-epidemiological patterns of HIV infection in STD patients in Ibadan. Afr. J. Med. Sci. 33:321-327.

Ekwere P D (1995) Immunological infertility among Nigerian men: incidence of circulating antisperm auto-antibodies and some clinical

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

observations: a preliminary report. Br. J. Urol. *76*: 366-70

El-Rociy, A., Valesini, J. Friberg, Y. Shoenfeld, R.C. Kennedy, A. Tincani, G. Balestrieri and N. Gleicher(1988) Autoantibodies and common idiotypes in men and women with sperm antibodies. Am J. Obstet. Gynecol. *158*: 596-603

Emembolu, J.D., D.O. Anyanwu and B. Ewa (1993). Genital Tuberculosis in infertile women in Northern Nigeria. West Afr. J. Med. *12*: 211-2

Favot, I.J. Ngalula, Z. Mgalia, A.H. Klokke, B. Gumodoka and J.T. Boerma (1997). HIV infection and Sexual behaviour among women with infertility in Tanzania a hospital-based study. Int. J. Epidemiol. *26*: 414-9

Fjallbrant B. (1996). Sperm agglutinins in sterile and fertile men. Acta Obstetricia et Gynecologica Scandinavica. *47*: 89-101

Figa-Talamanca, I.V. Dell'Orco, A. Pupi, F. Dondero, L. Gandini, A. Lenzi, F. Lombardo, P. Scavalli and G. Mancini (1992). Fertility and semen quality of workers exposed to high temperatures in ceremics industry. Reprod. Toxicol. *6*: 517-23.

Francaville, F, R. Romano, R. Santucci, V. Marronic and G. Corrao(1992) Failure of intrauterine insemination in male immunological

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

infertility in cases in which all spermatozoa are antibody-coated Fertil Steril *58* 587-92

Franco J G Jr , M Scimberni, P J Rojas, I Moretti Rojas and S C Stone(1989) Reproducibility of the indirect immunobead assay for detecting sperm antibodies in serum J Reprod Med *34* 259-263

Fuller, P J., H G Burger and H W Baker(1992) Concurrent hyperprolactinemia and sperm autoimmunity in a man with secondary infertility Fertil Steril *58* 202-4

Gilbaugh, J H and L I Lipshultz(1994) Non surgical treatment of male infertility An update Urol Clin North Am *21* 531-548

Giwercman, A E Carlsen, N Keiding and N E Skakkeback (1993) Evidence for increasing incidence of abnormalities of the human testis a review Environ Health Perspective *101* Suppl 2 65-71

Gonzales, F F., G Koncbani, A B Mazzoli (1992) Effect of isotypes of antisperm antibodies on semen quality Int J Androl *15* 220-8.

Gregoriou, D , N Vitomtos, C Papadias, G Gregoriou, P A Zournas (1989) The role of chlamydia serology in infertile and subfertile men Eur J Obstet Gynaecol Reprod Biol *30* 53-8

Gregoriou, D N Vitoratos, N Legakis, G Gregoriou, P A Zournas (1990)

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Detection of sperm-bound antibodies in male partners of infertile couples using immunobead test. Eur. J obtet. Gynecol. Reprod Biol. 33. 235-9.

Gupta, G S., R.G. Kinsky and J. Couderc(1992) Modulation of T-cell functions by sperm specific lactate dehydrogenase fluouresccence analysis of immune competent cells and local graft versus host reaction. Indian J. Biochem. Biopys. 29. 271-6.

Guzick, D.S., I. Grefenstette, K. Baffione, S.L. Berga, J.S. Krasnow, D.W. Strovall and G.J. Naus (1994) Infertility evaluation in fertile women: a model for assessing the efficacy of infertility status. Human Reprod. 9: 2306-10.

Haas, G.G. Jr (1987) Antibody mediated causes of male infertility. Urol. Clin. North Am. 14 539-50.

Hameed, N., T.A. Ahmed, N. Sultana, D.A. Khan, N.A. Khan and W. Hassan (1995). The role of antisperm autoantibodies in the management of patients with primary infertility. JPMA 45: 203-5.

Hargreave, T.B., M. Torrance, H. Young et al (1982) Isolation of Ureaplasma Urealyticum from seminal plasma in relation to antisperm antibody levels and sperm motility. Andrologia 14: 223-7.

Hargreave, T.B., J. Harvey, R.A. Elton, (1984) Serum agglutinating and

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Inmobilizing sperm antibodies in men attending a sexually transmitted disease clinic. Andrologia *16*: 111-5.

Heidenreich, T., R. Bonlig, D.M. Wilbert, W.L. Strohmaier and U.H. Engelmann (1994). Risk factors for antisperm antibodies in infertile men. Amer. J. Reprod. Immunol. *31*: 69-76.

Hekman, A. and Ph. Rumke(1976) in Text book of Immunopathology (P.A. Micscher and H.J. Muller-Eberhard eds.) Grune and Stratton, New York. 947-962.

Heilstrom, W.J.G., J. Schater and R.L. Sweet(1987) Is there a role for Chlamydia Trachomatis and genital Mycoplasma in male infertility? Fertil. Steril. *48*: 337-9.

Hendry, W. F. (1992). The significance of antisperm antibodies. Measurement and Management. Clin. Endocrinol. *36*, 219.

Holland and , M.H. and W. Kruse (1992). Semen analysis and fertility prognosis in andrological patients. Int. J. Androl. *15*: 473-84.

Hull, M.G.R., C.M.A. Glazener, N.J. Kelly, D.I. Conway, P.A. Foster, R.A. Hinton, C. Coulson, P.A. Lambert, E.M. Watt and K.M. Desai (1985). Population study of causes, treatment and outcome of infertility. B.M.J. *291*: 1693-7.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Humpheys-Behcr, M G , P W Garnison and R E Blackwell(1990) Detection of Antigalactosyl-Transferase antibodies in plasma from patients with antisperm antibodies Fertil Steril *54* 133-7

Ilesanmi, A O (1995) Endometrial dating correlated with multiple luteal progesterone levels in confirming ovulation and luteal function in infertile Nigerian women West African Journal of Medicine *14* 152-6.

Ilesanmi, A.O., J.A. Adeleye and B O Osotimehin (1995) Comparison of single serum progesterone and endometrial biopsy for confirmation of ovulation in infertile Nigerian women African Journal of Med & Med Sci *24* 97-101

Ilesanmi, A O, W Omoniiah, A Bankole and O A Ojengbede (1996) The semen of infertile Nigerian men A statistical analysis of 1662 cases Afr J Med Med Sci *25* 195-202

Ingersley, H J , S Walter, J T Anderson, P Brandenhoff, J Eldrup, J P Geerdsen, J Scheibel, N Tromholt, H M Jensen and T Hjort(1986) A prospective study of antisperm antibody development in acute epididymitis J Urol *136* 162.

Isahakia, M A (1988) Characterisation of Baboon testicular antigens using monoclonal antisperm antibodies Biol Reprod *39* 899-99

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Isidori, A., F. Dondero and A. Lenzi (1998). Immunobiology of male infertility. Human Reprod. 3: 75-7.

Isojima, S., R.M. Graham, and J.B. Graham (1959). Sterility in female guinea pigs induced by injection with testis. Science 129:44.

James, K and T.B. Hargreave(1984) Immunosuppression by seminal plasma and its possible clinical significance. Immunology Today 5: 357-363.

Jennings, M.G., M.P. McGowan, H.W.G. Baker(1985) Immunoglobulins on Human sperm validation for screening test for sperm autoimmunity. Clin. Reprod. Fertil. 3:35-342.

Jones, W.R (1980) Immunologic infertility-fact or fiction. Fertil. Steril. 33: 577-58.

Kaplan, P. and R.K. Naz(1992) The Fertilization Antigen-1 does not have proteolytic acrosin activity but, its monoclonal antibody inhibits sperm capacitation and acrosome reaction. Fertil. Steril. 58: 386-402.

Katz, D.F., L. Diel, J.W. Overstreet (1982). Differences in the movements of morphologically normal and abnormal human seminal spermatozoa. Biol. Reprod. 26:566-570.

Khoo D, S. L. Feigenbaum and R.D. Mcclure (1991). Screening Assays for

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

immunologic infertility A comparison study Am J Reprod Immunol 29 11-16

Koksal, I , G K Ozgur, M Ceylan, A Sivrikaya and F Aker (1991) Effect of antisperm antibodies in male infertility Mikrobiyoloji Bulteni 25 160-6

Koyama K, K Kameda, N Nakamura, K Kubota, M Shigeta, S Isojima (1991) Recognition of Carbohydrate antigen epitopes by Sperm-immobilizing antibodies in sera of infertility woman Fertil Steril 56 954-959

Kaufman, D G and H M Nagler (1987) Specific Non-surgical Therapy in male infertility Urol Clin Nor Am 14 489-498

Kuku, S (1995) African endocrine infertility A review Afri J Med Sci 24 111-123

Ladipo, O A (1986) The epidemiology of infertility Dokita 16 1-5

Ladipo, O A , D A Olatunbosun and O A Ojo(1978) Biophysical and Biochemical analysis of semen in fertil and infertile Nigerian males Int J Gynaecol Obstet 16 58-60

Ladipo, O A , A O Osoba (1978b) Ureplasma Urealyticum(T mycoplsma)

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

and male infertility in tropical countries Afr J Med Sci 7: 187-190

Landsteiner, K (1899) Zur-Kenmnis derspezifisch auf blutkorperchen wirkenden sera Zentralblatt fur Bakteriologie 25 546

Law, H Y., W F Bodmer, J D Mathews and D C G Skegg(1979) The immune response to vasectomy and its relation to HLA system Tissue Antigens 14 115-39

Lee, H Y and members of vasectomy study project(1980) Observations of the results of 300 vasovasostomies J Androl 1 11

Lehman D., B Temminck, D DaRugna, B Leibundgut, A Sulmoni and H Muller (1987) Role of immunological factors and serological evidence Laboratory Investigation 57 21-8

Leke, R J., J A Oduma, S Bassol-Mavagoitia, A M Baelia and K M Grigor (1993) Regional and geographical variations in infertility effects of environmental, cultural, and socio-economic factors Environmental Health Perspectives 101 Suppl 2 73-80

Lipshultz, L I (1994) Male infertility (preface) Urol Clin North Am 21 xi

Marana R., A Lucisano, F Leone S Antonio, S Dell'Acqua, S Mancuso (1990) High prevalence of silent Clitamydia Colonization of the

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

tubal mucosa in fertile women. Fertil steril. 53: 354-356.

Liu, D.Y., G.N. Clarke and H.W. Baker(1991) Inhibition of human sperm-zona pellucida and sperm-oolemma binding by antisperm antibodies. Fertil. Steril. 55: 440-2.

Margalioth E.J., G.W. Cooper, F.H. Yancy, G.M. Scholl and D.L. Rosenfeld (1992) Capacitated sperm cells react with different types of antisperm antibodies than fresh ejaculated sperm. Fertil. Steril. 57: 393-8.

Marinho, A.O. (1986) Aetiological factors in infertility. A review of 277 infertile Nigerian couples. W. Afr. J. Med. 5:69-74.

Martin-Villa, J.M., J.De-Juan, J.L. Vicario, I.M. Luque, E. Alvarez, J. Cortes-Prieto and A. Arnaiz-Villena (1993) HLA class I, class II and class III antigen sharing is not found in couples with unexplained infertility. Int. J. Fertil. Menopausal stud. 38: 280-8.

Mazzoli, A.B. and C. Battera (1989) Role of the immune response in male infertility. Medicina 49: 232-8.

Mcclure R.D. (1987) Endocrine Investigation and Therapy. Urol. Clin. Nor. Am. 14: 471-488.

Meisel, M.T., Romer and R. Lober (1994) Sperm antibodies- a suitable parameter for diagnosis of fertility? Zentralblatt fur Gynakologie 116: 107-9.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Mellinger, B and M Goldstein (1987) New Areas of Research in male infertility. Urologic Clinics of North America *14*: 619-632

Menge, A C (1971) Effects of isoimmunisation and isoantisera against seminal antigens on fertility process in female rabbits. Biol Reprod *4*: 137-144

Meniru, G I (1996) Premarital conception in married couples: the Nigerian Igbo experience. West Afr J of Medicine *15*: 177-80

Merino, G S. Carmona-Lira, J.C. Martinez-Chequer J C., E Barahona, C Moran and J A Bermudez (1997) Hyperprolactinemia in men with asthenozoospermia, oligozoospermia, or azoospermia. Archives of Andirol *38*: 201-6

Metalnikoff, S (1900) Etudes sur la Spermotoxine Ann Inst Pasteur *14*: 577-589

Metchnikoff, E (1899) E tudes sur la resorption des cellules Ann Instit Pasteur *13*: 737

Micil S, Petrovic S, Dotlic R (1990) Seminal antibodies and genitourinary infection Urol *35*: 54

Moskowitz, M O and B C Melliger (1992) Sexually Transmitted Diseases and their relation to maleinfertility Urologic Clinics of North America *19*: 35-45

Naz, R K (1990) The effects of sperm-reactive antibodies present in human

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

infertile sera on fertility of female rabbits J Reprod Immunol *18* 161-77

Nkponsong, E O , J Lawani, S O Osanyintuyi and O A Awojobi(1982) Semen analysis in infertility in Ibadan Nig Med J *12* 181-86

Obafunwa, J O , S O Elesha and E O Odunjo(1993) Morphological changes found in the testis of 177 Nigerian males investigated for infertility Afr J Med Med Sci *22* 35-40

Ogbimi, A O , G O Oyeyinka and A E Omu(1986/1987) ABO blood group incompatibility and infertility in Nigerian couples Immunology letters *14* 299-301

Ogbinii, A O , G O Oyeyinka and A E Omu (1988) Antibodies to spermatozoa in serum and cervical mucus of infertile women in Benin City, Nigeria Singapore J Obstet Gynae *19* 32-36

Ogunbanjo, B O , A O Osoba and J Ochei(1989) Infective factors of male infertility among Nigerians Afr J Med Med Sci *18* 35-38.

Ojo, O A.(1968) Male factor in infertile marriages in Nigeria W Afr Med J *17* 210-211

Okonofua, F E , K A Ake Nai and M D Dighitoghi (1995) Lower genital tract infection infertile women compared with controls Genitourinary Medicine *71* 163-8

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Okonofua, F E , R C Snow, Alcmnji G A , A Okoiuwa and C O Ijaware (1997) Serological and Clinical correlates of gonorrhoea and syphilis in fertile and infertile Nigerian women Genitourinary Medicine *73* 1947

Oldereid, N B, Thomassen Y, Attramadal A, Olaccon B, and K Purvis (1993) Concentrations of lead Cadmium and Zinc in the tissues of reproductive organs of men J Reprod Fertil 99 421-5

Omoriah, W E , GN Egbunike and O A Ladipo(1985) Classification of the semen of the male partners of infertile Nigerian couples Andrologia *17* 257-261

Osoba, A O , A Onifade, K O Alausa (1975) Genital Infection and reproductive failure in developing countries Nig Med J 5 401-406

Ouahes, N A T Qureshi and A R Ahmed (1997) Infertility in women with pemphiligus Vulgaris and other autoimmune diseases J Amer Aca Dermatol *36* 383-7

Overstreet, J W and D F Katz (1987) Semen Analysis Urol Clin Nor Am *14* 441-449

Oyeyinka, G O, C S S Bello, O Y Elegba and B I Nsofor(1987) Levels of some complement component in azoospermic, oligospermic and

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

IBADAN UNIVERSITY LIBRARY

Normospermic Nigerian men. J. Reprod. Med. *32*: 774-776.

Patrizio, P., I. Mortti-Rojas, T. Ord, J. Balmaceda, S. Silber and R.H. Asch (1989) Low incidence of Sperm anitbodies in men with congenital absence of the vas deferens. Fertil. Steril. *52*: 1018-21.

Patrizio, P., T. Ord, S.J. Siber, A.T. La, F.J. Rojas, R.H. Asch(1992a) Repetitive attempts of sperm aspiration and in vitro fertilization in men with congenital absence of the vas deferens on development of antisperm antibodies. Fertil. Steril. *58*: S22 Absr. 0-048.

Patrizio, P., T. Ord, S.J. Siber, A.T. La, F.J. Rojas, R.H. Asch (1992b) Testicular origin of immunobead reacting antigens on human sperm. Fertil. Steril. *57*: 183.

Parker, M.M.F.L. Humoller, D.J. Mahler(1967) Determination of copper and Zinc. Clin. Chem. *13*: 40-48.

Parslow, J. M (1987) Characterisation of sperm antigens reacting with human antisperm antibodies. Clin. Exp. Immunol. *69*: 179-87.

Pattinson, H.A. and D. Mortimer(1987) Prevalence of sperm surface antibodies in the male partners of infertile couples as determined by immunobead screening. Fertil. Steril. *48*: 466-9.

Peleg, B.A. and M. Ianconescu(1966) Spermagglutination and spermadosorption

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

duc to myxoviruses. Nature *215*: 484-487.

Pelouze, P.S. (1941) Gonorrhoea in Male and Female. Philadelphia: W.B. Saunders. 240.

Primakoff, P.W. Lathrop and R. Bronson (1990). Identification of human sperm surface glycoproteins recognised by autoantisera from immune infertile men, women, and vasctomized men. biol. Reprod. *42*: 929-42.

Quesada, E.M., C.D. Dukes, G.H. Dean, R.R. Franklin(1968) Genital infection and sperm agglutinating antibodies in infertile men. J. Urol. *99*: 106-108.

Requeda, E., J. Charron, K.D. Roberts, A. Chapdelaine and G. Bleau(1983) Fertilizing capacity and sperm antibodies in vaso-vasostomized men. Fertil. Steril. *39*: 197-203.

Rogoza A, Mierzejewski W., Puzo M. (1994) Detection and treatment of hyperprolactinemia in male infertility. Ginekologia Polska *65*: 75-9.

Rose, N.R., F. Hjort, P. Rumke, M.J.K. Harper, O. Vjazov(1976) Techniques for detection of iso and autoantibodies to human spermatozoa. Clin.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Exp Immunol 23 175- 199

Ross, P., F.W. Kan, P. Antaki, N. Vigneault, A. Chapdelaine and K.D. Roberts (1990) Protein synthesis and secretion in the human epididymis and immunoreactivity with sperm antibodies Mol. Reprod. Dev. 26:12-23.

Ruijs G.J., F.M. Kauer, S. Jager, P.F. Schroder, J. Schirm and J. Kremer (1990) Is serology of any use when searching for correlations between Chlamydia trachomatis infection and male infertility? Fertil. Steril. 53: 131-136.

Rumke, P. (1954) The presence of sperm antibodies in serum of two patients with oligozoospermia. Vox Sang. 4: 135.

Rumke, P. (1974) The origin of immunoglobilins in semen antibodies. Clin Exp Immunol 17: 287-97.

Rumke, P. and G. Hellinga (1959) Auto antibodies against spermatozoa in sterile men. Am. J. Clin Path. 32: 357-63.

Saeed, S., F.A. Khan, S.B. Rahman, D.A. Khan, M. Ahmed M (1994) Biochemical parameters in evaluation of oligospermia. JPMA - J. of the Pakistan Med. Association 44: 137-40.

Shahmanesh, M., J. Stedronska and W.F. Hendry (1986) Antispermatozoal antibodies in men with urethritis. Fertil. Steril. 46: 308-11.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Shai, S. and Y. Naot(1992) Identification of human sperm antigens reacting with antisperm antibodies from sera and genital tract secretion. Fertil. Steril. *58*: 593-8.

Shulman, S. (1986) Sperm antigens and autoantibodies: effects on fertility. Am J. Reprod. Immunol Microbiol. *10*: 82-9.

Silber, S.J. (1994). A modern view of male infertility (review). Reprod. Fertility & Development 6: 93-103. Discussion 103-4.

Sigma, M. and M.L. Vance (1987). Medical Treatment of Idiopathic infertility. Urol. Clin. Nor. Am. *14*: 459-469.

Skakkebaek, N.E., A. Giwercman and D. de Wetser (1994). Pathogenesis and Management of male infertility. Lancet *343*: 1473-1478.

Silverberg, K.M, T. Dey, D.L. Olive and R.S. Schenken (1992) Sperm antibodies facilitate the acrosome reaction in human spermatozoa in vitro. Fertil. Steril. *58*: S21 Abstr.0-047.

Smith, D. R. (1972) General Urology. California: Lange Medical Publications.

Soffer, Y., R. Ron-El, A. Golan, A. Herman, E. Caspi and Z. Samra (1990) Male genital mycoplasma and Chlamydia Trachomatis culture: its relationship with accessory gland function, sperm quality, and autoimmunity. Fertil. Steril. *53*: 331-336.

Speroff, L., R.H. Glass and N.G. Kase (1989) Clinical Gynaecologic

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Endocrinology and Infertility. 4th edn. Baltimore: Williams and Williams.

Stankovic, M. and D. Mikac-Devic (1976) Zinc and copper in human semen. Clin. Chim. Acta 70: 123-126.

Takahashi, K. Ogawa, H. Tobai, T. Kojima, K. Shida, M. Isula and K. Sato (1992). Clinical significance of direct immunobead test to detect antisperm antibody (Japanese) Nippon Sanka Fujinka Gakkai Zasshi-Acta Obstetrics and Gynaccologica Jiponica 44: 779-86.

Tayler, C.J., O.A. Vandcipuye, J.A. McIntyre and W.P. Faulk (1990) Lactoferrin binding molecules in human seminal plasma. Biol. Reprod. 43: 712-7.

TaylorRobinson, D. and R.J. Manchee (1967) Spermadsorption and spermagglutination by Mycoplasmas. Nature 215: 484-487.

Thomas, W.E.G., M.J. Cooper, G.A. Crabe, G. Lee and R.C.N. Williamson (1984) Testicular exocrine malfunction after torsion. Lancet 2: 1357-60.

Thompson, S.T. (1994). Prevention of male infertility: An Update. Urol. Clin. Nor. Am. 21: 365-376.

Tomlinson, M.J., C.L. Barratt, I.D. Cooke (1993). Prospective study of leukocytes and leukocyte sub-populations in semen suggests they are not a

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

cause of male infertility. Fertil Steril 60: 1069-75

Tung, K S K., W D Cooke Jr., T A McCarthy, P Robitaille (1976) Human sperm antigens and antisperm antibodies II Age-related incidence of antisperm antibodies Clin Exp Immunol 25 73-9

Tung, K S K., A Okada, R Yanagimachi (1980) Sperm autoantigens and fertilization Effects of antisperm antibodies on rouleaux formation, viability and acrosome reaction of guinea pig sperm Biol Reprod 23 877-1980

Turek and L I., Lipshultz (1994) Immunologic infertility Urol Clin North Am 21:447-68

Uheling, L D (1971) Secretory IgA in Seminal fluid Fertil Steril 22 769-773

Ulcova-Gallova, Z and T Hjort (1992) Immunoreactivity of Mycoplasma HominisPG 21 antigens and human sperm antigens Cesk-Gynekol 57 418-21

Unlu, C S Bohmer and D H Maas(1990) Immunological factors in extracorporeal fertilization The role of antibodies against spermatozoa and zona pellucida antigens Fortschr Med 108 450-3

Upadhyaya M., B M Hibbard and S M Walker (1984a) Antisperm antibodies and male infertility Brit J Urol 56 531-536

Verheyen, S., H Tournaye, C Janssenswillen, P Hendrix, P Devroey and A S

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

Van (1994) The effect of pentoxifylline on in-vitro fertilization in the presence of antisperm antibodies J Rep Immunol 27 187-97

Verderame M (1986) CRC Handbook of Hormones, Vitamins, and Radiopaques Florida CRC Press, Inc p2

Weinbauer, G F and E Nieschlag (1995) Gonadotrophin control of testicular germ cell development (Review) Advances in Experimental Med and Biol 377 55-65

Weiss, V (1987) Immunolgic aspects in disorders of male infertility Immun Infekt 15 56-61

Wilson, L (1954) Spermagglutinins in human semen and blood Proc Soc exp Biol 85 652

Wingate, E W, R T Patrick, S Mathur (1993) Antigens in capacitated spermatozoa eliciting autoimmune responses J Urol 149 1331-7

Witkin, S S (1988a) Mechanisms of active suppression of the immune response to spermatozoa Am J Reprod Immunol Microbiol 17 61-4

Witkin, S S and A Chaudhry (1989) Relationship between circulating antisperm antibodies in women and autoantibodies on the ejaculated sperm of their partners Am J Obstet Gynecol 161 900-3

Witkin, S S and A Toth (1983) Relationship between genital tract infections,

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

sperm antibodies in seminal fluid and infertility. Fertil. Steril. *40*: 805-808.

Witkin, S.S. and G. Zelikovsky (1986) Immunosuppression and sperm antibody formation in men with prostatitis. J. Clin. lab. Immunol. *21*: 7-10.

Witkin, S.S., G. Zelikovsky, R.A. Good, and N.K. Day (1981) Demonstration of IIS IgA antibody to spermatozoa in human seminal fluid. Clin. Exp. Immunol. *44*: 368-74.

Witkin, S.S, G. Zelikovsky, A.M. Bongiovanni, R.A. Good, N.K. Day (1982) IgA antibody to spermatozoa in seminal and prostatic fluids of a subfertile man JAMA. 247: 1014.

Wolff, H. (1995). The biologic significance of white blood cells in semen (Review) Fertil. Steril. 63: 1143-57.

World Health Organisation (1987) Laboratory Manual for the examination of human semen and semen-cervical mucus interaction Cambridge University Press 67.

Yanagimachi, R. (1988) Mammalian Fertilisation In Knobil E., Neil J.D., editors. The physiology of reproduction. New York: Raven Press 135-86.

Yanagimachi, R., A. Okada, K.S.K. Tung (1981) Sperm auto-antigens and

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT

fertilisation II. Effects of anti-guinea-pig serum antibodies on sperm-ovum interactions. Biol. Reprod. 24: 512-8

Yeboah, E.D. and J.L. Marmar (1994). Urethral Stricture and semen quality. Int. J. Fertility & Menopausal Studies. 39: 310-5.

Zavos, P.M., and G.D. Kolinas (1992)Antisperm Antibodies (1992) Treatment mode of level of ASA following Test Yolk Buffer treatment and filtration via the sperm Prep T_m method. Fertil. Steril. 58: 522 Abstr. 0-049.

Zenilman, J.M. (1992) Update on Bacterial Sexually Transmitted Diseases. 19: 25-34.

Zouari, R. and M. De-Almeida(1993) Effect of sperm associated antibodies on human sperm ability to bind to zona pellucida and to penetrate zona-free hamster oocytes. J. Reprod. Immunol. 24: 175-86.

UNIVERSITY OF IBADAN LIBRARY

AFRICA DIGITAL HEALTH REPOSITORY PROJECT