THE INCIDENCE OF MORPHOLOGICAL ABNORMALITIES OF SPERMATOZOA IN SEMEN OF INFERTILE MEN AND SOME CAUSES OF VARIATIONS IN ZARIA. NORTHERN NIGERIA

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**A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL
AHMADU BELLO UNIVERSITY, ZARIA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE**

**DEPARTMENT OF HUMAN PHYSIOLOGY
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA**

JUNE, 1999

DECLARATION

I hereby certify that I carried out the research in this thesis by myself in the Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria, under the supervision of Professor Victor Olugbade Sekoni, Head of Animal Reproduction Department, National Animal Production Institute, Shika and Professor M.A. Ali of the Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria.

Where use has been made of the published work of other investigators, this is acknowledged and the source of information appropriately referenced. No part of this thesis has been submitted or accepted elsewhere for a higher degree or diploma.



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CERTIFICATION

This thesis entitled *"INCIDENCE OF SPERMATOZOA MORPHOLOGICAL ABNORMALITIES IN HUMAN SEMEN AND SOME CAUSES OF VARIATIONS IN ZARIA, NORTHERN NIGERIA"* by KOLAWOLE Victor Olorunshola meets the standard and regulations governing the Degree of Master of Science of Ahmadu Bello University and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This work is dedicated to:

the memory of my father, Chief J. Kolawole Odofin, the Odofin of Omuo - Ekiti,

my wife, Agnes Anike

and

my children, Adebanjo, Yasmin and Megan.

ACKNOWLEDGEMENT

I sincerely acknowledge the Grace and Guidance of the Almighty God for the successful completion of this study.

I wish to express my appreciation to my major supervisor Professor O.V. Sekoni for his guidance, constructive criticisms and invaluable assistance. The laboratory skills learnt under his guidance are invaluable and highly appreciated. The contributions of my second supervisor, Professor M.A. Ali are also immensely acknowledged.

My profound gratitude to my friend, Dr. Rotimi Aluko of the University of Guelph, Canada for helping with reprint of current journals in the fields of male reproduction.

I am highly indebted to the entire staff of the Human Physiology Department, Faculty of Medicine for their cooperation and assistance particularly Mr. R.R. Ettarh, and Mr. Daniel Daruwana.

I appreciate the words of encouragement from the Head of Department, Human Physiology, Dr. A.B. Adelaiye and the Dean of Faculty of Medicine, Prof. Ilyasu Mohammed.

I also appreciate the assistance of Professor G.C. Onyemeluke, Head, Department of Medicine for allowing me to use the facilities in the immunology unit, Ahmadu Bello University Teaching Hospital, Zaria.

Lastly, I am, grateful to Miss Abimbola Oguntolu of Salama Hospital, Zaria for helping in the typing of the thesis.

KOLAWOLE VICTOR OLORUNSIIOLA

ABSTRACT

This project consists of two studies (Study I and Study II). Study I (Methodological study) was used to study the effect of preparation, fixation in buffered formal saline. Storage at $+4^{0C}$ for one month and staining techniques on sperm morphological abnormalities.

Semen from 150 men in Zaria, were wet fixed in buffered formal saline and examined with phase contrast microscope. Semen smears stained with Eosin-nigrosin and carbol fuchsin (William Stain) were also examined using light microscope. The same ejaculates were stored for a month at $+4°^{c}$ after fixation in buffered formal saline and the above examination repeated.

There was no significant difference in the means of most of the sperm morphological abnormalities obtained using the three methods of examination. Also, there was no significant difference in the means of the sperm morphological abnormalities before and after storage at -+ 4^{0C} for one month. However, there was increase in the mean values of detached sperm heads in the stained smears and higher mean values of proximal cytoplasmic droplets for fixed semen examined with phase contrast microscopy. This study indicates that a combination of phase contrast microscopy and examination of stained smears using light microscopy is ideal for obtaining the most reliable results in sperm morphological abnormalities evaluation of human semen.

The study also demonstrated that buffered formal saline is a good fixative for human sperm.

Study II was carried out to establish values for different sperm morphologic characteristics in semen of adult men in Zaria, and to determine the possible influence of some factors such as age, diseases, social habits such as cigarette smoking and alcohol consumption and season on sperm morphology.

The Medical history and physical examination including examination of the genitalia of 150 men followed by semen collection was done between January to December 1995. The color, volume and density of each ejaculate were noted. Also the presence of any abnormality such as blood, pus etc if any was noted. Thereafter, sperm morphological studies for different abnormalities were done using fresh semen fixed in buffered formal saline and examination by the phase contrast microscope for abnormalities of acrosome, midpiece and sperm tail, while semen smears prepared from the fresh semen were stained using Williams (carbol fuchsin) and eosin-nigrosin stains and examined with the light microscope for abnormalities of sperm heads.

The meteorological data for Zaria for the year 1995 was used to study the influence of season on human sperm morphology.

Detached normal head, detached abnormal head, midpiece abnormalities and total sperm morphologic abnormalities had significantly higher means ($P<0.05$) during the dry/hot partly harmattan season as compared to the rainy season. The means obtained for proximal cytoplasmic droplets, distal cytoplasmic droplets, sperm tail abnormalities and head abnormalities were higher during the rainy season than in the other two seasons.

The total sperm morphological abnormalities in the study groups was found to be 43.69% which is clearly above the 25% suggested in fertile men.

Age and cigarette smoking was found not to affect sperm morphological abnormalities. Midpiece and sperm tail abnormalities were significantly higher ($P<0.05$) in the group with past or present sexually transmissible disease (STD) than the group without STD.

Higher mean values for sperm morphological abnormalities evaluation in subjects with genital abnormalities such as cryptorchidism, varicocele and hydrocele were also obtained. The total sperm morphological abnormalities in these groups were significantly higher than values obtained in fertile men without genital abnormalities.

From study II, it is concluded that season of the year, alcohol consumption, infection with sexually transmissible diseases and genital abnormalities such as bilateral undescended testes, varicocele and hydrocele have adverse effect on human sperm morphology.

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TABLE OF CONTENT

Contents	Page
Title Page	i
Declaration	ii
Certification	iii
Dedication	iv
Acknowlegement	v
Abstracts	vi
Table of Contents	ix
List of Table	xiii
List of Figures	xiv
List of Appendices	xv
Chapter One - Introduction	
1.1 General Introduction	1
1.2 Statement of Research Problem	3
1.3 Objectives	3
Chapter Two - Review of the Literature	
2.1.0 Major Causes of Infertility	4
2.1.1 Infections	4
2.1.2 Varicocele (Ditalations/Tortuosity of Spermatic Veins)	5
2.1.3 Endocrine Disorders	5

2.1.4 Cryptorchidism 6
2.1.5 Ductal Obstruction 6
2.1.6 Ejaculatory Disturbances 7
2.1.7 Trauma 7
2.1.8 Diseases and Genetic Factors 8
2.1.9 Immunological Causes of Infertility 8
2.1.10 Effects of Drugs and Chemicals on Male Fertility 10
2.1.11 Effect of Irradiation 10
2.2 Sperm Morphology and Infertility 12
2.3 Normal Sperm Morphology 12
2.4 Methods of Preparation and Examination of Human Sperm Morphology . . 16
2.5 Some Factors which Alter Normal Sperm Morphology 16
2.5.1 Origin of Sperm Defects 17
2.5.2 Genetic Factors 18
2.5.3 Frequency of Ejaculation 18
2.5.4 Osmotic Pressure 19
2.5.5 Ambient Temperature 19
2.5.6 Effect of Season on Sperm Morphology 20
2.5.7 The Effect of Age on Sperm Morphology 21
2.5.8 Effects of Diseases on Sperm Morphology 22
2.5.9 Influence of Drugs and Chemicals on Sperm Morphology 22
2.5.10 Staining Techniques 26

2.6 Relationship between Sperm Morphology and Other Semen Characteristics 23

Chapter Three - Survey of Materials and Methods

3.1 Research Design 24

3.2 Study 1 25

3.2.2 The Preparation of Stained Smears with Eosin nigrosin and William's Stain 25

3.2.3 Laboratory Examinations 26

3.3 Study II 29

3.3.1 Introduction 29

3.3.2 Examination of Semen with Phase Contrast Microscope 29

3.3.3 The Preparation of Stained Smear with Eosin Nigrosin and William's Stains 29

3.4 Methods of Statistical Analysis 30

Chapter Four - Results

4.1 The Effect of Preparation, Fixation in Buffered Formal Saline and Staining Techniques on Human Sperm Morphology 32

4.1.1 Pathologic Head 32

4.1.2 Acrosome Abnormalities 34

4.1.3 Proximal Cytoplasmic Droplets 35

4.1.4 Distal Cytoplasmic Droplets 35

4.1.5 Midpiece Abnormalities 36

4.1.6 Sperm-tail Abnormalities 37

4.1.7 Detached Normal Head 37

4.1.8 Detached Abnormal Head 37

4.2 The Incidence of Sperm Morphological Abnormalities in Human Semen in Zaria with Spects on Some Causes of Variations 40
4.2.1 The Influence of Age on Sperm Morphology 42
4.2.2 The Effect of Sexual Transmissible Diseases on Sperm Morphology 45
4.2.3 The Influence of Season and Months on Human Sperm Morphology 47
4.2.4 Influence of Smoking on Human Sperm Morphology 68
4.2.5 The Influence of Alcohol Consumption on Human Sperm Morphology . . . 70
4.2.6 The Influence of Genital Abnormalities on Human Sperm Morphology . . . 74
4.2.7 Effect of Varicocele and Hydrocele on Human Sperm Morphology 74
4.2.8 The Influence of Inguinal and Scrotal Surgery on Human Sperm Morphology75

Chapter Five - Discussion

5.1 Methodological Studies (Study I) **78**
5.2 Incidence of Sperm Morphological Abnormalities in Zaria with Aspects on Some Causes of Variations (Study II) 80
General Conclusions 86
References 88
Appendices 98

LIST OF TABLES

Table		Page
1.	The Principle for Evaluation of Human Sperm Morphology	15
2.	Percentage of Sperm Abnormalities in 150 Adult Males Using Three Methods of Preparations and Staining on day one and at a month after fixation and storage at 4^{oc}	33
3.	Values for sperm morphological characteristics in human semen in Zaria	41
4.	Percentage mean of sperm morphological abnormalities in two age groups	43
5.	Percentage means of sperm morphological abnormalities in subjects with sexually transmissible diseases	45
6.	Meteorological Data, Samaru, Zaria 1995	47
7.	Percentage means of sperm morpholoical abnormalities during the three seasons	50
8.	Percentage means of sperm morphological abnormalities in smokers and non smokers	69
9.	Percentage means of sperm morphological abnormalities in alcohol and no alcohol ingesters	71
10.	Percentage means of sperm morphological abnormalities in subjects with undescended testes	75
11.	Percentage means of sperm morphological abnormalities in subjects with varicocele and hydrocele	76
12.	Percentage means of sperm morphologicval abnormalities in subjects who had inguinal and scrotal surgeries	77

LIST OF FIGURES

Figure			Page
1.	Monthly variations of proximal cytoplasmic droplets		55
2.	Monthly variations of distal cytoplasmic droplets		56
3.	Monthly variations of midpiece abnormalities		57
4.	Monthly variations of sperm tail abnormalities		58
5.	Monthly variations of sperm head abnormalities		59
6.	Monthly variations of acrosome abnormalities		60
7.	Monthly variations of detached normal heads		61
8.	Monthly variations of detached abnormal head		62
9.	Monthly variations of total sperm morphological abnormalities		63
10.	(a)	Morphology of Normal Human Spermatozoan	64
10.	(b)	Photomicrograph of Human Spermatozoan Morphology	65-67

LIST OF APPENDICES

Appendix		Page
ia-ih.	Results for Methodological Studies	98
ii.	Total Sperm Abnormalities for 150 Subjects	142
iii.	*1995* Monthly Mean Values for Sperm Morphological Abnormalities	147
iv.	Storage and Staining Solution	138
vi.	Research Protocol	140

THE INCIDENCE OF MORPHOLOGICAL ABNORMALITIES OF SPERMATOZOA IN HUMAN SEMEN AND SOME CAUSES OF VARIATIONS IN ZARIA NORTHERN NIGERIA

INTRODUCTION

1.1. GENERAL INTRODUCTION

Reproductive disorders in couples which result in the inability to have normal pregnancies resulting in the births of normal children are common problems which could present clinically as various grades of infertility to complete sterility (WHO Scientific Group on Epidemiology of infertility 1976).

Defects in the reproductive systems in either the male or both sexes could be involved, indeed, complaints of infertility could be as high as 10 per cent in family planning or gynecology clinic (Nasah, 1978). The social implications of infertility are varied and sometimes severe. Marital instability, divorce, polygamy, prostitution and suicide, in extreme cases are common consequences of infertility (Nasah, 1984). Belsy (1976), Olatunbosun *et al,* (1979), David and Vocas (1981) have shown that the incidence of infertility in Nigeria is as high as 23 per cent. Ojo (1970), Chukwudebelu (1974), Belsy (1976, 1979) WHO (1976), Chukwudebelu *et al.* (1979), Olusanya (1980) and Vassen (1983) showed that infertility is a common problem world wide and even more common in the Sub-Saharan Africa.

Most traditional African cultures place a high social value on fertility, particularly as a demonstration of the consummation of the marriage and as an important expression of the couples social roles in the community. One can therefore understand the social stress to which the infertile couple is subjected. Also, in most traditional African culture

the woman is usually blamed as the cause of the childlessness, but Chukwudebelu (1974), Nasah (1978), Willson (1979) and Ajabor *et al.* (1981) have shown that 40% to 50% of infertility in Africa is due to male factors. In a study of 200 cases of sterile marriage, Meaker and Vose (1940) found the male to be at fault alone in 8%, the female alone in 14% and both at fault in 78%. When both were at fault, the male was chiefly to blame in 12%, the female in 10% and both sexes in 51%.

In male animals as well as human beings there are many well documented causes of infertility/sterility. These includes the production of poor quality semen associated with the malfunction of the reproductive organs (Chukwudebelu 1974; Mefo 1981; Jecht *et al.*, 1982; Sekoni and Gustafsson 1987). A relationship between sperm morphological abnormalities and infertility is well established in man (Macleod and Gold 1951; Joel 1955; Dublin and Amelar 1971; Ladipo 1980; Bostofle *et al.*, 1982). Elevated incidence of sperm morphological abnormalities beyond normal values in men are highly correlated with infertility/sterility (Moench 1931a; Macleod and Gold, 1951; Ladipo, 1980; Bostofle *et al.*, 1982; Osegbe and Amaku (1985 and 1987) and Yue *et al.*, (1995).

Bostofle *et al.*, (1984) in analyzing the clinical fertility of 1077 men who underwent evaluation showed decreasing fertility highly correlated with elevated percentage of morphological abnormal spermatozoa, decreasing sperm count, increasing number of immotile sperm and poor motility.

1.2. STATEMENT OF RESEARCH PROBLEMS

In contrast to Nigeria and particularly Northern Nigeria; Considerable investigations have been carried out in developed countries linking sperm morphological abnormalities to infertility in human (Moench, 1931a, b; Meaker and Vose, 1940; Macleod and Gold 1951; Nistal and Paniagua 1984).

1.3 OBJECTIVES OF THE STUDY

The objectives of this study are:

a. To study and compare the accuracy of various techniques and stains in the evaluation of sperm morphological abnormalities in human semen.

b. To establish values for different sperm morphological characteristics in semen of men in Zaria, Northern Nigeria.

c. To study the possible influence of some factors such as age, diseases, social habits like Cigarette smoking and alcohol consumption and season on sperm morphology in semen of men in Zaria.

CHAPTER TWO

REVIEW OF THE LITERATURE

2.1 MAJOR CAUSES OF INFERTILITY IN THE MALE.

Poor sperm quality in terms of the volume, concentration, morphology and function can lead to male infertility and may arise from the following:

2.1.1. Infection

Testicular degeneration due to infections could lead to impairment or cessation of spermatogenesis. Ladipo and Adelusi (1981) incriminated Herpes Simplex Virus type 2 infection in one series of male infertility in Ibadan. Similarly, Mefo (1981) found a history of present or past urethritis in 88% of patient with oligospermia and multiple sperm morphological defects in Yaounde. Osegbe (1991), Merino *et al* (1995) and Piacentino *et al* (1995) also demonstrated decreased testicular functions and infertility following unilateral and bilateral bacterial infections.

Asymptomatic passage of bacteria in the seminal fluid following prostatic infection may cause poor sperm motility, agglutination and immune reaction in the female partner. There are also cases of infertility caused by sperm agglutination in the cervix due to incompatibility of couples(Nasah 1984). Postpubertal mumps associated with orchitis, testicular degeneration resulting in the production of poor quality semen (e.g. elevated sperm morphological abnormalities, oligospermia/azoospermia) has been implicated as a causative agent of infertility/sterility by many workers (Moench, 1931a; Stengel, 1936;

Charles, 1950). Hydrocele caused by infections such as filariasis may also be an important factor in some cases of male infertility.

2.1.2 Varicocele (Dilatation/tortuosity of spermatic veins)

It has been shown that varicocele may cause elevated production of abnormal spermatozoa/oligospermia by increasing the scrotal temperature or due to venous congestion or by regurgitation of toxic substances from the renal vein. Nasah (1978) has shown higher prevalence of varicocele in an infertile population (between 20% to 30% as opposed to 10% in the fertile population). Rodninquer *et al* (1981) demonstrated increased sperm morphological abnormalities in varicocele in line with the findings of Jecht *et al* (1982).

2.1.3. Endocrine Disorders

Male infertility caused by the production of poor quality semen due to disorders of the hypothalamus, pituitary, adrenal, thyroid glands and abnormal seminal prostaglandin $F_2\ \alpha$ ($PGF_2\ \alpha$) levels have been documented (Dublin and Amelar 1971; Consentino *et al.*, 1984). The semen of the infertile men were characterized by oligospermia, poor spermatozoa motility and elevated incidence of spermatozoa morphological abnormalities.

Male infertility due to idiopathic factors in men with hyperprolactinemia associated with hypogonadism, impotence, galactorrhea has also been reported. Other etiological factors implicated included hypothalamic dysfunction, pituitary adenoma and

ty. The prostate glands of the infertile men were hypoplastic while their semen actrerized by low volume, oligospermia and impaired spermatozoa motility *t al.*, 1976).

Cryptorchidism

Cryptorchidism has been shown to cause seminiferous tubular damage and result infertility (Dubin and Amelar, 1971; Lipshults, 1976). Nistal and Panigua (1984) in study of 23 infertile adult men with no other apparent cause of infertility than lateral retractile testes, reported Oligospermia and elevated incidence of spermatozoa morphological abnormalities (narrow sperm heads and proximal droplets) which ranged between 60-80%.

2.1.5 Ductal Obstruction

Congenital absence of both vas deferens and or seminal vesicles has been shown to lead to infertility. Makler and Hampel (1975) reported the case of a 26 years old childless male who was married for three years. He was found to be azoospermic and testicular biopsy showed tubular atrophy and impaired spermatogenesis which terminated in spermatides formation. Further investigation showed congenital obstruction between the tests and epididymis (absence of vasa efferentia).

Dubin and Amelar (1971) in the study of aetiologic factors in 1294 consecutive cases of male infertility found 7.4.% of the cases to have some form of ductal obstruction. In this group, 4.4.% was epididymal obstruction, secondary to epididymitis

with gonorrhea or tuberculosis as aetiology. Dublin and Amelar (1971) also found absent vasa differentia in 1.88% of the cases. Voluntary sterilization by vasectomy is by far the commonest cause of ductal obstruction today and an important form of male contraception.

2.1.6 Ejaculatory Disturbances

Failure to deliver sperm into the vagina as a result of ejaculatory disturbance such as lack of ejaculation, premature and retrograde ejaculation are documented causes of male infertility (Dubin and Amelar 1971), Ejaculatory disorders resulting from epispadias, hypospadias, phimosis and Extropy of the bladder have also been reported. (Dubin and Amelar 1971, Chan *etal* 1982).

2.1.7 Trauma

Surgical trauma especially in the inguinal and perineal area, may lead to male infertility/sterility. Mefo (1981) in the study of male infertility discovered that 14% of cases of severe oligospermia/Azoospermia were linked to herniorrhaphy. Iatrogenic injury to the Vas deference or the epididymis was also reported in orchidopexy, varicocelectomy, hydrocelectomies and vasography (Chan *et al* 1982).

Extensive operations for pelvic malignancies account for a minor percentage of cases of male infertility. The injury could be caused by direct severance of the ductal system, vascular, neurological damage or inflammatory. Dubin and amelar (1971)

discovered 1.4.% of the cases of male infertility to be caused by surgical injury to the testes.

2.1.8 Diseases and Genetic Factors

Impotence which could be organic (e.g. from diabetes mellitus) or psychosomatic will also lead to male infertility. Emotional stress and genetic factors such as Klinefelter syndrome are also well known cases of male infertility.

2.1.9. Immunological causes of infertility

The role of antisperm antibodies in the aetiology of infertility has been reported by earlier workers. Landsteiner (1899), Metchnikoff (1899) and Jones (1980) described the finding of sperm antibodies in mammalian serum. Kibrick *et al.*, (1952) also reported the presence of circulating antibodies in guinea pigs immunized with autologous testicular homogenate and Isojima *et al.*, (1968) suggested a possible antisperm autoimmunization in spontaneous diseases in man. Wilson (1954) demonstrated sperm agglutin in the blood and semen of humans. Fuchs and Alexander (1982) recently have demonstrated in post vasectomy patients that as sperm agglutinating antibody titres increased, pregnancy rate decreased. They also noted that increased levels of seminal plasma sperm agglutinating antibody level were associated with significantly reduced pregnancy rates. Ross. (1983) suggested serum antibody studies in patients who have sperm agglutination on repeated semen analysis or an abnormal in-vitro cross species fertilization test and other unexplained infertility cases. Franklin and Dukes (1964)

demonstrated high level of antispermatozoa antibody in a study of group of men with unexplained infertility.

Also in the study of etiologic factors in 1294 consecutive cases of male infertility, Amelar and Dubin (1971) found 10 cases with severe sperm autoagglutination.

2.1.10. Effects of drugs and chemicals on male infertility

Drugs and exogenous chemicals may affect the testis directly or indirectly. Direct acting compounds include flutamide, cyproterone acetate, spironolactone and cimetidine. Van Thiel *et al.* (1979) showed Cimetidine, to have antiandrogen effect, inhibits the response of luteinizing hormone to luteinizing hormone releasing factor, prevention of normal gonadotrophin response to provocative clomiphene-stimulation and reduction in sperm concentration. Prolonged sultasalazine administration has been shown to cause oligospermia and decreased sperm motility in rat and man (Van-Thiel *et al.*, 1979 and Hoyt *et al.*, 1995). Ross (1983) also observed patient in whom reduced sperm motility developed coincidentally with administration of phenylbutazone. *Cannabis Sativa* (india hemp) have been shown to cause decrease in sperm concentration and semen testosterone level with frequent and continuous use. (Kolodny *et al.* 1974.

The widespread use of alkylating agents and antimetabolites in the treatment of malignancies and immunologically mediated diseases account for growing numbers of infertile males (Sherins and Devita 1973). Of the heterocyclic compounds, nitrofurantoin is known to impair testicular function in high doses but have little effect when doses of less than 10mg/kg body weight are used, it causes depression in spermatogenesis by

interfering with carbohydrate metabolism in the germinal epithelium to produce an arrest at the primary spermatocyte stage. (Nelson and Stein-Begar 1952). Reports show that chronic ingestion of larger amount of ethanol (alcohol) or its metabolite, acetaldehyde, can directly impair synthesis of Testosterone in man and animals by increasing testosterone metabolic clearance rates, by suppressing gonadotropin and reduction in testicular weight and sizes (Mandelson *et al.*, 1977). These result in the decline of libido and impairment of spermatogenesis (Vanthiel *et al.*, 1975; Mandelson *et al.*, 1977). Adegoke and Onwuchekwa (1998) reported decreased sperm motility and destruction of leydig cells in the testes of Sprague-Dawley rats fed with Indomethacin for two days. These effects were due to inhibition of Prostaglandins.

2.1.11. Effect of Irradiation

Rowley *et al.* (1974) showed the effect of ionizing irradiation on the testes to be dose dependant and no significant change in sperm density followed exposure of the testis to either 8 or 20 rads, but azoospermia was observed in men who received more than 70 rads to testis. Prepubertal testes seem to be more susceptible to radiation injury than post pubertal testes. The spermatogonia are more radio-sensitive.

2.2. SPERM MORPHOLOGY AND INFERTILITY

Several workers agree that a relationship exist between abnormal sperm morphology and male infertility (Carry 1946, Williams, 1946; Macleod and Gold, 1951, Eliasson, 1971, Osegbe and Amaku, 1987 and Yue *et al.*, (1995).

It has been shown that normal semen samples contain no more than 20% abnormal spermatozoa (Moench, 1931 a, b); Williams 1946 and Macleod and Gold (1951); though Eliasson (1971) suggested that up to 30-40% of all spermatozoa present in human semen possess an abnormal morphology and when this percentage increases to greater than 50%, infertility results.

Moench (1931a), one of the foremost proponents of the implication of abnormal spermatozoa as a causative factor of male infertility, states that a maximum of 20% abnormal forms is found in the semen of fertile men and that when this figure exceed 25% infertility/sterility is probable. Williams (1946), stated that where the percentage of abnormal forms exceeds 40% the semen of that individual should be considered infertile/sterile.

Macleod and Gold (1951) also studied semen samples which had elevated incidence of spermatozoa morphology with consequent failure to produce conceptions or causes of habitual abortions and miscarriages in the patients partners. Bostofte *et al.* (1982) showed a significant correlation between increasing numbers of abnormal spermatozoa and decreasing chance of live births and that many abnormalities also seemed to be related to a longer time interval to the first pregnancy achieved. Spermatozoa morphological abnormalities of various types in human semen have also been shown to cause infertility/sterility, for example, round headed spermatozoa lacking acrosome (Schirren *et al.*, 1971), sperm-tail defects (Baccetti *et al.*, 1975); multiple tails (Nistal *et al.*, 1977); double form (Baccetti *et al.*, 1979), axonemal abnormalities (Afzelius and Eliasson, 1979) and detached abnormal sperm heads (Perotti *et al.*, 1981).

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2.3. NORMAL SPERM MORPHOLOGY

Eliasson (1971) presented a table of principles for evaluation of sperm morphology which was a result of workshop for an inter-laboratory cooperation organised in Stockholm in 1969. The main objectives for the classification of different forms of spermatozoa were (a) to be simple with high repeatability/accuracy, (b) to make a standardization possible and (c) to make it easy to instruct other persons. The whole spermatozoa should be evaluated (head, mid pieces and tail) and only the spermatozoa with tails should be counted and classified (WHO 1980). (See Table 1 and Figure 10a and b).

2.4. METHODS OF PREPARATION AND EXAMINATION OF HUMAN SPERM MORPHOLOGY.

There is a wide variety of sperm staining techniques in use (Williams *et al.*, 1934; Macleod and God 1951).

Some stains and methods employed for morphological studies produce artifacts and inaccurate results because some of the procedures employed for preparing stained semen smears seem detrimental to the acrosome (Harasmowycz *et al.*, 1969; Larsson *et al.*, 1976). Little investigation has been done on the techniques employed for human semen evaluation particularly in Nigeria.

The commonly used technique is that of Kaufman which consists essentially of smears air dried at room temperature and fixed in 10% formaldehyde for one minute and stained with simple Meyers hematoxylin (Kaufman 1946). The Kaufman technique is

rapid, gives a clear background and most of the structures of the spermatozoa are clearly delineated. Campbell *et al.* (1960) showed that bluish eosin-nigrosin gave inaccurate results as far as morphological studies are concerned. Supravital stains such as eosin-nigrosin is used for the differential staining and the determination of the % live/dead spermatozoa. Dead spermatozoa are stained dark reddish while live spermatozoa are unstained/colourless (Eliasson and Treichi, 1971 and Bryan, 1975). Williams stain (carbolfuchsin) developed by William (1920) and modified by Lagerlof (1934) gives a clear morphologic characteristic of the sperm head. Bryan (1975) found using Eosin-fast green naphthol yellow mixture stain to be reliable and accurate for the differential staining of cytologic components in many mammalians species.

Harasmowycz *et al.* (1969) reported that highest repeatability in evaluation of morphological features were obtained by counting 200 spermatozoa in wet preparation of unstained, fixed semen samples using phase contrast microscopy as compared to evaluation of stained dry smear using bright field microscopy. The unstained wet preparation were in buffered glutaraldehyde and formal saline. The most accurate results were in the wet preparations, formal saline was the most reliable.

Dott and Foster (1975) using the semen of different species found that formal saline or formal citrate were reliable for the preservation of differential staining of spermatozoa kept at 4^{oc}-40^{oc} for periods of three weeks.

Parrish *et al.* (1978) recommended that a minimum of 200 spermatozoa should be studied under the microscope at x400 or under oil immersion at x 1000-1250 for the evaluation or morphological abnormalities and the detection of the presnce of other cells

such as white blood cells and bacteria. However, Macleod and Godl (1951) suggested the examination of 300 cells in each smear for the morphological classification.

Sekoni *et al.* (1981) suggested that a combination of phase contrast microscopy and light microscopy seem ideal for obtaining the most reliable results in sperm morphologic evaluations. Head abnormalities could be studied with stained preparations of eosin-nigrosin or William's stain while other morphological abnormalities could be evaluated in wet fixed semen with phase contrast microscope.

Table 1: **The Principle for Evaluation of Sperm Morphology**

Normal	**Length**	**Width**	**Remarks**
	μ	μ	
Head	3.0-5.0	2.0-3.0	Regular oval shape border line forms have to be counted as normal.
Midpiece Tail	5.0-7.0 Approx. 45	Approx. 1	
Abnormal Heads			
Too small (micro)	>5	>3	As long as the shape
Too small (micro)	<3	<2	Approximates to an oval is to be counted primarily.
Tapering (narrow)	>5	>3	According to its size (too small)
Amorphous Duplicate (double) Pear shaped	<5	<2	Too large tapering counted under amorphous or tapering.
Tailless Head			Borderline forms are regarded as normal.
Abnormal Midpiece		>2.0	Cytoplasmic droplet is included here when it is larger than half of the sperm head.
Tail defects e.g. broken or coiled (not bent nor asymmetrical insertion.			

Source: From Eliasson (1971) Standards for Investigation of Human Semen. Andrologie 3:49-64.

The investigators also demonstrated that buffered formal saline is a good fixative for the bulls sperm morphology. It was also shown that semen wet fixed in buffered formal saline and kept at +4^{oc} could still give an accurate result for the live/dead differential staining and morphological evaluations at one month after semen collection and preservation. Therefore, semen fixed in buffered formal saline and stored at +4^{oc} for several days could still be transported to localities where there are ideal semen laboratory for accurate evaluation.

Menchini-Fabris *et al.* (1986), suggested that very good preparations may often be obtained with prestained slides. These slides are manufactured with a thin layer of stain (methylene Blue N and crystal violet acetate) covering the glass. Mechini- Fabris *et al.* (1986), showed that prestained slides (Testsimplets) worked very satisfactorily, rapidly and with minimal errors in handling, they also observed that the percentage of abnormal spermatozoa do not vary significantly in different samples from the same man.

2.5. SOME FACTORS WHICH ALTER NORMAL SPERM MORPHOLOGY

2.5.1 Origin of sperm defects

Williams (1920) and Lagerlof (1934, 1936) thoroughly investigated the occurrence of pathological spermatozoa in bulls with diminished or arrested fertility and by experimental research showed that the occurrence of pathological spermatozoa is connected with disturbances in spermatogenesis due to degenerative changes in the epithelium of the seminiferous tubules and proved conclusively that many morphologically abnormal spermatozoa resulted from testicular dysfunction.

Largerlof (1934) also indicated that maturation of the spermatozoa occur in the epididymis. Largerlof (1936), Parish *et al.* (1978) and Menchini-Fabris *et al.* (1986) confirmed that spermatozoa with cytoplasmic droplets suggest a failure of maturation of spermatozoa in the epididymis and epididymal dysfunction. Swanson and Boyd (1962) also showed that coiled and bent tails result from epididymal malfunction. Gustafsson *et al.* (1974) reported two cases of epididymal dysfunction with high incidence of sperm tail abnormalities. Cupps and Briggs (1965) gave supplementary estrogen to four bulls that subsequently produce large numbers of spermatozoa with coiled or bent tails as a result of epididymal epithelium degeneration which was corrected by administration of supplementary testosterone. Thus impairment of the epididymis causes the occurrence of some sperm morphological abnormalities.

2.5.2.Genetic factor

Bane (1954) working on identical twin bulls found genetic influence on the frequency and total number of abnormal sperm heads and cytoplasmic droplets. Menchini-Fabris *et al.* (1986) suggested that the sperm morphological abnormalities described by Schirren *et al.* (1971) acrosome; Bacetti *et al.* (1975) tail defects; Nistal *et al.* (1977) multiple tails; Bacetti *et al.* (1979) double forms; Afzelius and Eliasson (1979) and Perotti *et al.* (1981) detached abormal heads are related to genetic disorders which could not be modified by any treatment. (See Figure 10).

2.5.3. Frequency of ejaculation

Large numbers of spermatozoa containing cytoplasmic droplets may indicate epididymal dysfunction or the result of frequent ejaculations (Macleod (1962); Gustafsson, 1972; Parrish *et al.,* 1978).

Wells *et al.* (1971) observed that a period of four weeks or more of sexual rest resulted in large increase in aged acrosomes (rough or irregularly surfaced), an ejaculation frequency of four times weekly reduced aged acrosomes and maintained significantly fewer than a frequency of once weekly.

2.5.4. Osmotic Pressure

Alteration in the osmotic pressure of seminal fluid will affect the morphology of the spermatozoa. Lagarlof (1936) showed that mixing of semen with water leads to slight bending of the tail ("walking stick handle"). Kollicker (1856), noted that treatment of mammalian sperm with water causes coiling and spiralization. The result was confirmed by Drevius (1963) on bull, man and rabbit. Dispiralization sets in after spiralization at a high degree of hypotonicity. Spiralization is a result of influx of ions or other low molecular weight substances into the medium causing disturbances in the *contraction relaxation mechanisms.*

Pulsey and Herman (1950) studied the effects of hypertonic and hypotonic solutions on the liveability and morphology of bovine spermatozoa. they found that the optimum range for sperm survival was 0.44 to .061 in terms of freezing point depression or 2.3% - 3.5% in terms of sodium dihydrate concentration. There was little difference

Hultnas (1959) observed significant annual variations for sperm with abnormal heads and narrow heads in young bulls of Swedish red and white breed tested in September and April. Igboeli and Rakha (1971) also found that in the hot season, the Angoni (short-horn Zebu) bulls have a higher incidence of secondary abnormalities and increased cytoplasmic droplets. The influence of season on sperm morphology in humans is yet to be investigated in Nigeria.

2.5.7. The Effect of Age on Sperm Morphology

Macleod and Gold (1954) suggested that no relationship exist between the percentage of morphologically normal cells and age in a study of semen quality in relation to age and sexual activity. Kliesch *et al.* (1996) also demonstrated that sperm concentration, sperm motility and normal sperm morphology in the adolescent patients did not show significant differences compared with adults. It is expected that there should be variation in the incidence of abnormal sperm morphology in the peripubertal, young, old and very old people due to testicular degeneration in the very old people.

In bulls, Hahn *et al.*, (1969) found that the percentage of morphologically normal spermatozoa in semen at the time of collection were lowest in the aged Holstein bulls. Rao (1971) also demonstrated that age has an effect in sperm morphology in bulls. The influence of age on human sperm morphology is yet to be studied in Nigeria.

2.5.8. Effect of Diseases on Sperm Morphology.

a. *Effect of Allergy.*

Macleod J. (1965) showed that an increase in the amorphous spermatozoa and sperm precursors (Spermatids) often indicated an allergic reaction.

b. *Effect of inflammation and infection*

Macleod (1965) has demonstrated that an increase in the abnormal midpiece seems to be related to inflammatory disease while the presence of tapered (narrow) heads and sperm precursors are frequently the result of viral and bacteriological infections especially in the presence of large numbers of white blood cells. Osegbe (1991) and Merino *et al.*, 1995) reported the deleterious effect of bacterial infections on sperm morphology.

Burges and Chenoweth (1975) found high incidence of midpiece abnormalities in bovine semen following experimental and natural cases of bovine ephemeral fever. Charles (1950) in a study of patients with mumps orchitis and testicular atrophy showed increased mean morphologically abnormal spermatozoa compared to the control group.

Mefo (1981) found a history of present or past urethritis in 80% of patient with oligospermia and tetratospermia in Younde, Cameroon.

The influence of infection on sperm morphology in human is yet to be investigated in Northern Nigeria.

c. *Varicocele*

Macleod (1965), Rodniquer *et al.* (1981), Jecht *et al.* (1982) and Porluondo *et al.* (1983) have demonstrated increased sperm morphological abnormalities in varicocele.

A high incidence of tapering forms is frequently observed. It is postulated that varicocele cause oligospermia and abnormal sperm by increasing the scrotal temperature or by regurgitation of toxic substances from the renal vein.

d. ***Cryptorchidism***

Nistal and Panigua (1984) in a study of 23 infertile men with no other apparent cause of infertility than bilateral retractile testes, reported oligospermia and elevated sperm morphological abnormalities ranged between 60-80% (consisting mainly of tapering, amorphous and immature forms).

e. ***Sickle Cell Disease***

Osegbe *et al.*, (1981), in the study of semen characteristics of men with sickle cell disease demonstrated significant decrease in sperm concentration, total sperm count, motility and sperm morphology compared with semen characteristics of fertile male controls.

f. ***Influence of Drugs and Chemicals on Sperm Morphology***

el-Shoura *et al.*, (1995) demonstrated the deleterious effects of khat addiction on semen parameters in general and sperm morphology in particular especially when khat consumption was for longer periods of time.

2.5.9 Staining Technique

There is no doubt that some stains employed for morphological studies and the methods employed produce artifacts and inaccurate results. Procedures for preparing smear is detrimental to the acrosome. Campbell *et al.*, (1960) showed that eosin-nigrosin

gave inaccurate result as far as morphological studies are concerned. Bryan (1975) found using Eosin-fast green naphthol-yellow mixture stain to be reliable and accurate for the differential staining of cytologic components in many mammalian species.

2.6. RELATIONSHIP BETWEEN SPERM MORPHOLOGY AND OTHER SEMEN CHARACTERISTICS

Several workers have demonstrated that all ejaculate/semen characteristics are correlated (Macleod and Hotchkiss, 1946; Macleod and Gold, 1951; Freund, 1962; Sherins *et al.*, 1977 and Bostofle *et al.*, 1984). Macleod and Gold in (1951) showed that there is a decreasing number of good morphologically normal spermatozoa with increasing ejaculate volume in both fertile and infertile men from 6ml semen volume and above. Up to a count level of 100 million/cc, there is a rise in the average percentage of normal cells with increasing count, but above the 100 million/cc level, the average percent normal remains about the same (Macleod and Gold, 1951).

Macleod and Gold (1951) demonstrated that in both fertile and infertile men the percentage of motile cells rises significantly with rising percentages of morpholoically normal cells. Bostofle *et al.* (1984) concluded that all characteristics of semen provide independent information about fertility potential and the maximum possible information cannot therefore be expressed by use of only one or two characteristics.

CHAPTER THREE

MATERIAL AND METHOD

3.1. RESEARCH DESIGN

A total of 150 adult males in Zaria and environments whose wives were attending fertility clinic in Ahmadu Bello University Teaching Hospital in Zaria participated in this research. After obtaining personal dat, the medical history of each subject was obtained. Previous or active infection with sexually transmissible diseases, noting types and form of treatment obtained, pervious history of mumps parotitis/orchitis/ other diseases, trauma or surgery especially in the inguinal, perineal or scrotal areas were also noted. History of infertility in the family were also noted where present. Social habits like cigarette smoking, alcohol ingestion and drug usage were also obtained. (Research Protocol A.)

Detailed physical examination was carried out. Examination of the reproductive organs included measurement of scrotal circumstances using a metric tape, presence or absence and number of testes/testis in the scrotum were noted. Scrotum and scrotal contents were palpated to detect any abnormality such as varicocele, orchitis, cryptorchidism etc (Research Protocol B).

The physical examination was followed by sperm collection which was by masturbation after at least 3 days of abstinence from sex. The semen samples were collected into a sterile dry graduated centrifuge bottle. The colour and volume were recorded. The presence of blood, pus etc if any were noted. Sperm concentration of the semen was determined using the Haemocytometer and a microscope at x400 objective as

described by Coffin (1953). (RESEARCH PROTOCOL C). Samples with many pus cells were subjected to bacteriological studies (gram staining, culture and sensitivity).

Morphological studies on the first objective are referred to as study I and on the second and third objectives as study II.

3.2. STUDY I.

I. Direct smear with eosin-Nigrosin from fresh semen was done for each ejaculate by touching a spreader slide to a drop of the mixture of stain and raw semen (5.1) or according to sperm concentration and then drawing the mixture across the surface of a prewarmed clean slide at 45° angle (EN1).

II. Direct smears were made on prewarmed clean slide and stained later (within 3 days) in the laboratory with modified William stains (WS1).

III. Zero point five (0.5ml of the fresh semen was transferred to a test tube containing 5 mls of bufferred formal saline of 37°c. The formal saline fixed semen was used for phase contrast microscopy (FS1) and for preparation of stained smears (EN30 and WS30) after 1 month of storage at 4°c also for phase contrast microscopy at the end of one month storage (FS30) (see introduction; objectives).

3.2.2. The Preparation of Stained Smears with Carbol Fuchsin Eosin According to the Method Described by Williams, 1920 and Modified by Lagerlof, 1934

William staining:

1. Preparation of semen smears.

2. Drying in air and fixation over heat.

3. Fixing three to four minutes in absolute alcohol.

4. One to two minutes treatment with 1/4% chloramine solution until the mucus disappears and smears become fairly clear.

5. Rinsing in distilled water followed by 96% alcohol. a little alcohol left floating on the slides.

6. Staining with carbol fuchsin eosin for six to ten minutes.

7. Rinsing in tap water.

8. drying in air.

3.2.3. Laboratory examination

Semen wet fixed in buffered formal saline immediately after collection was handled and examined according to the following:

a. examined in phase contrast microscope shortly after the fixation (1-3 days and after a month's storage at 4^{oc}).

Wet preparation under small coverslip were made by placing a drop of the fixed semen on the centre of clean slide and coverslip was put on the drop. Care was taken to ensure that no bubbles and immersion oil got under the coverslip. The wet preparation under small coverslip was then examined and counted in a regular sequence with phase contrast microscopy at a magnification of x 1000 for the following abnormalities. Abnormal acrosome, detached normal heads, detached abnormal heads, proximal cytoplasmic droplet, distal cytoplasmic droplets,

abnormal midpiece and sperm abnormalities. A total of 400 well spaced spermatozoa were carefully examined in each preparation by scanning.

The morphologic characteristics studied were the following:

Cytoplasmic Droplets

I. Proximal

II. Distal.

Sperm Tail Abnormalities

I. Simple Bent

II. Coiled

III. Coiled around the head.

IV. Others.

Tailess Head

I. Normal head morphology

II. Abnormal head morphology.

Midpiece abnormalities

I. Swollen

II. Double

III. Rough

IV. Abaxial attachment

V. Coiled

VI. Other

Acrosome (Apical Cap

I. Detached Acrosome

II. Rough.

III. Irregularly surfaced.

IV. Others.

The study of Head Morphological Abnormalities

The stained smear (Eosin-Nigrosin and Williams stains) were used for the study of head abnormalities. Five hundred spermatozoa were counted systematically in each smear at a magnification of x 1000 under oil immersion.

The sperm Head-morphological abnormalities studied were the followings:

1. Narrow (tapering)
2. Narrow at the base
3. Pear shaped
4. Abnormal contour
5. Small normal (Micro)
6. Small abnormal
7. Short broad
8. Undeveloped/Immature forms (Sperm Precussors)
9. Giant
10. Double
11. Tailess pathological
12. Others.

3.3. STUDY II

3.3.1. Introduction

To establish values for different sperm morphologic abnormalities and some cause of variations such as age, seasons, sexually transmissible diseases, cryptorchidism, surgery and social habits like cigarette smoking and alcohol consumption, etc on human semen in Zaria. (see Research Protocol A and B).

A total of 150 subjects from Zaria and its environments participated in this study. The age range of the subject is from 20 years to 50 years and the research period was from January,1995 to December, 1995.

Immediately after semen collection 0.5ml was transferred to a glass vial containing 0.8ml of buffered formal saline solution. The semen samples were studied with phase and light microscopy within two weeks after collection, fixation and storage at $+4^{oc}$.

3.3.2 Examination of Semen with the Phase Contrast Microscope

Semen wet fixed in buffered formal saline immediately after collection were examined exactly as described for study I.

3.3.3 The Preparation of Stained Smears with Modified Williams Stains

Decantation of buffered formal saline was done and smears were made from the fixed semen.

Smears were stained and head morphological abnormalities studied exactly as done in study I. Microscopical examination were done to study the morphological abnormalities of the sperm head, cytoplasmic droplets, abnormal acrosome, detached normal heads, detached abnormal head, abnormal midpiece and abnormal sperm tail. When the sperm count was less than 20 million/ml, the semen sample was centrifuged for 15 minutes at 2000 RPM and the semen smear was prepared from the sedimented material.

The meteorological data for Zaria for the year 1995 was collected from the meteorological unit of the Institute for Agricultural Research (IAR), Ahmadu Bello University (ABU), Zaria to study the influence of season on human sperm morphology.

3.4 METHODS OF STATISTICAL ANALYSIS

To establish whether there are differences in the result of sperm morphological abnormalities using the three different methods of examination, analysis of variance was used. To establish if there is any effect of no storage (day one) and storage for one month on sperm morphological abnormalities, pair comparison test for result of day one and after 30 days was used. The effect of the three methods of examination of sperm morphology and the effect of fixation and storage at $+4^{oc}$ for one month combined was analysed by a randomized block two way analysis of variance. Means that had a probability of 0.05 or less from the same population were reported as significantly different.

Student t-test was used to examined the variation of maximal mean temperature and relative humidity of Zaria for the study period of one year (1995).

Z-test (probability test) was used to examine the effect of age, season, smoking, alcohol ingestion and sexually transmissible diseases on sperm morphological abnormalities.

Means with different superscripts in the tables are significantly different.

CHAPTER FOUR

RESULTS

4.1. THE EFFECTS OF PREPARATION, FIXATION IN BUFFERED FORMAL SALINE AND STAINING TECHNIQUES ON HUMAN SPERM MORPHOLOGY.

4.1.1 Pathologic Head

The results for abnormal sperm heads studied with stained smear preparations of eosin Nigrosin and Williams stain and studied with only light microscope are presented in table 2 and Appendix 1a.

Fresh semen smears stained with eosin Nigrosin on day one (EN1) and studied with the light microscope had a mean of 10.67% head abnormalities. Semen fixed in buffered formal saline stored for a month at +4^{oc} and stained with eosin Nigrosin had a mean of 10.54% head morphological abnormalities. A month later, after fixation in buffered formal saline and storage and staining with Williams stains and study with light microscope (WS3O) a mean of 11.36% head abnormalities was obtained which is not significantly different from the mean of 11.07% obtained on day one. (WS1).

The results obtained on day zero for fresh semen smears stained with eosin Nigrosin and Williams stain did not differ significantly from those obtained a month after the semen had been fixed in buffered formal saline and stored at 4^{oc}. There is no significant difference in the result obtained for the three methods of observation and also between no storage and storage at +4°C for one month.

Table 2. **Percentage of sperm abnormalities in 150 adult males using three methods of preparations and staining on day one and at a month after fixation and storage at +4°c.**

Comparsions	FS1	FS30	EN1	EN30	WS1	WS30
No. of subjects	150	150	150	150	150	150
ABNORMALITIES						
Head abnormalities	-	-	10.67 (0.41)	10.54 (0.38)	11.07 (0.45)	11.37 (0.46)
Acrosome Abn.	6.2 (0.48)	6.4 (0.48)	6.1 (0.43)	6.4 (0.43)	6.15 (0.44)	6.24 (0.44)
Prox. Cyto. Droplets	2.6 (0.28)	2.5 (0.25)	2.38 (0.23)	2.23 (0.21)	2.01 (0.20)	2.4 (0.21)
Dist.Droplets	0.92 (0.14)	0.94 (0.13)	0.80 (0.10)	0.80 (0.09)	0.92 (0.09)	1.11 (0.11)
Midpiece Abnormalities	6.28 (0.31)	6.36 (0.33)	6.37 (0.34)	6.54 (0.34)	6.85 (0.34)	6.96 (0.32)
Tail Abnorm.	9.13 (0.44)	9.07 (0.44)	9.20 (0.46)	9.34 (0.48)	8.61 (0.46)	8.71 (0.45)
Detached Normal Head	2.74* (0.30)	2.85+ (0.29)	3.20* (0.30)	3.95+ (0.31)	3.25* (0.25)	3.63+ (0.26)
Detached Abnormal Head	4.70*	4.82*	5.27	5.30	5.40	5.62

* Means were significantly different ($P<0.05$)

FS_1 Semen fixed in buffered formal saline on day one and studied with phase microscope.

FS_{30} Semen fixed in buffered formal saline after a month storage at +4°C and studied with phase microscope.

EN_1 Fresh semen smears stained with eosin-nigrosin on day one and studied with the light microscope.

EN_{30} Smears made from semen fixed in buffered formal saline and stained with eosin-nigrosin after a month storage at +4°C and studied with the light microscope.

WS_1 Fresh semen smears stained with Williams stain on dayone and studied with the light microscope.

WS_{30} Smears made from semen fixed in buffered formal saline and stained with Williams stain after a month storage at +4°C and study with the light microscope.

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4.1.2 Acrosome Abnormalities (rough, irregularly contoured, detached)

The mean obtained for acrosomal abnormalities for semen fixed in buffered formal saline and studied on day one with the phase contrast microscope was 6.2% while a month later after fixation and storage at $+4^{oc}$, the mean was 6.4%. The result for day one and one month were not significantly different. Fresh fixed semen smears stained on day one with eosin Nigrosin and studied with light microscope had a mean of 6.1% a month later after fixation in buffered formal saline and storage at $+4^{oc}$ the mean was 6.4% which is not significantly different from that of day one. Fresh semen stained with Williams stain on day one had a mean of 6.15% while after fixation in buffered formal saline and storage at $+4^{oc}$ for a month, the mean was 6.24 when studied with the light microscope. The two results are not significantly different. The mean of 6.4% obtained with the phase contrast microscope for acrosome abnormalities in semen fixed in buffered formal saline and stored at $+4^{oc}$ for a month did not differ significantly from the mean of smears of semen fixed in buffered formal saline and stained with Eosin Nigrosin after a month storage at $+4^{oc}$ and studied with the light microscope. There was no significant difference between the results obtained for all the methods of observation, also there was no significant difference between day one and a month of storage for all the methods. The results are summarized in Table 2 and Appendix 1b.

4.1.3 Proximal Cytoplasmic Droplets

The highest percentage mean of proximal cytoplasmic droplets was recorded under the phase contrast microscope in semen fixed in buffered formal saline immediately after semen collection.

The result obtained with the phase microscope (2.6%) however, did not differ significantly from 2.38% obtained with fresh semen stained with eosin Nigrosin and from 2.10% from fresh semen smears stained with Williams stain and studied with light microscope immediately after semen collection.

After one month fixation in buffered formal saline at a temperature of $+4^{\circ c}$ the mean percentage for proximal Cytoplasmic droplets for phase contrast microscopy of unstained sample was 2.5% which is not significantly different from the result obtained from Williams stain (2.4%). There is no significant difference from the results obtained for proximal cytoplasmic droplets using the three methods of observation and between no storage and storage for one month at $+4^{\circ c}$. The results are summarized in Table 2 and Appendix 1c.

4.1.4 Distal Cytoplasmic Droplets

See Table 2 and Appendix 1d for results. The percentage of distal cytoplasmic droplets observed with phase contrast microscope in semen fixed in buffered formal saline and studied immediately after collection had a mean of 0.92% which was not significantly different from the mean of 0.80% obtained with fresh semen stained with Eosin Nigrosin and 0.82% obtained with fresh semen stained with Williams stain. A

month later, after fixation in buffered formal saline and storage at +4°c, the wet fixed semen in buffered formal saline studied with the phase microscope had a mean of 0.94% which is not significantly different from 0.80% for semen smears stained with Eosin-nigrosin after a month's fixation in buffered formal saline and storage at +4°c. The value (0.94%) did not also differ significantly form 1.11% for semen smears stained with Williams stain after a month fixation in buffered formal saline and storage at +4°C. The results obtained at day one did not differ significantly from that obtained at a month after storage at +4°c and evaluation with the phase microscope. Also, the result obtained using the three methods of observations did not differ significantly.

4.1.5 Midpiece Abnormalities (double, coiled, rough, swollen)

See Table 2 and Appendix 1e for results. The results obtained on day one were not significantly different from those obtained a month later after fixation and storage at +4°c. The mean for the fixed semen in buffered formal saline on day one studied with the phase microscope was 6.28% midpiece abnormalities while a month later following storage at +4°c in formal saline a mean of 6.36% was obtained. Fresh semen smear stained with eosin nigrosin on day one and studied with the light microscope had a mean of 6.37% midpiece abnormalities, fixed semen smears stained with eosin Nigrosin after a month storage at +4°c had a mean of 6.54% while fresh semen smears stained with Williams stain and studied with the light microscope on day one had a mean of 6.85% midpiece abnormalities while following fixation in buffered formal saline and a month storage at +4°c the mean was 6.96% for smears stained with Williams stain and studied

with the light microscope. The results obtained for the three methods of observation were not statistically significant. Similarly, the results obtained on day one and after a month fixation and storage at $+4^{oc}$ were not significantly different.

4.1.6 Sperm-Tail Abnormalities

See table 2 and Appendix 1f for the result obtained. The mean for semen fixed in buffered formal saline immediately after collection on day one with the phase microscope was 9.13% tail abnormalities while a month later following fixation and storage at $+4^{oc}$ the mean was 9.07% tail abnormalities. Fresh semen smears stained with eosin Nigrosin on day one and studied with the light microscope had a mean of 9.20% for tail abnormalities, wet fixed semen smears stained with eosin Nigrosin after a month storage at $+4^{oc}$ had a mean of 9.34% tail abnormalities with light microscope. Fresh semen smears stained with Williams stain and studied with the light microscope on day one had a mean of 8.61% tail abnormalities while following fixation and a month storage at $+4^{oc}$ the mean was 8.71% tail abnormalities. The results obtained on day one were not significantly different from those obtained a month later after fixation in buffered formal saline and storage at $+4^{oc}$. Similarly, the results obtained using the three methods of observation for sperm tail abnormalities were not significantly different.

4.1.7 Detached Normal Sperm head

The result obtained for detached normal sperm heads is also presented in Table 2 and Appendix 1g. On day one, detached normal sperm heads in semen fixed in

Higher mean values were also obtained for detached normal heads with semen smears obtained with Eosin Nigrosin and Williams stain when examined with light microscope than for fixed semen examined with the phase contrast microscope for both before and after fixation and storage at +4^{oc} for one month.

4.1.8 Detached Abnormal Head

The results are summarized in Table 2 and Appendix 1h. The mean for semen fixed in buffered formal saline immediately after collection on day one with phase microscope was 4.70% for detached abnormal heads which is not significantly different from 4.82% obtained a month later after fixation in buffered formal saline and stored at +4^{oc}. Fresh semen smears stained with Eosin Nigrosin on day one and studied with the light microscope had a mean of 5.27% for detached abnormal head, this is not significantly different from 5.30% obtained after fixation and a month storage at +4^{oc} and smears stained with Eosin Nigrosin when examined with light microscope. Fresh semen smears stained with Williams stain and studied with light microscope had a mean of 5.40% for detached abnormal heads which is not significantly different from 5.62% obtained after fixation and storage at +4^{oc} for one month. The values obtained for detached normal heads (4.70% and 4.82%) before and after storage respectively when examined by phase microscope is significantly different ($P<0.01$) from values (5.27% and 5.30%) before and after fixation and storage for a month at +4^{oc} respectively when semen smears are stained with eosin Nigrosin and examined with light microscope respectively, and significantly different ($P<0.01$) from mean values of 5.40% and 5.62%

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obtained for detached abnormal heads before and after fixation and storage for month at $+4^{oc}$ respectively. Higher mean values of detached abnormal heads were also obtained with the stained smears than values from fixed semen examined with phase contrast microscope.

4.2 THE INCIDENCE OF SPERM MORPHOLOGICAL ABNORMALITIES IN HUMAN SEMEN IN ZARIA WITH ASPECTS ON SOME CAUSES OF VARIATION

Values for sperm morphological characteristics in human semen.

The mean percentage abnormalities found in the human semen in the year 1995 are presented in Table 3. Detailed result obtained for all the 150 subjects are presented in appendix II.

There were 150 subjects in this study, medical history, physical examination and sperm morphological abnormality evaluations were done as described for study II. (See survey of materials and methods). From this study, morphologically abnormal sperm head constitute the highest (11.1%) of the total sperm abnormalities followed by Tail abnormalities (9.13%), midpiece has a mean of 6.28% while acrosome abnormalities accounted for 6.18%. The lowest sperm abnormality in the study is the Distal Cytoplasmic droplets with a mean of 0.29%. The total sperm abnormalities in the study has a mean of 43.69%. From the study group of 150 subjects, only 24% (16) has total sperm abnormalities of 25% and below. While majority (76%) had total sperm number of 26% and above. (See figure 10a, b, c, d, e and f).

Table 3: **Total Sperm Morphological abnormalities (% Mean)**

ABNORMALITIES N= 150	% Mean	(SEM)
Acrosome	6.18	(0.48)
Detached Normal Head	2.74	(0.29)
Detached abnormal Head	4.70	(0.33)
Proximal Cytoplasmic Droplets	2.62	(0.28)
Distal " "	0.92	(0.14)
Midpiece abnormalities	6.28	(0.36)
Tail abnormalities	9.13	(0.44)
Head abnormalities	11.10	(0.46)
TOTAL	43.69	(1.29)

SEM - Standard Error of Mean.

4.2.1 The Influence of Age on Sperm Morphology

The semen of one hundred and fifty (150) adult subjects were analysed in 1995. Their age ranged from 20 years to 55 years. They were divided into 2 age groups. The young adults were aged 18 years to 30 years while the middle age adult were aged between 30 years to 55 years. The result of sperm morphological abnormalities evaluated as in Study II are presented in Table 4.

RESULT

Acrosome Abnormalities

The mean percentage of acrosomal abnormality for the young adults was 6.06 which is not significantly different from 6.27% in the middle aged adult.

Detached Normal head

The mean of detached head in the young adult is 3.2% while in the middle aged adult the mean of detached normal head is 2.42%. The two results are not significantly different.

Detached Abnormal Head

There is no significant difference between the mean of detached abnormal head between the young adults (4.9%) and 4.57% in the middle aged adult.

Table 4: % Mean of Sperm Morphological Abnormalities in Two Age Groups

	Young Adult < 30 years (n=62)		Middle aged adults >30yrs (n= 88)	
	Mean	SEM	Mean	SEM
Acrosome	6.06	(0.76)	6.27	(0.62)
Detached Normal Head	3.02	(0.58)	2.42	(0.28)
Detached abnormal Head.	4.09	(0.54)	4.57	(0.42)
Prox.Cyto.Drroplets	2.27	(0.34)	2.87	(0.41)
Dist. Cyto.droplets	0.90	(0.24)	0.94	(0.16)
Moidpiece	6.87	(0.49)	5.86	(0.41)
Tail abnormality	8.80	(0.59)	9.37	(0.62)
Head abnormality	11.54	(0.64)	10.8	(0.63)
Total	44.54	(1.99)	43.09	(1.70)

Proximal Cytoplasmic Droplets

Proximal cytoplasmic droplets has a mean of 2.27% in the young adult and this is not significantly different from 2.87% in the middle aged adults.

Distal Cytoplasmic Droplets

The mean of Distal cytoplasmic droplets in the young adults is 0.90% which is not significantly different from 0.94% in the middle aged adult.

Midpiece Abnormalities

The mean of midpiece abnormalities in the young adults is 6.87% which is not significantly different from the 5.86% in the middle aged adults.

Tail Abnormalities

There is no significant different between the mean of 8.80% tail abnormalities found in the young adults and 9.37 in the middle aged adults.

Pathologic Head

The abnormal sperm head has a mean of 11.54% in the young adult while the value in the middle aged adults has a mean of 10.8%. The two values are not significantly different.

Total Sperm Abnormalities

The mean value of total sperm morphological abnormalities in the young adults was found to be 44.54% which is not significantly different from 43.09% total sperm morphological abnormalities in the middle aged adults.

4.2.2 Effects of Sexually Transmissible Diseases on Sperm Morphology in Humans

Out of the one hundred and fifty subjects (150) that participated in the study, 47.3% (71) had past or present history of infections of the genital tract, urethritis, orchitis, epididymitis and syphilis while 48.3% had no history of STD, six subjects were not sure. The causative agents were Neisseria gonorrhea, staphlococus aureus, coliform spp. and syphilis (treponema pallidum). The six subjects who were not sure of their history of previous sexually transmissible disease were excluded. Comparison was made between the two groups by Z-test and the results obtained is presented in Table 5.

Table 5: **% Mean Sperm Morphological Abnormalities in Subjects with and those without Sexually Transmissible Dieases.**

	STD(n=71)		No STD(n=73)	
Abnormalities	Mean	SEM	Mean	SEM
Acrosome	5.95	(0.67)	6.39	(0.68)
Detached Nor. Head	2.86	(0.47)	2.64	(0.36)
Detached Abnor.Head	4.83	(0.52)	4.60	(0.42)
Prox. Cyto.Droplets	2.34	(0.36)	2.88	(0.42)
Dist.Cyto. Droplets	0.94	(0.22)	0.91	(0.17)
Midpiece	7.06*	(0.47)	5.58*	(0.42)
Tail	10.33*	(0.68)	8.06*	(0.54)
Head.	11.94	(0.69)	10.35	(0.60)
TOTAL	46.25*	(1.90)	41.39*	(1.73)

* Significantly different means ($P < 0.05$)

Acrosome, Detached Head, Cytoplasmic Droplets and Head Abnormalities

From the results obtained, there is no significant different between the mean values for acrosome abnormalities, detached normal head, detached abnormal head, proximal cytoplasmic droplets, distal cytoplasmic droplets and head abnormalities between the subjects with sexually transmissible diseases and the subjects without sexually transmissible diseases.

Midpiece Abnormalities

The mean value for midpiece abnormalities for the group with history of sexually transmissible disease is 7.06% which was significantly higher. ($P<0.05$) from 5.58% for the groups without STD.

Tail Abnormalities

The mean for tail abnormalities in the STD group was found to be 10.33% which is significantly higher ($P<0.05$) from 8.06% in the group without STD.

Total Sperm Morphological Abnormalities

The mean total sperm morphological abnormalities for the group with sexually transmissible disease was found to be 46.25% which is not significantly different ($P<0.05$) the 41.39% in the group without STD.

4.2.3 The Influence of Season on Human Sperm Morphology

In determining whether there are seasonal and monthly variations on human sperm morphology, semen samples collected from 150 subjects who participated in the study from January 1995 to December 1995 were evaluated according to the procedure described in Study II.

The meteorological data for Zaria for the year 1995 is presented in Table 6. The results of the monthy variations for the sperm morphological abnormalities are presented in Figure 1-9 and Appendix III. Zaria has three distinct seasons (dry/hot partly harmattan; rainy, and dry/cold partly harmattan. The result for morphological studies for the three seasons is presented in Table 7.

Table 6. **Meterorological Data Samaru, Zaria 1995**

Month	Temperature			Rainfall		Relative Humidity
	Max.	Min.	Mean	(MM)	Days	m%(1000)
Jan.	28.2	10.5	19.4	-	-	31.4
Feb.	30.5	13.3	31.9	-	-	28.5
Mar.	35.8	18.4	27.1	-	-	26.3
Apr.	36.2	19.4	27.8	90.5	4	41.2
May	34.2	17.0	25.6	45.3	4	68.0
June	32.4	15.6	23.5	125.4	12	70.6
July	29.8	21.7	25.8	155.6	13	79.5
August	28.7	20.1	24.4	258.6	22	81.8
Sept.	29.9	20.4	25.2	159.1	16	81.3
Oct.	32.0	19.0	25.5	32 .5	3	74.3
Nov.	28.4	15.6	22.0	-	-	43.7
Dec.	32.9	14.1	23.5	-	-	20.7

The mean maximum temperature for the dry/hot, partly Harmattan season in 1995 was 32.7°C which was significantly higher ($P<0.05$) than 31.3°C for the rainy season and also significantly higher ($P<0.05$) than 30.8°C for the dry/cold and partly Harmattan season. Also, the mean temperature of 26.6°C for the dry/hot and partly harmattan season was significantly higher ($P<0.05$) than 24.8°C for the rainy season and significantly higher ($P<0.05$) than 24.1°C for the dry/cold and partly harmattan season. However, the mean relative humidity for the dry/hot partly harmattan season in 1995 was 31.85% which is significantly lower ($P<0.05$) than 74.98% for the rainy season and significantly lower ($P<0.05$) than 55.0% for the dry/cold and partly harmattan season of the same year.

Acrosome Abnormalities

The result for acrosome abnormalities during the three seasons is summarized in Table 7. The mean for acrosome abnormalities during the rainy season was 6.74% which is significantly higher ($P<0.05$) than the mean of 5.21% obtained during the dry/cold and partly harmattan season.

The mean of 6.74% though numerically higher, did not differ significantly from the mean of 6.0% obtained during the dry/hot partly harmattan season. The incidence of acrosomal abnormalities showed two peaks in the months of May and July and gradually decreased to the lowest in October. (See Figure 6 and Appendi III).

Detached (tail-less) Normal Head

The mean of 3.28% obtained for detached normal head during the dry/hot partly harmattan season is significantly higher ($P<0.05$) than the mean of 2.28% and 2.61% obtained during the rainy and dry/cold partly harmattan seasons respectively (See Table 7). The incidence of the detached normal head was highest in April, August and November while the lowest incidence was obtained in February, June and September (see Figure 7 and Appendix III).

Detached (tailless) Abnormal Head

The mean of 3.88% obtained for detached abnormal head during the rainy season is significantly lower ($P<0.05$) than the mean of 5.57% obtained during the dry/hot partly harmattan season and also significantly lower than the mean of 5.58% obtained during the dry/cold partly harmatan season. (The result is summarised in Table 7). The incidence of detached abnormal head decreased from a high level (6.93%) in January gradually until June and July when the lowest incidence was obtained (2.3%). There was a sharp rise in incidence in August (6.93%) and a gradual rise from September to December. (See Figure 8 and Appendix III).

Table 7: **Percentage Mean Sperm Morphological Abrnomalities during Three Seasons in Zaria, 1995.**

Season	1	2	3
Abnormalities	Dry/Hot Partly Harmattan. (Jan.-April) n=46	Rainy (May-August) n=71	Dry/Cold partly Harmattan (Sept.-Dec.) n=33
Acrosome	6.00	6.74+	5.21+
Detached Normal Head	3.28+	2.28	2.61
Detached Abnormal Head	5.57+	3.88	5.86+
Proximal Cytoplasmic Droplets	2.74	3.05	1.81
Distal Cytoplasmic Droplets	0.77	1.02*	0.79
Midpiece	6.70	5.8 +	7.09
Tail	9.52	10.03+	8.46
Head	10.52	11.76+	9.84
Total	45.89+	44.55+	42.02+

* - Higher Means Values

\+ - Significantly different means ($P<0.05$).

● - Mean T° = 32.7°C > 31.3°C 30.8°C

◆ - Relative Humidity = 31.85% 74.98% 55.0%

Proximal Cytoplasmic Droplets

A summary of the results for proximal cytoplasmic droplets in the three seasons is presented in Table 7. The mean for proximal cytoplasmic droplets during the dry season was found to be 3.05% which is not significantly different ($P<0.05$) from the mean of 2.74% and 1.81% obtained during the dry/hot partly harmattan and dry/cold partly harmattan seasons respectively. The high incidence of proximal cytoplasmic droplets obtained in the month of January (3.34%) gradually declined through the month of April. The incidence of proximal cytoplasmic droplets had two other peaks in the months of May (3.18%) and July (4.13%) and declined gradually towards the end of the year (see Figure 1 and Appendix III).

Distal Cytoplasmic Droplets

The result for the seasonal variations is presented in Table 7. Numerically, the mean of 1.02% obtained during the rainy season is higher than the mean of 0.77% obtained during the dry/hot partly harmattan season and the mean of 0.79% obtained during the dry/cold partly harmattan season. The three means are not significantly different. There was gradual increase in the incidence of Distal cytoplasmic droplets from January (0.38%) to March (1.09%). Distal cytoplasmic droplets also had two peaks in the months of July (1.71%) and October, (1.68%), (See Figure 2 and Appendix III).

Midpiece Abnormalities

A summary of the results for midpiece abnormalities for the three seasons is presented in Table 7. The mean of midpiece abnormalities during the dry/hot partly harmattan season was 6.7% which is not significantly different from the mean of 7.09% obtained during the dry/cold partlyharmattan season. The mean of 5.8% obtained during the rainy season is significantly lower ($P < 0.05$) than the means obtained during the other two seasons. The incidence of midpiece abnormalities declined from a peak of 8.63% in January to 5.13% in April. The mean value of Midpiece abnormalities remains fairly stable until towards the end of the year when it increased from 5.75% in September to 7.83% in December (See Figure 3 and Appendix III).

Tail Abnormalities

A summary of the results for seasonal variation is is presented in Table 7. The mean of 9.52% obtained during the dry/hot partly harmattan season is not significantly different from the mean of 10.03% obtained during the rainy season. The two means are significantly higher ($P<0.05$) than the means of 8.46% obtained during the dry/cold partly harmattan season. There was a decline in the incidence of sperm tail abnormalities from 11.35% in January to 8.21% in March. There was a sudden rise in the incidence of sperm tail abnormalities from 6.8% in June to 13.0% in August. The level of sperm tail abnormalities fell to the lowest level of 6.58% in September and rose gradually to 10.28% in December (See Figure 4 and Appendix III).

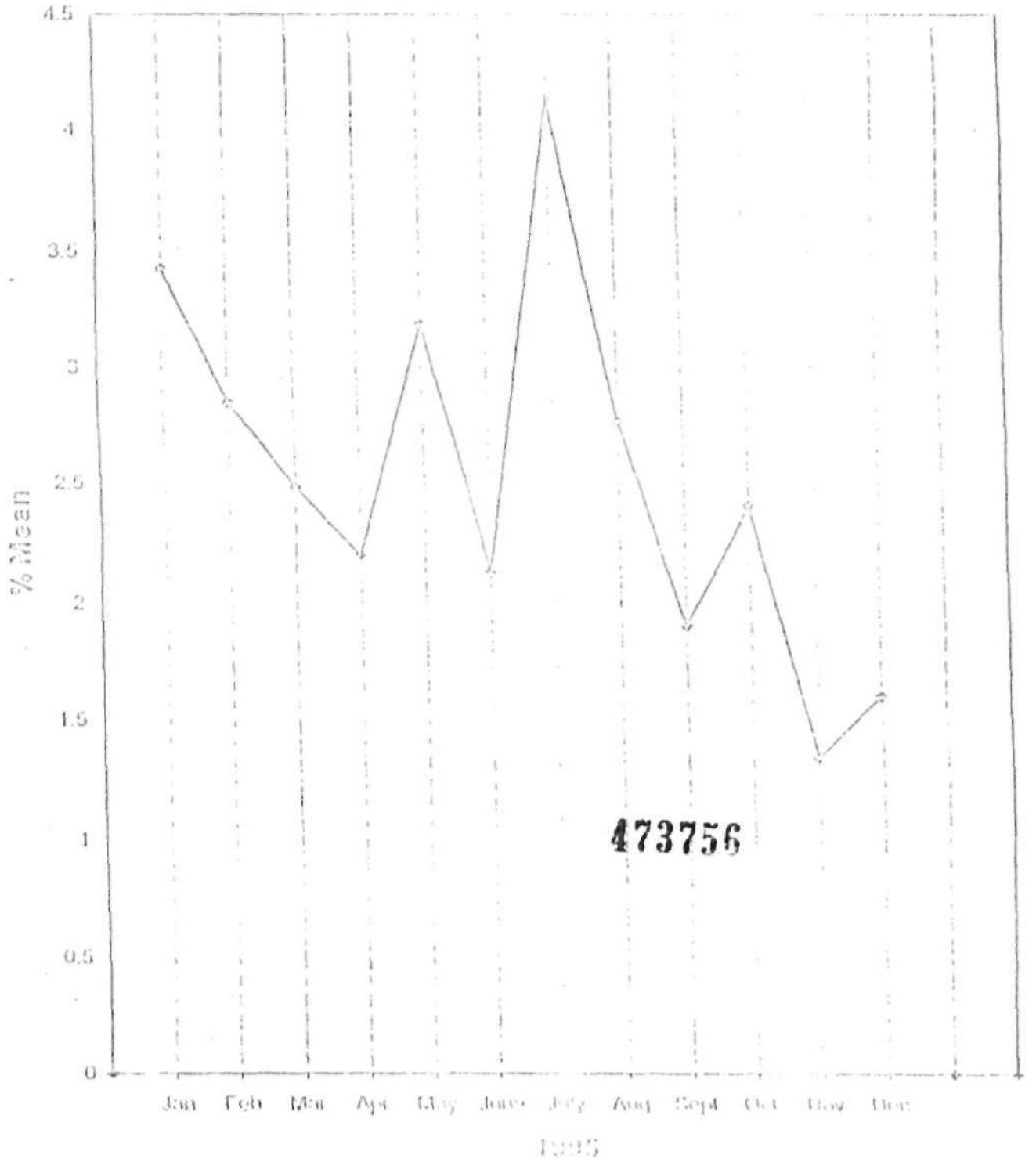
Head Morphological Abnormalities

The result obtained for the head morphological abnormalities for the three seasons is summarised in Table 7. The mean for sperm head morphological abnormalities during the rainy season was 11.76%. This value though higher, is not significantly different from the mean of 10.52% obtained during the dry/hot partly harmattan season. The mean of 11.76% obtained during the rainy season is significantly higher ($P<0.05$) than the mean of 9.84% obtained for sperm head morphological abnormalities during the dry/cold partly harmattan season (See Table 7). Sperm head abnormalities had high values during the months of May (13.35%) and July (13.55%) and the lowest incidence was obtained during the months of March (7.33%) and September (7.48%). The results are presented in Appendix III and Figure 5.

Total morphological abnormalities

The summary for the total morphological abnormalities for the three seasons is presented in Table 7. The mean for total sperm morphological abnormalities during the dry/hot partly harmattan season was found to be 45.89% which is significantly higher ($P<0.05$) than the mean of 44.55% obtained during the rainy season and significantly higher ($P<0.05$) than the mean of 42.02% obtained during the dry/cold partly harmattan season. The incidence of total sperm abnormalities showed significant monthly variations ($P<0.05$). There was a significant decrease ($P<0.05$) in the incidence from 55.02% in January to 37.34% in March. Two peaks periods in the incidence of total sperm morphological abnormalities were observed in April (44.85%) and August (48.15%). There was also a gradual increse in the incidence from 33.16% in September to 46.25% in December (See Figures 6 to 9 and Appendix III).

Figure 1 : Monthly Variations of Proximal Cytoplasmic Droplets



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Figure 2 : Monthly Variations of Distal Cytoplasmic Droplets.

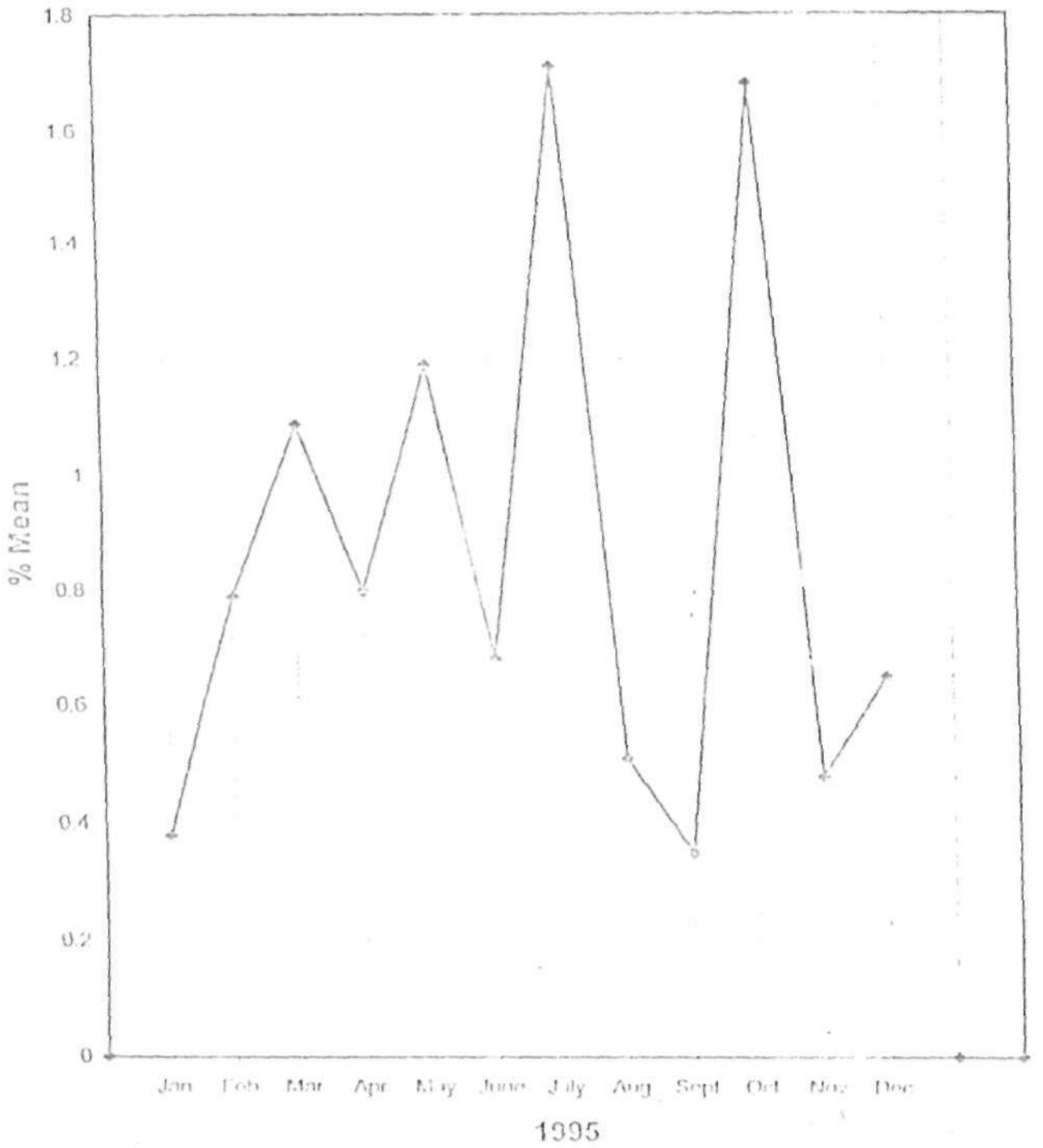


Figure 3 : Monthly Variations of Midpiece Abnormalities.



Figure 4 : Monthly Variations of Sperm Tail Abnormalities.

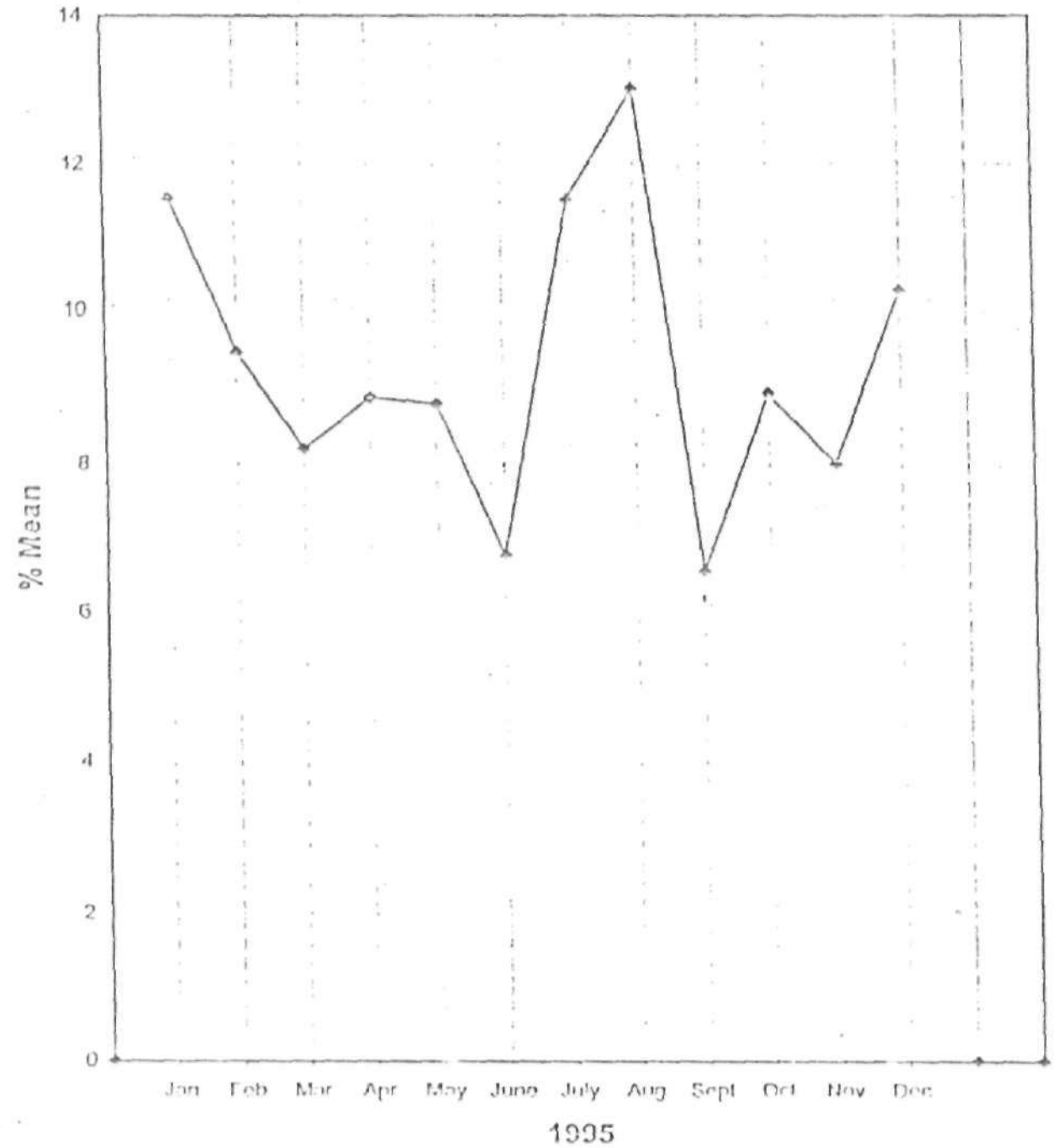


Figure 5 : Monthly Variations of Sperm Head Abnormalities.

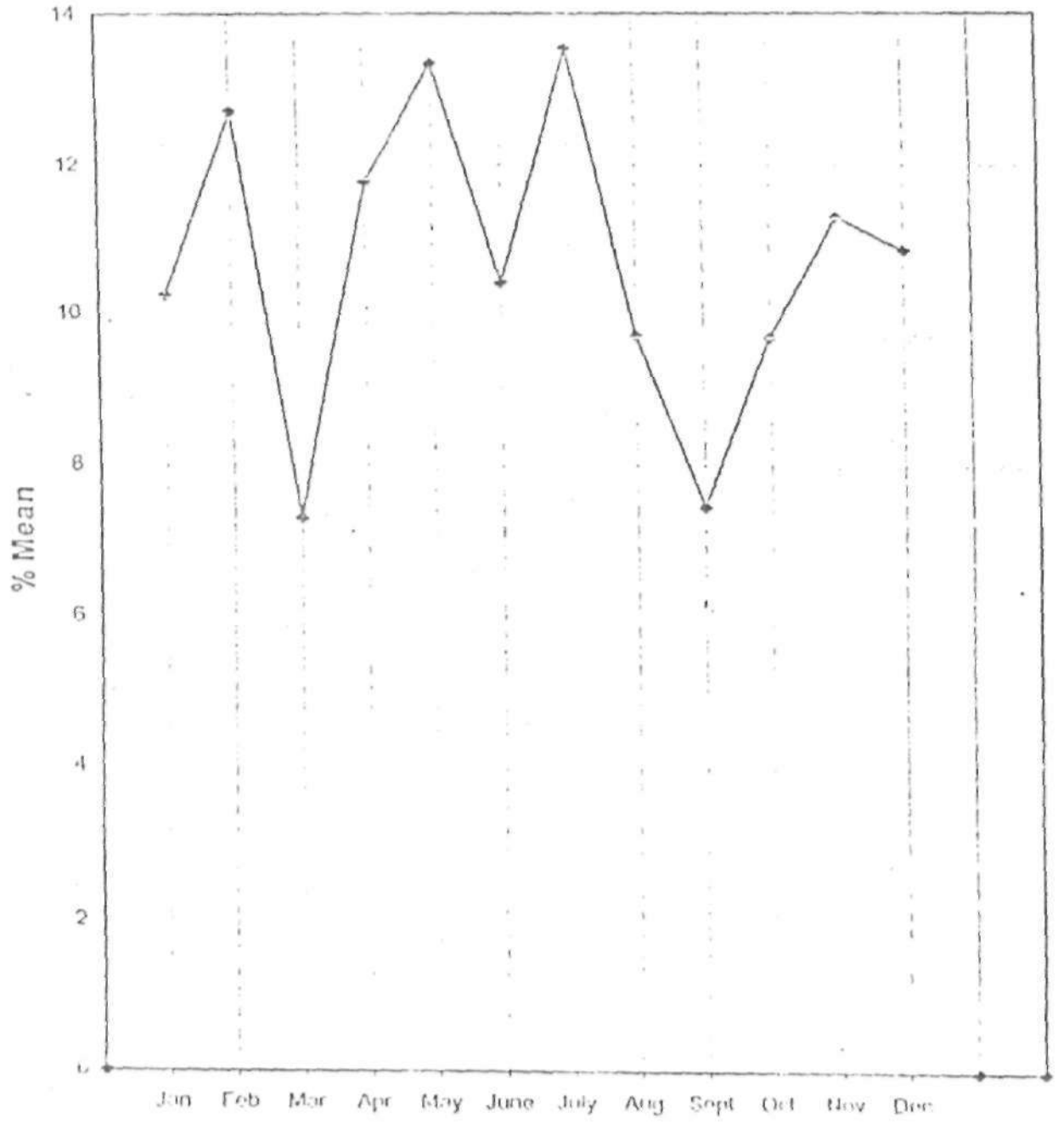


Figure 6 : Monthly Variations of Acrosomal Abnormalities.

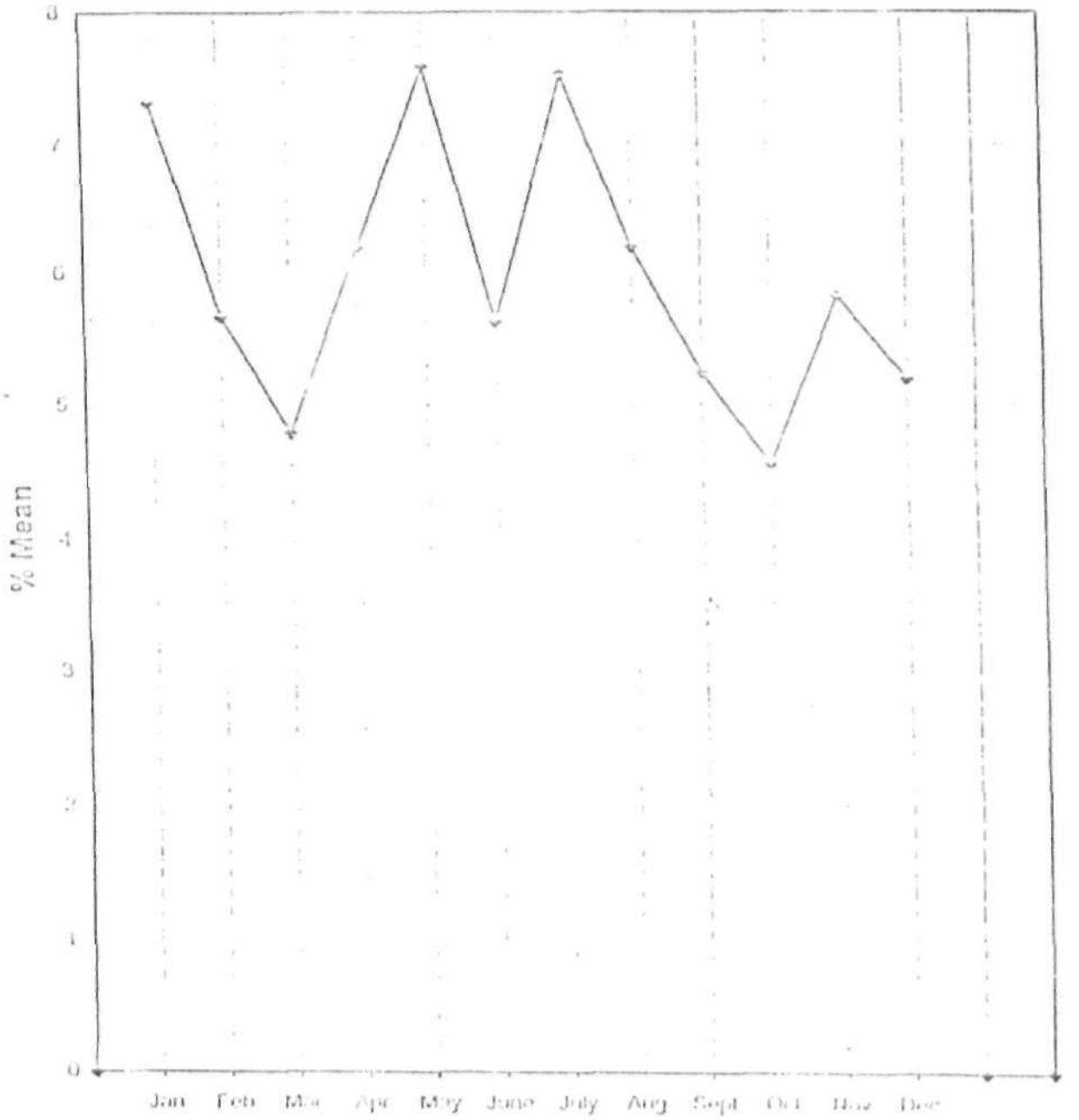


Figure 7 : Monthly Variations of Detached Normal Heads.

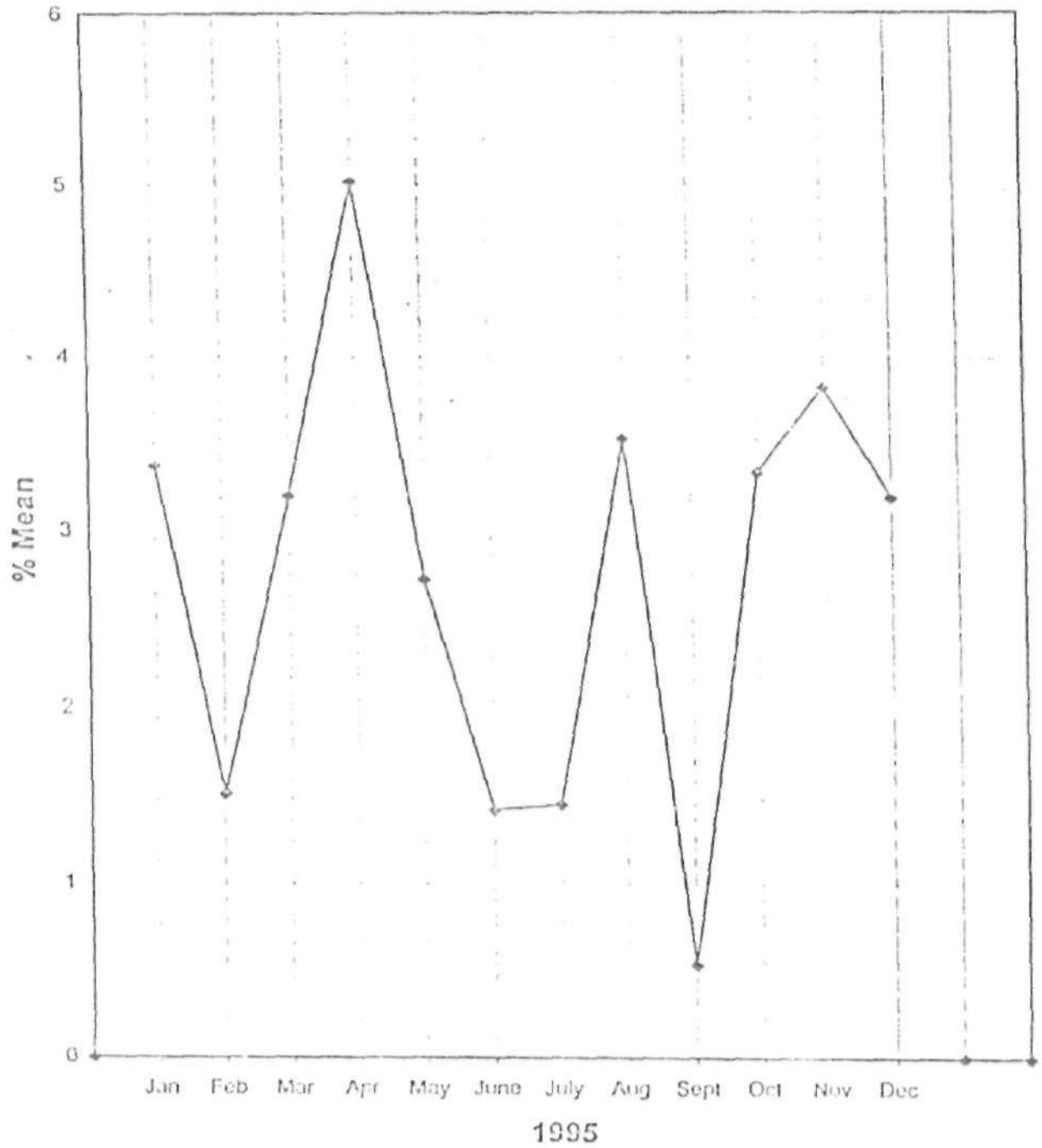


Figure 8 : Monthly Variations of Detached Abnormal Head

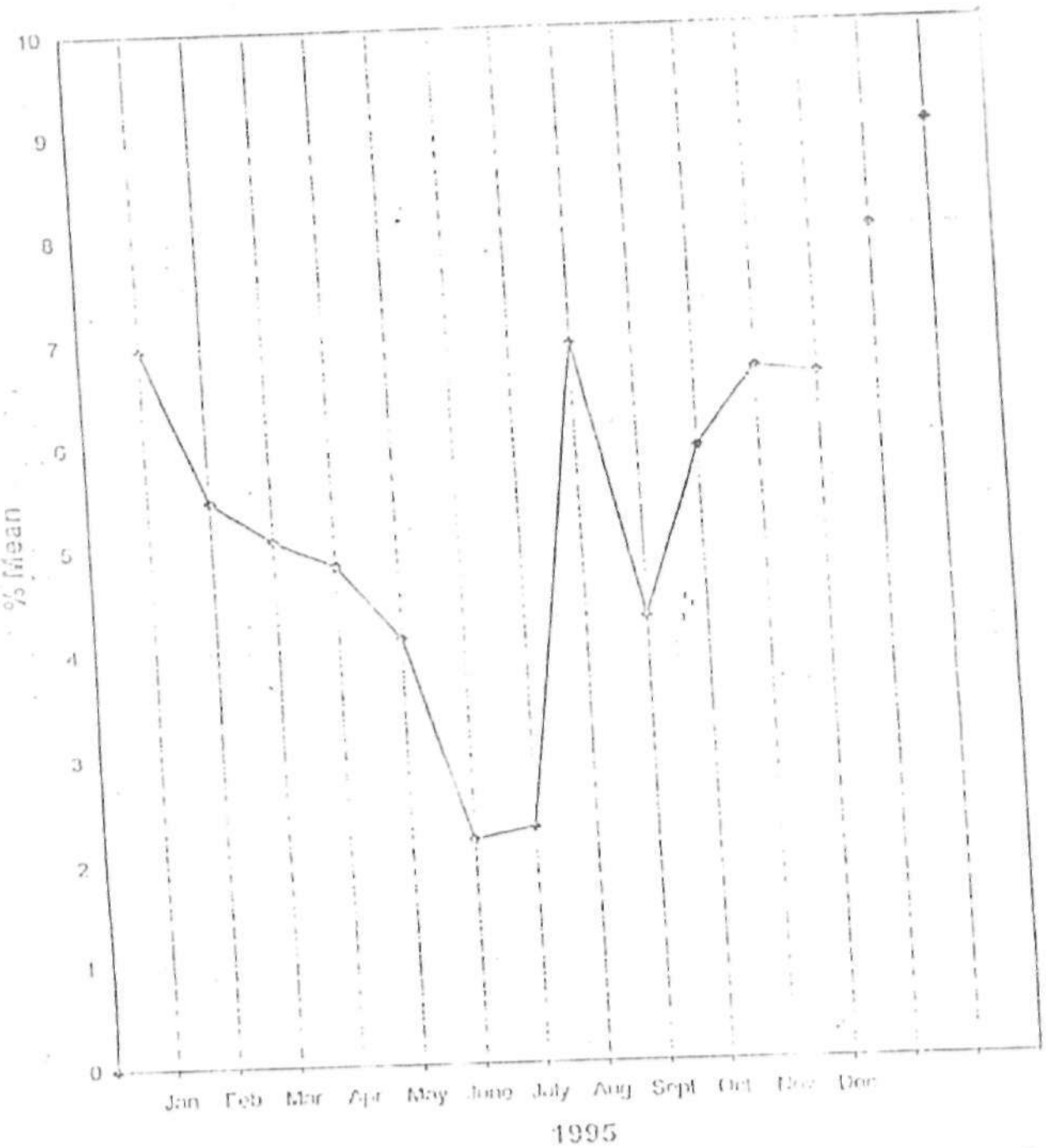
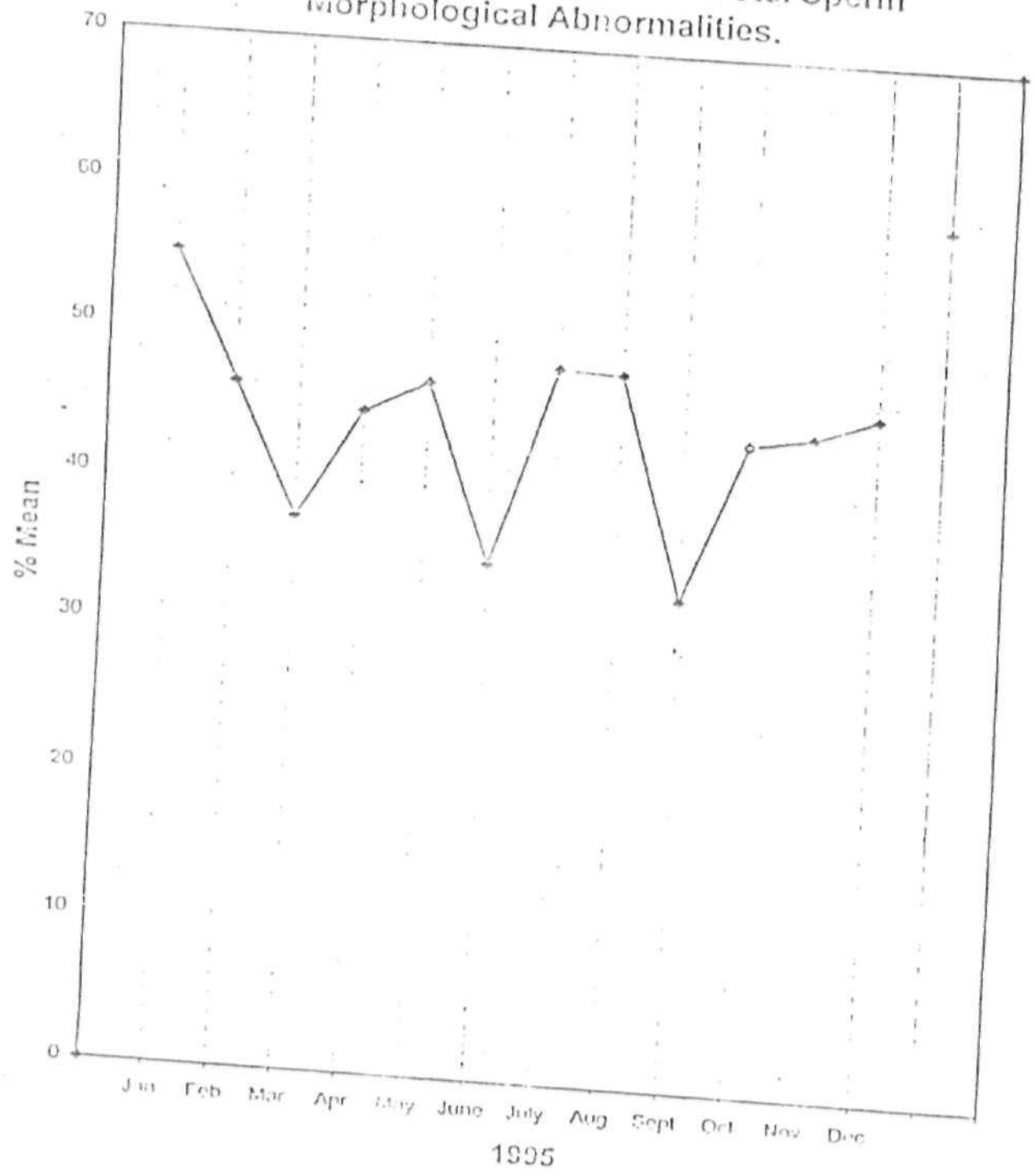


Figure 9 : Monthly Variations of Total Sperm Morphological Abnormalities.



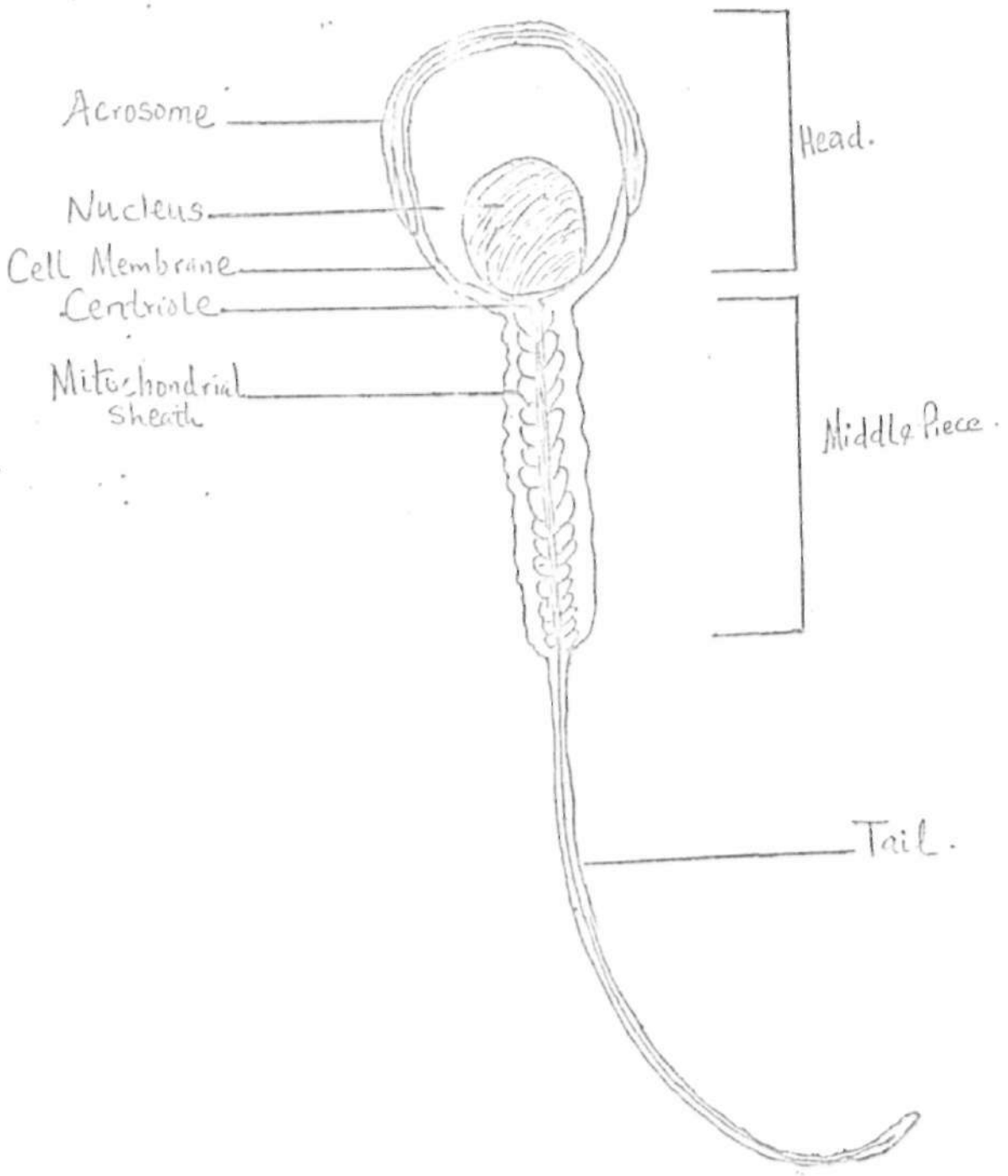


Figure 10a: The Morphology of Normal Human Spermatozoan.

PLATES: PHOTOMICROGRAPHS: SPERMS MORPHOLOGICAL ABNORMALITIES

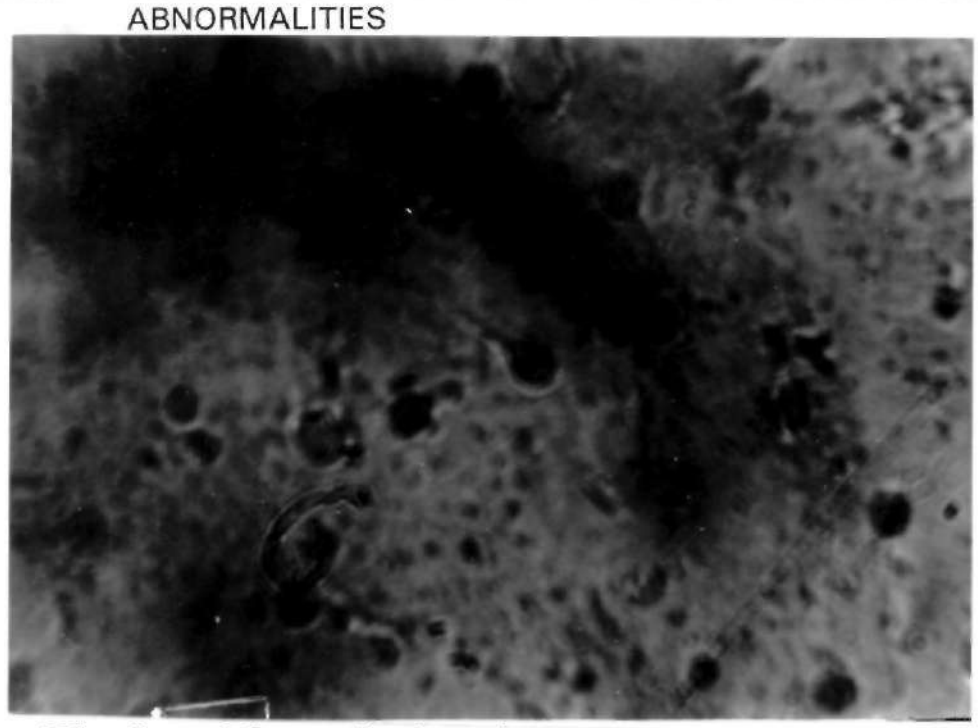


Figure 10b: Normal Sperm Heads and acrosome (Williams Stains x 1000)

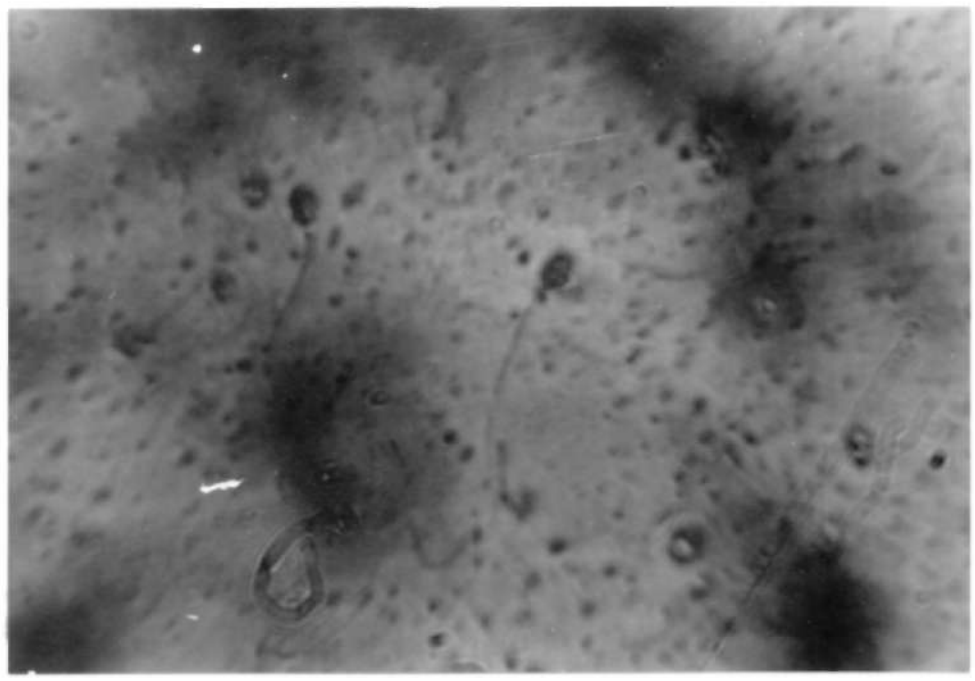


Figure 10c: Normal Sperm Morphology (Williams Stains x 400).

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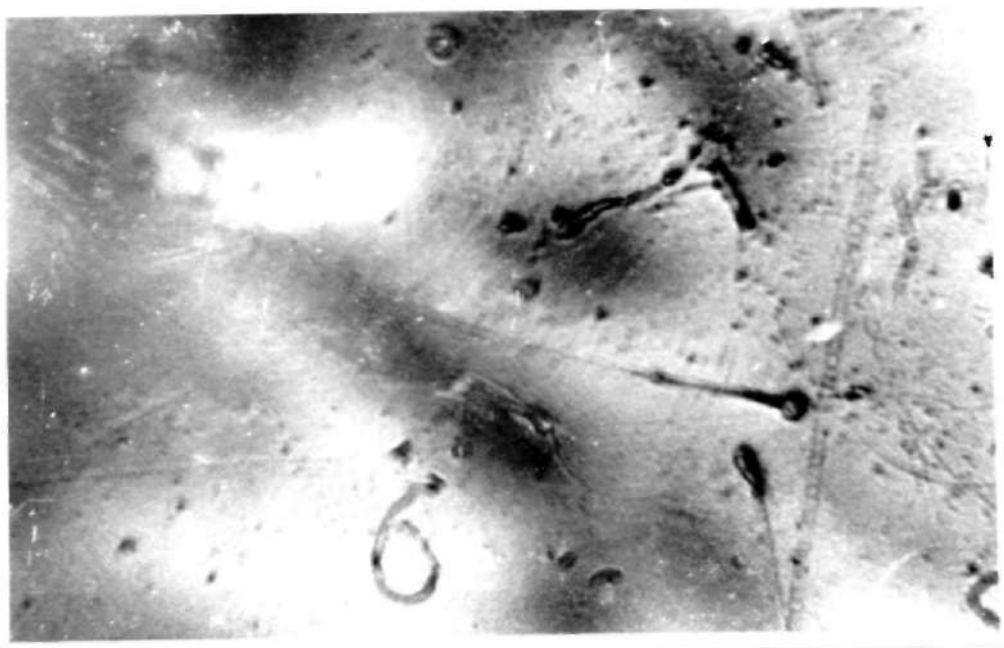


Figure 10d: Short, broad head with Distal Cytoplasmic Droplet (lying transversely). (Unstained Fixed in Buffered Formal Saline x 400).

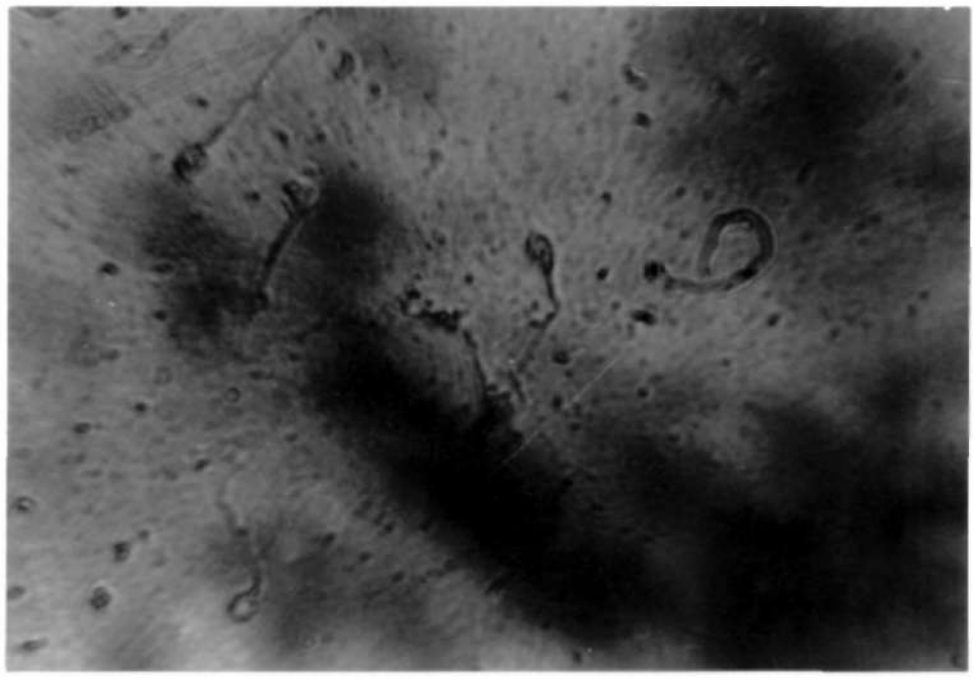


Figure 10e: Sperm Tail Abnormalities: Coild and Bent. (Eosin-Nigrosin x 400).

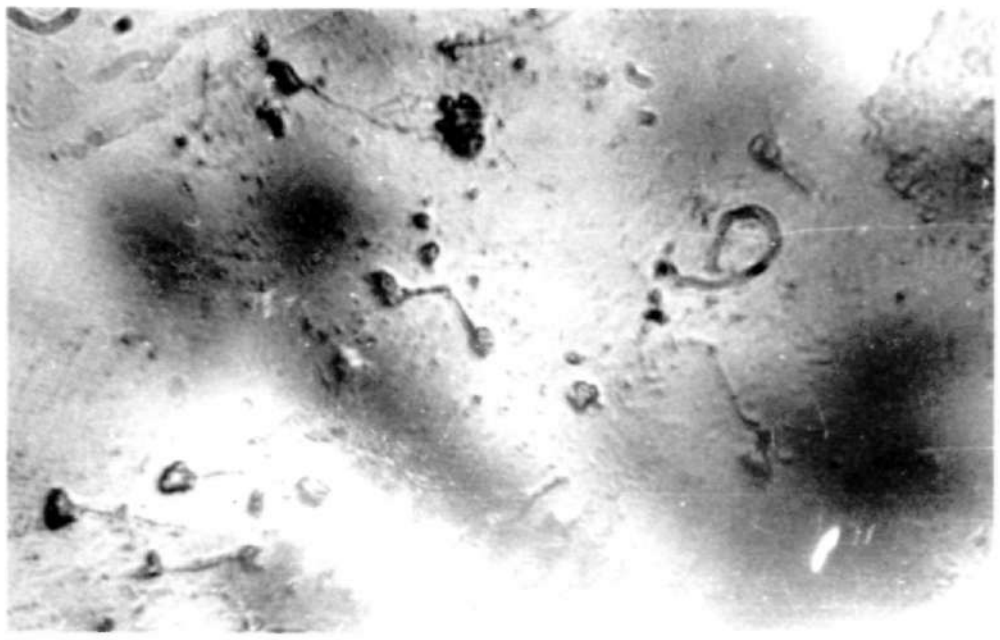


Figure 10f: Multiple Morphological Abnormalities: Abaxial middle piece attachment (Top) Detached Heads. Broad Heads. (Eosin-Nigrosin x 400).

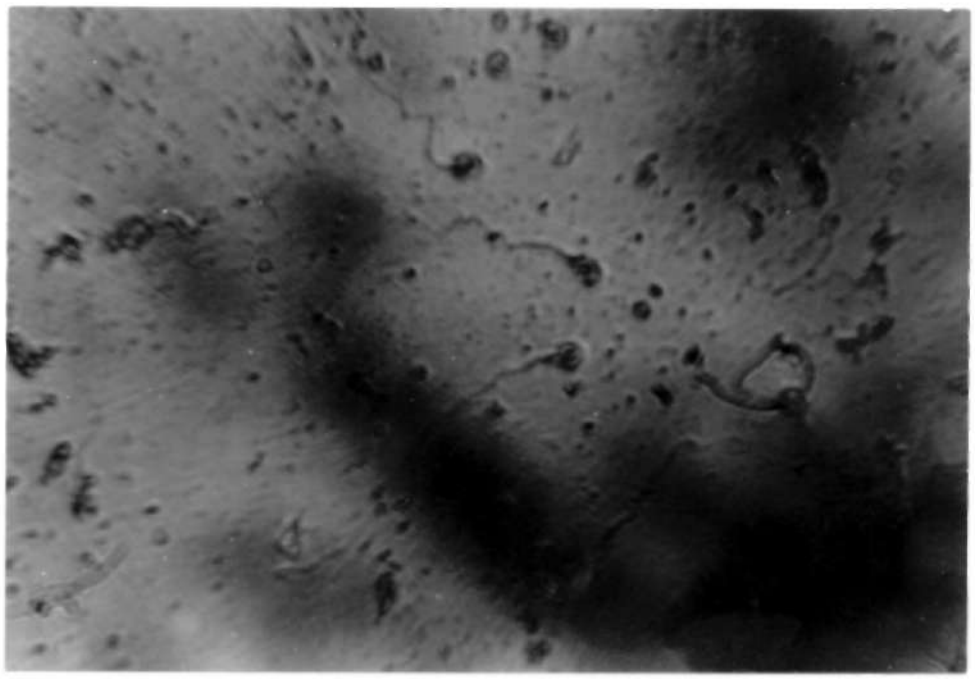


Figure 10g: Acrosome Abnormalities: (Irregular, rough) (Williams Stains x 400).

4.2.4 The Influence of Smoking on Human Sperm Morphology

The result of evaluation of sperm morphological adnormalities as described in Study II for adult cigarette smokers and non smokers is presented in Table 8 and Appendix III.

Acrosome Abnormalities

The mean for acrosomal abnormalities among smokers was 5.66% which is not significantly different drom 6.30% obtained for non smokers. Detailed resuls are shown in Table 8.

Detached Normal Head

The mean for detached normal head for smoker was 4.30% which is not significantly different from 2.38% obtained for non smokers (see Table 8).

Detached Abnormal Head

There is no significant difference between the mean of detached abnormal head of 5.37% in smokers and 4.55% in non-smokers (See Table 8).

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Table 8: **% Means of Sperm Morphological Abnormalities in Smokers and Non-Smokers**

Abnormalities	**Smokers (n=28) % Mean & SEM**	**Non-Smokers (n=122) % Mean & SEM**
Acrosome	5.66 (1.13)	6.30 (0.53)
Detached Nor. Head	4.30 (1.0)	2.38 (0.27)
Detached Abnor. Head	5.37 (0.94)	4.55 (0.35)
Prox. Cyto. Droplets	2.69 (0.62)	2.61 (0.31)
Dist. Cyto. Droplets	0.72 (0.22)	0.97 (0.97)
Midpiece	6.11 (0.80)	6.32 (0.35)
Tail	9.54 (0.83)	9.04 (0.50)
Head	11.92 (1.11)	10.92 (0.05)
TOTAL	46.30 (2.97)	43.09 (1.44)

Z test - means not significantly different ($P<0.05$)

SEM - Standard error of mean.

Proximal Cytoplasmic Droplets

There is no significant difference between the mean of proximal cytoplasmic droplets of 2.69% obtained for smokers and 2.61% among non smokers (See Table 8).

Distal Cytoplasmic Droplets

The mean of distal cytoplasmic droplets obtained in smokers was 0.72% while 0.97% was obtained in non smokers. There is no significant difference between the two results (See Table 8).

Midpiece Abnormalities

The mean of 6.11% midpiece abnormalities in smokers is not significantly different from the mean of 6.32% obtained for non smokers (see Table 8).

Sperm Tail Abnormalities

The mean of 9.54% tail abnormalities in smokers is not significantly different from the mean of 9.04% in non smokers (see Table 8).

Head Morphological Abnormalities

The mean of head abnormalities for smokers was 11.92%, while the mean of Head abnormalities among non smokers was 10.92%. The two results are not significantly different (see Table 8).

Total Sperm Morphological Abnormalities

Smokers had a higher mean of 46.30% than non smokers that had a mean of 43.09%, the two values did not differ significantly (see Table 8).

4.2.5 The Influence of Alcohol Consumption on Human Sperm Morphology

The detailed results of the influence of alcohol consumption on human sperm morphology are presented in Table 9.

Table 9: **% Mean of Sperm Morphological Abnormalities among Alcohol and Non-Alcohol Ingesters**

Abnormalities	Alcohol ingesters n=63	Non alcohol ingesters n=87
	% Mean & SEM	% Mean & SEM
Acrosome	5.70 (0.76)	6.53 (0.61)
Detached Normal Head	2.92* (0.46)	2.61 (0.38)
Detached Abnormal Head	4.72* (0.56)	4.69 (0.41)
Prox. Cyto. Droplets	2.56* (0.48)	2.67 (0.33)
Distal Cyto. Droplets	1.25* (0.30)	0.69 (0.09)
Midpiece	6.67* (0.50)	5.99 (0.41)
Tail	9.94* (0.67)	8.55 (0.57)
Head	12.11* (0.77)	10.37 (0.55)
TOTAL	45.88* (1.86)	42.10 (1.76)

* Higher Mean Values.

Proximal Cytoplasmic Droplets

There is no significant difference in the mean of 2.56% obtained for proximal cytoplasmic droplets among the alcohol ingesters and 2.67% among non-alcohol ingesters (Table 9).

Acrosome Abnormalities

The mean acrosomal abnormalities among the group that ingested alcohol was 5.70 which is not significantly different from the mean of 6.53% in non alcohol ingestion group (see Table 9).

Detached Normal Head

The mean of detached normal head among the alcohol ingesters was 2.92% and 2.16% in non alcohol ingesters. The two results are not significantly different.

Detached Abnormal Head

The mean of detached abnormal head among the alcohol ingesters was 4.72% which was not significantly different from 4.69% obtained among the non-alcohol ingesters (Table 9).

Distal Cytoplasmic Droplets

The mean of distal cytoplasmic droplets in the alcohol ingesters was higher (1.25%), and not significantly different from the means of 0.69% obtained among the non-alcohol ingesters (Table 9).

Midpiece Abnormalities

The mean of midpiece abnormalities among alcohol ingesters was 6.67% which was higher than the mean of 5.99 obtained for non-alcohol ingesters. The two results are not significantly different (Table 9).

Sperm Tail Abnormalities

The mean of sperm tail abnormalities among the alcohol ingesters was 9.94%, though higher, is not significantly different from the mean of 8.55% sperm tail abnormalities obtained from semen of non-alcohol ingesters (Table 9).

Pathological Sperm Heads

The mean of sperm head abnormalities was higher in alcohol ingesters. The mean obtained is 12.11% which is not significantly different from the mean of the 10.37% obtained from the semen of non-alcohol ingesters (Table 9).

Total Sperm Abnormalities

The mean for total sperm abnormalities was higher among the alcohol ingesters. The mean obtained was 45.88% which is not significantly different from the mean of 42.10% total sperm abnormalities obtained from the semen of non-alcohol ingesters (Table 9).

4.2.6 Influence of Genital Abnormalities on Human Sperm Morphology

In the course of this study, some subjects were noticed to have genital abnormalities such as cryptorchidism (undescended testes), varicocele and hydrocele. One subject had bilateral cryptorchidism, four had unilateral undescended testes and four had *varicocele* in one scrotal sac and hydrocele of the other scrotal sac. The results of the sperm morphological abnormalities are presented in Tables 10 and 11.

The semen of subject serial no 101 with bilateral undescended testes had elevated total spermatozoa morphological abnormalities of 55.4%. Detailed results are presented in Table 10. There were also no systemic or genital infection at the time of semen evaluation. The result of sperm morphological abnormalities evaluation for four subjects (serial numbers 119, 123, 124, 135) with no other complaints or illness except unilateral undescended testis is also presented in Table 10. Mean total sperm morphological abnormalities was 44.4%.

4.2.7 Effect of Varicocele and Hydrocele on Human Sperm Morphology

The mean of total sperm abnormalities in this group was found to be 47.45% which is obviously higher than mean values reported by other workers in normal subjects. Detailed results are shown in Table 11.

4.2.8 Influence of Inguinal and Scrotal Surgery on Human Sperm Morphology

During the one year period of the studies, twenty-six subjects were discovered to have had surgical operations in the inguinal and scrotal areas with no other illness or abnormalities except complains of infertility. Thirteen had herniorrhaphy, six had varicocelectomy, two had hydrocelectomy and five had orchidopexy. A detailed study of their sperm morphological evaluation was done. Detailed result of the sperm morphological abnormalities in the semen of the men are presented in Table 12.

Table 10: **% Mean of Sperm Morphological Abnormalities in Subjects with Undescended Testis**

Abnormalities	**Bilteral Undescended Testes** $n=1$	**Unilateral Undescended Testis** $n=4$
	% Mean & SEM	**% Mean & SEM**
Acrosome	5.8	5.8 (1.27)
Detached Normal Head	0.4	2.05 (0.82)
Detached Abnormal Head	8.4	4.73 (1.60)
Prox. Cyto. Droplets	2.6	2.26 (1.30)
Distal Cyto. Droplets	0.8	0.85 (0.39)
Midpiece	10.8	5.83 (2.18)
Tail	17.2	10.95 (3.26)
Head	9.4	11.95 (2.79)
TOTAL	55.4	44.4 (8.17)

SEM - Standard Error Mean.

Table 11: **% Mean of Sperm Morphological Abnormalities in Subjects with Varicocele and Hydrocele n=4.**

Abnormalities	**% Mean**	SEM
Acrosome	6.95	(3.66)
Detached Normal Head	3.85	(2.33)
Detached Abnormal Head	6.95	(2.46)
Prox. Cyto. Droplets	0.45	(0.26)
Distal Cyto. Droplets	0.20	(0.14)
Midpiece	8.95	(1.30)
Tail	6.95	(1.13)
Head	13.35	(2.47)
TOTAL	47.45	(2.93)

SEM - Standard Error Mean.

Table 12: **The % Mean and Standard Error of Mean (SEM) of Sperm Morphological Abnormalities of Subjects who had Inguinal or Scrotal Surgeries**

Sperm Abnormalities	Herniorraphy n=13	Varicocelectomy n=6	Hydrocelectomy n=2	Orchidopexy n=5
	% Mean & SEM	% mean & SEM	% Mean & SEM	% Mean & SEM
Acrosome	9.88 (2.37)	4.93 (1.17)	1.3 (1.3)	8.68 (1.17)
Det. Nor. Head	2.06 (0.69)	0.68 (0.34)	2.55 (2.55)	3.2 (0.72)
Det. Abnor. Head	4.03 (1.27)	5.97 (1.94)	3.8 (1.12)	3.4 (1.29)
Prox. Cyto. droplets	3.48 (1.15)	4.93 (3.32)	2.15 (0.65)	2.09 (1.36)
Dist. Cyto. Droplets	1.25 (0.78)	0.73 (0.48)	1.5 (1.1)	0.27 (0.08)
Midpiece	6.05 (0.84)	7.1) (1.25)	7.6 (3.6)	6.38 (1.60)
Tail	9.58 (1.09)	10.37 (2.01)	8.75 (3.75)	7.36 (1.82
Head	11.86 (1.63)	10.3 (2.60)	4.7 (2.9)	13.6 (2.48)
Total	48.20 (4.96)	45.0 (6.78)	32.35 (0.55)	44.78 (6.52)

SEM - Standard Error of Mean

CHAPTER FIVE

DISCUSSION

5.1 METHODOLOGICAL STUDIES (STUDY I)

The results obtained in the methodological study showed that wet fixed semen samples examined with the phase contrast microscope gave higher percentage of proximal droplets than did the stained preparation studied with the light microscope, though the difference was not significant statistically. This decrease in the values of proximal cytoplasmic droplets obtained with stained preparation is likely due to droplets lost during the staining procedures. However, the means of 2.62% proximal cytoplasmic droplets in the studies is very low. There were more detached normal sperm heads and detached abnormal sperm heads in the stained preparations before and after a month fixation in buffered saline and storage at +4°C. Detached normal sperm heads counted in semen smears stained with eosin-nigrosin and Williams stain and studied with the light microscope was significantly different ($P<0.05\%$) than wet fixation method with phase microscope. The increase in the values of detached heads could have resulted from the process of making smears and staining. There was no significant difference in the values recorded for acrosome abnormalities using the three methods of evaluation and also before and after storage. Any or a combination of the methods can be used to assess acrosome abnormalities.

From the result obtained in the methodological studies, there was no significant difference in the values for the various sperm morphological abnormalities between the

three different methods of observations except for the detached sperm heads which were significantly higher in the stained smears (eosin-nigrosin and Williams stain). A combination of phase contrast microscopy and light microscopy seem ideal for obtaining the most reliable results in sperm Morphological evaluation. There is the possibility of droplets loss during the process of preparing semen smear with slide and during staining. There is also the pssibility of increased acrosome abnormalities in stained smears as a result of damage or distortion of the acrosome when preparing smear for staining. Head abnormalities could be studies with stained preparations of eosin-Nigrosin or Williams stain while other morphological abnormalities could be evaluated with wet fixed semen with the phase microscope. (Largerlof, 1934; Campbell *et al.*, 1960; Sekoni *et al.*, 1981).

The methodological study also demonstrated that buffered formal saline is a good fixative for the human sperm which if kept at the room temperature or a temperature of +4°C could still give accurate result for morphological studies. These findings agree with earlier workers findings in other mammalian species (Harasmowycz *et al.*, 1969; Dott and Foster, 1975; Sekoni *et al.*, 1981). Human semen fixed in buffered formal saline and stored at 4°C to 40°C for several days could still be transported to centres where there are ideal semen laboratories and experts to carry out accurate evaluations.

5.2 INCIDENCE OF SPERM MORPHOLOGICAL ABNORMALITIES IN ZAIRA WITH ASPECTS ON SOME CAUSES OF VARIATIONS. (Study II)

Macleod and Gold (1951) suggested that normal semen should contain not more than 20% abnormal spermatozoa. This is clearly lower than the 43.69% found in this study. Eliasson 1971 has suggested that up to 30-40% of all spermatozoa present in human semen possess an abnormal morphology and when this percentage increases to greater than 50% infertility result. Eliasson is probably more generous. Moench etal (1931ab) stated that a maximum of 20% abnormal form is found in semen of fertile men and that when this figure exceeds 25% sterility is probable. This 20% value is far lower than the 43.69% obtained in this study. Williams (1946) concluded that where the percentage of abnormal forms exceeds 40% the semen of that individual should be considered infertile/sterile. This is in agreement with the findings of the present study. The men whose semen were studied in this investigation had reproductive problems manifested by the inability to impregnate their wives. Their semen characteristics had elevated sperm morphological abnormalities.

Subjects no 52 had a history of urethritis and orchitis 2 weeks before examination, his semen samples showed a lot of pus-cells and had the highest number of midpiece abnormalities (16.4%) of the total 60.8%. The culture of his semen produced heavy growth of *staphylococcus aureus*. This findings is supported by Macleod (1965) that demonstrated an increase in the abnormal midpiece to be related to inflammatory diseases. Varicocele was found in subjects no 83,140,142 and 145. Their semen had

spermatozoa with elevated sperm head abnormalities of the tapering (narrow) type, this findings agree with those of several earlier workers (Macleod, 1965;' Rodniquer *et al.*, 1981; Jecht *et al.*, 1982; Porluondo *et al.*, 1983).

The classification of sperm morphological abnormalities in this study agree with that suggested by Eliasson (1971) for human spermatozoa except that cytoplasmic droplets no matter how small were evaluated as separate abnormalities from midpiece abnormalities.

The results obtained from this study demonstrate that the semen of the subjects were of poor quality as far as sperm morphology was concerned (the mean total sperm morphological abnormality was found to be 43.69% which is above the value suggested by several workers (Moench, 1931 a, b; Williams, 1946; Macleod and Gold, 1951; Eliasson 1971).

From the investigation, the results showed that there was no definite influence of age on sperm morphology in humans. This finding support the finding of Macleod and Gold (1954) that suggested that no relationship exist between the percentage of morphologically normal cells and age in a study of semen quality in relation to age and sexual activity in humans. However, Hahn *et al.* (1969) found that percentage of Morphologically normal spermatozoa in semen at the time of collection were lowest in the aged Holstein bulls. The result is also in contrast to Rao (1971) who demonstrated that age has an effect on sperm morphology in bulls. The difference in their findings and that of this study is not surprising because their studies were conducted in animals over

large age range from very young bulls to very old bulls. It will be interesting to study sperm morphological abnormalities in very young and old sexually active humans.

The results of this investigation also showed that there is a definite influence of sexually transmissible diseases on sperm morphology. The sperm abnormalities mostly affected were those of the midpiece and tail. This findings agree with Macleod (1965) who demonstrated that an increase in the abnormal midpiece is related to inflammatory disease.

The results also showed that there is no doubt that there are seasonal variation in the morphology of the spermatozoa of the 150 human semen examined in 1995. The result agree with the findings in bovine sperm morphology by Igboeli and Rakha (1971), Fayemi and Adegbite (1982) Saxena and Tripathi (1984) and Sekoni *et al.* (1988).

The months of dry season (partly harmattan, dry and hot) that is January, February, March and April were the hottest period of the year and could be detrimental to sperm morphology. During this period, there were more detached (tailess) head (normal and abnormal) abnormal midpiece, abnormal tail and total sperm abnormalities than in the rainy season. The hottest period of the year appeared to have the most adverse effect on the morphology of human spermatozoa. The explanation for the high abnormalities during the dry season is most likely due to heat stress experienced by humans during this period. The fact that some sperm morphological abnormalities such as acrosome abnormalities, tail abnormalities, cytoplasmic droplets and head abnormalities were higher during the rainy season than during the dry season may be due to the abilities of humans to adjust to the environmental temperatures by such

mechanisms as cooling, change of accommodation and even that of environment. The present investigation supports the view that elevated environmental temperature accompanied with dry air impairs testicular function.

The months of dry season (January, February, March, April and May) were the hottest period of the year and there were more total sperm morphological abnormalities during this season than during any other time.

This agree with the findings of Hultnas (1959) and Wells *et al.* (1971) and Sekoni and Gustafsson (1987), who found significant seasonal and monthly variations in the morphology of bovine spermatozoa.

From the results obtained, it is clear that smoking does not have effect on the sperm morphological abnormalities in humans. There has not been previous investigation on the effect of smoking on human sperm morphology especially in Nigeria. Kolodny *et al.* (1974) reported *Cannabis sativa* (Indian Hemp) to cause decrease in sperm concentration and semen testosterone level with frequent and continuous use. The effect of smoking cannabis was not investigated on the sperm morphology in this study. There was no subject who volunteered any information on past or present use of cannabis in this study.

The results showed that the highest means of sperm morphological abnormalities were obtained among the subjects that ingest alcohol. Specifically, the means of detached normal head, detached abnormal head, Distal cytoplasmic droplets, Midpiece abnormalities, sperm tail abnormalities, head abnormalities and total sperm abnormalities were higher in the alcohol ingesters group than the group that did not use alcohol.

Although the direct effect of alcohol on human sperm morphology has not been previously studied, reports show that chronic ingestion of larger amount of ethanol (alcohol) or its metabolites such as acetaldehyde can directly impair synthesis of testosterone in man and animals by increasing testosterone metabolic clearance rates, by suppressing gonadotropin and reduction in testicular weight and sizes and thereby impairing spermatogenesis. (Mendelson *et al.*, 1977).

From the results obtained in both bilateral and unilateral cases of undescended testes, it could be deduced that undescended testes due to testicular pyrexia had detrimental effect on sperm morphology. The highest abnormalities occurred on the heads, tails and midpieces of the spermatozoa. The above findings support the view that undescended test lead to severe sperm morphological abnormalities. Cryptorchidism has been shown to cause tubular damage and result into infertility (Dublin and Amelar, 1971 and Lipshults, 1976). Nistal and Paniqua (1984) in the study of 23 infertile adult men with no other apparent cause of infertility than bilateral retractile testes reported oligospermia (2-10 million per ml) and sperm morphological abnormalities ranged between 60-80% (tapering/narrow heads, amorphous and immature forms) in addition to focal histologic lesions in the testes in 20%.

The results obtained indicate that varicocele and hydrocele definitely have detrimental effect on human sperm morphology. This findings support the observation of other researchers who demonstrated increased sperm morphological abnormalities invaricocele. (Rodniquer *et al.*, 1981 and Jecht *et al.* 1982). Nasah (1978) has shown the prevalence of varicocele in an infertile population to be between 20% to 30% as

opposed to 10% in the fertile population. It was postulated that varicocele may cause oligospermia and elevated incidence of abnormal sperm morphology by increasing the scrotal temperature or by regurgitation of toxic substances from the renal vein (Nasah, 1978).

It can be deduce from the result obtained that surgical operation in the inguinal or scrotal region had detrimental effect on sperm morphology. Sperm head, sperm tail, midpiece and acrosome were elevated and were mostly affected. It was not certain whether the sperm morphological abnormalities resulted from genital abnormalities that necessitated the surgical intervention or from the surgery. One can only ascertain this if there is an opportunity to do morphological studies before and after operation. Mefo (1981) in the study of male infertility discovered that 15% of cases of severe oligospermia/Azoospermia were linked to herniorrhaphybut the investigator did not comment on the morphology of the sperm in his investigation. Iatrogenic injury to the vas deference or the epididymis was also reported in orchidopexy, varicocelectomy, hydrocelectomies and vasography (Chan *et al.* (1982).

Conclusion of study II

Study II demonstrated that season, alcohol, genital abnormalities such as cryptorchidism and sexually transmissible diseases had detrimental effect on testicular/epididymal functions which caused the elevation of sperm morphological abnormalities and infertility in humans. It also showed that age and smoking does not have effect on human sperm morphology in this study.

GENERAL CONCLUSIONS

The methodological study demonstrated that buffered formal saline is a good fixative for human's semen for the study of spermatozoa morphological abnormalities.

Semen fixed in buffered formal saline and kept at a temperature of $+4^{oc}$ could still produce reliable and accurate results for morphological studies at 4 weeks after storage.

The combination of the phase contrast microscope and the light microscope gave more accurate results than when the light microscope was used alone. Some abnormalities such as acrosomal and cytoplasmic droplets could only accurately evaluated with the phase microscope.

Many aspects of sperm analysis could be done in a specialised laboratory and not in a remote setting without necessary facilities if spermatozoa were fixed in buffered formal saline and stored at +4°C.

The second aspect of the investigation showed that season of the year had an effect on sperm morphology, with the hottest season being the most detrimental.

Alcohol ingestion, cryptorchidism, varicocele, hydrocele and sexually transmissible diseases also have detrimental effects on sperm morphology.

Age and cigarette smoking does not have any effect on sperm morphology. Morphologically abnormal spermatozoa definitely lead to infertility/sterility as revealed in this study.

The mean value for total morphologically abnormal spermatozoa for 1995 in the study groups of 150 subjects examined was highly elevated (43.69%). This value is more than double that found in normal, healthy human beings.

Further studies on the etiological agents of elevated morphologically abnormal spermatozoa and infertility in humans are suggestive from the findings in the present investigation.

Certain diseses which may impair normal reproductive function and cause elevated spermatozoa abnormalities could necessitate surgery, (e.g.) some of those mentioned in this study. The question is does surgery generally have untoward effect on sperm morphology? Yamamoto *et al.*, (1996) in assessing the outcome of surgical operation for subclinical varicocele on fertility and/or semen parameters concluded that subclinical varicocelectomy has some effect on spermatogenesis but no beneficial effect on pregnancy rate. Osegbe, (1991) also reported an improvement in the sperm concentration and sperm motility but no significant improvement in the sperm morphology following Sclerotherapy for Hydrocele.

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APPENDIX FOR STUDY I
(RESULTS FOR METHODOLOGICAL STUDIES)

APPENDIX 1A

PATHOLOGIC SPERM HEADS

S/N	WS1	WS30	EN1	EN30
1	22.6	21.8	18.4	18
2	9.4	10.1	13.6	13.8
3	11.2	12.4	12.1	12
4	16.4	16.3	1.4	7
5	5.4	5.2	9.2	9.5
6	17.2	18.1	10.6	10
7	25	26.2	23	22.7
8	11.2	10.9	11	9.8
9	10.4	11.4	13.2	11.2
10	16.2	18.1	18.7	15.7
11	20.6	17.9	21.3	20.8
12	17.6	18.2	19.4	19
13	12	10.85	10.8	10.2
14	20.8	22.1	16	15.7
15	26.6	26.4	20.9	19
16	22	23.1	19	17.8
17	2.5	2.4	4.6	6.4
18	11.4	11.8	12	13.2
19	13.4	14.3	11.4	12
20	6.4	7.2	4.8	5.2
21	17	15.8	19	20
22	4.9	4	6	8.1
23	12.8	12.3	14	13.7
24	10	11	12	12.8
25	1.7	3	3.9	4.2
26	0.8	1.2	2.4	3
27	1.4	1.6	3	0
28	22	18	17	18.2
29	1.8	2.1	3.6	2.5
30	0.6	1	2.2	1
31	15	17.3	18.4	18.6
32	2.2	5.2	3.8	4.3
33	4	3.75	2.5	3.2
34	16	18.4	12.5	10.7
35	8	8	9.1	8.5
36	15.2	15.25	14	15.2
37	15.8	15.1	17.4	17
38	22.2	24	24	20.6
39	11.6	9.4	14	14.3
40	12.2	10	13.75	13

41	14.2	14.7	13	12
42	4.8	5	7.4	8.3
43	9.8	11.3	8	9.4
44	9.2	9.15	7.8	7
45	14.2	14.3	13	14
46	7.4	6.25	9.6	9
47	11.8	10.4	10	10.1
48	8.6	8	11	12
49	8.6	9.6	13.5	13
50	9.8	12	12.2	12
51	9	7.4	11.5	12.7
52	7.2	8.3	9.4	10.2
53	3.6	5	2	0
54	10	9.2	13	12.4
55	8	6.9	11	11.2
56	4.6	5.1	7	5.6
57	8.8	9.2	10.5	8.4
58	12	11.25	7.8	7
59	4.8	5.15	10	10.5
60	8	8.4	6.4	6.8
61	8.2	10.2	7	7.2
62	5.6	4.8	6.75	5.3
63	8.4	8	7.6	7
64	16.6	18.3	14	13.3
65	12.2	12.8	9.6	9
66	9.6	10.2	8.2	8.4
67	6	4.8	4.3	4
68	10.4	11.4	8.4	7.4
69	12.2	13.6	14.5	14
70	7.6	7.4	9.2	9
71	14.2	9	8.4	8.8
72	15.2	11.25	12.1	12.4
73	4.8	0	2.6	3
74	8	8.6	9	8.7
75	13.2	15	10.4	10.2
76	10.6	7.4	13.2	13.2
77	13.2	12.8	10	10.4
78	11	13	11.75	11
79	4.6	10.4	6.8	6.2
80	19.6	18.5	20.4	21
81	11.2	14.9	12	13.4
82	5.2	11	14.25	14.8
83	6.2	13	11.8	11.2
84	15	15	12	13.4
85	14.2	7.4	9	10.5
86	14.4	12.2	12.4	13.4

87	12	9.8	8.9	6.8
88	7.8	10.4	8.4	8
89	11.6	12.2	13	13.2
90	7.2	8.7	10.2	10
91	8.2	9.4	10.8	9.6
92	11.6	10.2	7	8.4
93	9.2	10.8	8.6	8
94	10.8	13	11.2	11.4
95	20.6	22	18.4	18.6
96	14.6	16.2	15.2	15
97	8.8	9.4	9	10.2
98	13.2	14.3	12.1	12.4
99	14.4	15.1	16.3	15.6
100	14.2	10.4	11.4	10.2
101	9.4	13	12.8	13.4
102	15.6	12	13.4	13
103	7.2	8.5	9	6.5
104	11	13	12.7	10.4
105	8	16.8	9.4	8
106	14	13.8	12	11.6
107	9	9.2	7	7.2
108	9.4	9	10.2	10
109	11.2	11.4	11	10.8
110	17.2	18	16	14
111	6.4	5	8	10
112	1.7	1.6	2	1.2
113	1.4	1	1	1
114	1.8	2	2	2.6
115	0.6	0.4	1.2	1
116	6.4	6	5.4	5
117	5.6	5	5.8	7
118	7.4	7.2	4.2	5
119	8.4	8	6	4
120	10	11.2	13	12.6
121	22	23	18	14
122	4.8	5	6	8.2
123	8	8	9.2	10.6
124	11.4	9.8	10.8	7
125	4	26	4	3.8
126	13	11	14	13.4
127	9	8.4	7	7.2
128	2.2	2	3.6	4
129	11.2	8	12	10
130	12	12.6	8	7.8
131	12.8	6.8	15	15.6
132	6.4	6	3	3.4

133	20.4	20	21	20
134	16	18	14	16.4
135	20	20	12	10
136	16	14.8	11	12.8
137	20	13.2	16.2	14
138	12	14	13	7.2
139	20	18	18	14
140	17	20.8	10	8
141	12	10.2	6.8	12.6
142	16.2	16	18	14.2
143	20.8	22	20	15
144	10.2	12	6.4	7
145	14	10	8	6
146	8.2	8	4	3.4
147	13	14	10.8	12
148	4.6	2	2	4.8
149	20	16	16.6	10
150	17	14	12.2	16
MEAN	11.072	11.357	10.673	10.539
S.E.M	0.452	0.463	0.41	0.379

KASHM IBRAHIM LIBR

ACROSOME ABNORMALITIES

S/N	FS-1	FS-30	EN-1	EN-30	WS-1	WS-30
1	3.4	4.2	2.6	2.9	3.1	3.2
2	13.8	13.6	10.4	10	11.4	11.4
3	6.2	6	4.8	5.4	7	5.8
4	7	7.5	9.2	10.1	12	12
5	4.8	5.1	3.6	4.2	6.8	5.9
6	4.6	3.4	4	5.3	8.2	7.3
7	3	2.8	4.5	4	2.7	2
8	7.6	7.6	5.2	6	11.5	10.75
9	6	6.4	5	4.8	8.2	8.5
10	6.2	5.8	4.6	6.2	4.6	5
11	5	7.2	3.8	4	10.25	9.6
12	11	10.8	8.5	9	3.6	2.4
13	24.4	26	20.4	21.4	15.4	15
14	19.4	20.2	18.2	19.6	11.6	11.8
15	6	5.4	0	4.8	3	2.1
16	12	4.8	10.3	12.2	9.6	11.8
17	1.2	4.2	5.8	6.1	0	1.8
18	18.5	19.1	17.2	18.2	16.8	13.2
19	1.25	2.8	3	2.75	0	1.5
20	0.5	1.6	0	1.8	0	0
21	4	4.3	6.2	5.2	1.2	1.2
22	8.6	9	8	9.1	7.3	6.9
23	5	5.4	2.75	3.2	1.8	2.2
24	3	3.6	1.8	2.5	1.6	1.9
25	2.6	1	0.6	1.4	1.3	2
26	0	0	1.4	2	0.8	1.6
27	0	4	0	1.8	0	0
28	6	5.9	4.6	5	7.3	0
29	0.25	1.2	1	1.75	0	0
30	0	2	0	3	0	3.1
31	1.4	1	3.2	1.8	2.5	1.7
32	0	1.5	0	0	0.25	0
33	0	0	0	1.75	1.2	1.32
34	25.8	26	18.4	19.2	20.4	20.6
35	31	29.8	24	22.9	27	28.1
36	5.8	5.4	6.8	8.2	6.3	7.2
37	3	3	11.2	12.5	4.3	4
38	0	0	2.5	1.8	0	1.3
39	5	4.8	6.4	7.2	4.5	5
40	1.8	23	0.4	1.2	1	1.7
41	0	1.6	0.25	1	0.25	0.5

KASIM IBRAHIM LIBRARY

42	7.4	5.9	8.25	8.4	3.4	4
43	4.8	5	3.8	6.4	5.2	5.2
44	18	14.9	16	15	14.9	15.3
45	0	0	0.78	1.15	0	0.2
46	6.4	6.4	9	10.25	6	6.1
47	0	0	0.5	0.75	0.5	0.7
48	7.6	8.6	6.8	7.8	7	6.52
49	0	0	1.8	2.1	0	0.75
50	6.4	5.2	5.8	7.2	6.2	6.2
51	10.8	9.5	11.4	10.2	10	9.75
52	6.6	6	8.4	8.6	5.8	5.3
53	0	0	0	1.75	0	0
54	11.6	11.65	14.2	16	8.9	8.61
55	0	0	0	0.25	0	0
56	7.6	8.4	9	10.5	5.3	4.8
57	6.2	6	4.8	5.4	7.4	7
58	4	5.1	3.8	4.05	3.2	4
59	0	0	1.5	0	0.25	3.5
60	12.6	13	11.9	12	10.5	11.25
61	0	4.6	3.8	4.1	0	0.25
62	13.4	14.1	15.3	15.3	12.1	12.3
63	10.8	11.2	9.4	9	10.2	10.2
64	0	0	2.5	3	0.25	0.25
65	9.6	10.2	11.4	13.6	9	9.5
66	2.6	2.4	3.1	4	1.8	0
67	3.8	3.8	4.2	3.6	4.9	5.2
68	0	0.2	1.5	0	0.25	0
69	5.4	5.2	4.2	2.75	3.6	4.2
70	0	0	0.25	1.4	0	0
71	2	1.8	3	3	4.25	5
72	16	15.9	14.2	14.6	13.8	13.6
73	6.6	6.6	7.2	7.8	7	6.9
74	0	1.2	0	0	1	0.5
75	6.2	5.9	6	5.6	5.6	7
76	0	0	0	0	0.25	0
77	10	9.8	8.6	8.4	12.6	12
78	2.6	2.8	1.25	1.5	3	3.4
79	3	2.2	3.4	3	2.5	2
80	0	0	1.2	1.5	2	1.75
81	10.8	10.6	11.4	11	12.2	13.4
82	4.8	4	3.9	3.4	5.2	5
83	0	0	0	0.5	0.75	1
84	12.2	12	13.4	13.1	13.5	13
85	8.2	8.6	7.3	9.4	6.4	8.2
86	6.33	6	5.75	4.6	5	5
87	0	0	2	1.25	1.5	2

88	0	1.2	0	0.25	0.75	1.5
89	21.2	21	19.8	20.9	19	22
90	15	15.8	14.2	12.8	14.5	15
91	3	2.8	1.5	0	0	0
92	0	0	0	0.75	1.25	0
93	13	13	15.2	15	17	14.5
94	10	9.6	11	10	12.2	12
95	7.4	7.2	5.8	5.5	6.1	6
96	4.6	5	3.4	3.5	2	1.9
97	4.2	4.2	5.1	5	0	0
98	6.4	6	7.5	7.25	8.2	7.4
99	5.4	5.8	4.25	4	6	8.2
100	8.4	8.4	6.4	6.1	7.5	7
101	5.8	5.6	1.8	2	3.5	3.9
102	3.2	3	2.6	2.5	1.8	2.25
103	7	7	4.3	4	2.1	1.5
104	0	1.2	0	0.25	0.75	1.2
105	14.4	15.6	13.4	11.6	0	3.5
106	5	6.2	4	4.2	.8	8.2
107	0	1	2	1.8	2.6	3
108	1.2	0	0	1	0	1.8
109	18.5	20	16	15.8	14.2	14
110	1.25	2	1	0	1	1.4
111	0.5	0.5	0	0.2	0	0.8
112	4	4.2	5	5	6.2	6
113	5	6	7	6.8	5.4	5.8
114	2.6	2	1.8	1	3.6	4
115	0.25	1	0	0.2	2	4.2
116	1.4	0.8	0	0	1.8	3.6
117	11.6	12	10.2	11	14	12
118	13.4	9.6	11.4	11.6	10	8.6
119	6.4	4.2	5	6	5	7
120	1.8	2	1.2	2.2	4	4.2
121	5	5.4	6	7	6.2	6
122	3	1.8	2.2	1.8	2.6	3
123	9	6	7.8	7	10	11.8
124	4.8	4	4.2	4	6.4	6
125	0	1	0	1.2	2	2.4
126	12	13	11.8	12	13.4	13
127	0.6	0.4	0.2	0	1.8	0
128	0.25	1	0	0.2	0	0
129	4.8	5	4	6	5.2	5
130	5	7.2	6	5	8	4.8
131	4.6	2	3.4	3	4.8	5.2
132	6.2	5.8	6	8	10	12
133	5	4	3.8	4.2	3.6	6

134	11.4	11	13.2	14.6	16.2	14
135	3	4	6	8	11	12
136	12	12	10.8	11	10	10.2
137	7	8.2	10	12	11.4	13
138	10	10	11.8	14	16	12.6
139	12	14	16	9	10	10.4
140	5.6	4	8	7	6.8	6.25
141	2	1.8	0.8	4	5.4	5
142	14.8	10	16	12.6	14.2	15
143	4.6	3.8	5.2	4	3	1.25
144	9	12	10.6	13.4	16	16.75
145	7.4	9	7.8	10	12.2	11
146	5	3.2	6.2	7	8.4	10.2
147	12.8	10	10.4	13	15	14
148	20.6	22	15	14	14.8	16.25
149	11	8	12	13	14	12.5
150	7.6	10	14	12	15	15.75
MEAN	6.183	6.368	6.084	6.404	6.145	6.25
S.E.M	0.478	0.482	0.434	0.43	0.445	

APPENDIX 1C

PROXIMAL CYTOPLASMIC DROPLETS

S/N	FS0	FS1	EN0	EN1	WS0	WS1
1	4.2	4.6	3.6	3	2.5	4.5
2	5	4.4	3	2.75	3.4	2.6
3	1	0.8	0.1	0.15	0.75	1.5
4	1.6	1.6	1	0.8	1.25	2
5	2.8	2.9	2.5	2.5	4	5.2
6	0.4	0.52	0.3	0.4	0	2.2
7	1.8	2	1	0.7	2.6	1.6
8	1.4	1.4	1.2	1.2	1.75	1.4
9	2	2.1	1.8	1.6	2.1	1.4
10	4.4	3.8	4.2	4	4	9
11	1.2	1.4	1	0.75	1.3	1.8
12	1.8	1.8	1.6	1.6	1.7	2.2
13	14	13.8	10.8	10.4	7.5	9.4
14	7.8	7	5.4	5.4	6.2	8.2
15	1.2	1.2	0.8	0.9	0	0.25
16	1.5	2.5	1.3	2	2.2	4.2
17	0.5	1	1.2	1.5	0.25	1.2
18	14.75	14.3	12.8	10.2	9.8	10.5
19	1.75	2	1.3	1	0.75	1.25
20	2	1.9	2	2.1	0	2.6
21	1.3	1.3	1.4	1	0	1.8
22	2.9	3	2.6	2.4	1.8	1.2
23	3.4	3.5	3.2	3.2	2.4	3.4
24	1.6	1	0.75	0.5	1.5	1.6
25	0	0.5	0.25	0.3	1	1.6
26	0.25	0.1	0	0	2.2	1.8
27	4	3.2	4	3.75	3	3.6
28	21	2.1	1.8	1.6	1.8	1.2
29	3	2.9	2.7	2.7	2	2
30	6	6.2	5.8	5.4	4.8	4.6
31	0.8	1.2	0.5	0.75	0	1.8
32	2.8	2.8	2.2	2.2	0	2.3
33	0.8	0.6	0.25	0.2	1.2	1.5
34	0	0	0	0	0.25	0.75
35	15.4	15.2	13.75	12.5	10.4	11.4
36	0.8	1	0.4	0.2	2	3.2
37	14.6	14.6	12.8	12.6	10.2	10
38	1	1.25	0.5	0.5	0.75	1.2
39	2.8	2.9	1.2	1	1.6	2
40	1.4	1.5	2	1.8	1.4	1.8
41	5.6	5.5	4.5	4.4	3.8	3.2

42	1.2	1.3	1	1.2	0.25	1
43	10.4	10.5	8.9	8.2	8.6	9
44	2	3	1.75	1.5	0.5	0.25
45	0	0.25	0	0	0	2.2
46	2.6	2.6	2	2.2	3.2	1.5
47	3.2	3	3.5	3.4	2.5	3
48	0	0	0	0	0.25	1.2
49	0	0	0	0	0	0.75
50	5.6	4.8	4.2	4.2	3.8	3.4
51	0	1.2	1	0.8	1	1.4
52	5.2	5.2	3.6	3.6	4.2	5
53	2.2	2.1	2	2	1.6	2.3
54	0	0	0	0	0.5	0.75
55	0	0	0.25	0.5	1.2	1
56	5.2	5	4.5	4.5	3	2.75
57	0	0.25	0.1	0.2	0	0
58	3.4	3.6	3	3.2	1.75	1
59	3.2	3	2.6	2	2.3	3
60	1.2	1	1	0.8	0.75	1.25
61	2.4	2.2	2.2	2	2.5	3
62	2.4	1.9	1.75	1.5	2	1.8
63	2	2.1	1.8	1.9	1.5	2.2
64	0	0	0	0	0.25	1
65	5.8	5.6	4.5	5	6.2	7
66	2.8	2.8	2.4	2.4	1.4	1.5
67	0	0.25	0	0	0	0.5
68	3	3	2.8	2.6	2	2.4
69	2.8	2.4	2.6	2.4	2.1	2.6
70	2.8	1.9	2	2	1.8	2
71	2	2.1	3.1	3	2.4	2.8
72	1.6	1.6	1.2	1.4	0.5	1.5
73	0.6	0.5	1.2	1.5	0.75	4.2
74	0	0	0	0	0	0
75	1.8	1.6	1	0.75	1.25	3
76	1.6	1.6	1.7	1.5	1.75	2.5
77	1.8	1	1.2	1.2	0	1.2
78	0	0	0	0	0	2.6
79	1.8	1.4	0.8	0.7	1	1.25
80	4.4	4.5	4	4.2	2.6	6.4
81	1.4	1.4	1.4	1.2	0.5	1.5
82	1.6	1.65	1.5	1.25	1	2
83	0	0	0	0	0.25	0.5
84	3.6	3.5	2.8	2.75	3	2.4
85	0	0	0	0	0	0
86	7.8	7.8	6.4	6.2	6	7
87	0	0	0	0	0	0.25

88	2.6	2.8	2.7	2.8	1.8	2
89	3.2	3	3	2.9	3.6	4.2
90	6.6	6.4	6.2	6	5.2	5.5
91	1.4	1.5	1.5	1.25	1.8	2
92	0	0	0	0	0	0.5
93	5.8	4.8	4.9	5	2.6	2
94	2.6	2.6	2	2.75	1.8	2.4
95	0	0	0	0	0.5	1
96	1.8	0.75	0.25	1	0.6	1.2
97	0.2	0.12	0.3	0.25	0	0
98	2	2	1.75	1.5	1.8	2
99	0	0	0.25	0.1	0	0.25
100	7	7.2	6.9	6.8	5.4	5.6
101	2.6	2.8	2.4	2.3	0.9	1.2
102	1	1.4	0.8	0.75	1.2	2
103	7.4	8.2	6.9	6.2	6.4	6
104	6.8	6.4	6.8	7	5.2	5.5
105	3.2	3.8	3.6	3.8	2.4	2.75
106	1.6	2	4	4.8	3	3
107	1	1.6	2	3	2	1.8
108	5	4.2	3.6	3.4	2.6	2
109	1.6	1.6	2	0	1.8	1
110	2.4	14	10.8	8	12	13
111	3.6	4	6	3	2	2.6
112	5.2	5	3.8	0	0	0.2
113	0	0.4	0	1	0	0.4
114	1.5	2	3	1.6	1	0
115	2.2	2	1.8	3	1.2	0
116	1.5	1.8	0	0	0.8	0
117	3	2.4	2	3	4.2	4
118	2	4	5.6	4	3	2
119	0.25	1	1.8	0	0	0
120	0.75	1.2	0	1	0	0
121	0	0.4	0	0	1	0
122	3.2	0	4	4.2	2	1.8
123	0.8	0	2	2.2	6	7
124	6	6	5	7	5	4
125	4	3	5.2	5	3	5.2
126	0	0.2	1	0.8	0	0
127	16	13	14.8	12	10.8	11
128	1.2	2	1.8	2.2	1	0.6
129	2.8	3	2	2	1.8	1
130	2	1.8	5	5	7	6
131	0.75	1	6	4.6	2	1
132	1	0	0.8	1	0.8	0.6
133	0	0.4	1.2	2	0.2	0.2

134	0	0	0.5	0	0	0
135	2	1.8	1.25	1	0.75	0.5
136	1.2	1.6	0	0.25	1	0.25
137	0.6	1	0.25	0.75	0.25	1
138	1.8	2	3	1.5	1	0
139	0.4	0	0	0.25	0	0
140	1	0.2	1	0	0	0.5
141	0	0.2	0	0	0.25	0
142	0	0	0.75	0.5	0.5	0.75
143	0.6	0	0	0	0.25	0
144	0	0	0	0.25	0	0.25
145	0.8	0.6	1	1	0	1
146	1	0.8	0.5	0.75	1	0
147	0	0	0.25	0	0	0
148	0.4	0.2	0	0.5	0	0
149	0	0	0	0.25	1.25	2
150	0	0.4	0.5	0	0	0.25
MEAN	2.62	2.53	2.384	2.23	2.01	2.39
S.E.M	0.278	0.251	0.229	0.208	0.195	0.21

APPENDIX 1D

DISTAL CYTOPLASMIC DROPLETS

S/N	FS0	FS1	EN0	EN1	WS0	WS1
1	0.62	0.5	0.25	0.75	1	1.25
2	0.6	0.6	0.4	0.25	0.35	0.5
3	0.42	0.4	0.2	0.5	0.25	0.25
4	0.47	0.45	0.3	0.15	0.5	0.25
5	1.4	1.3	1	0.75	1.25	1
6	0.2	0.1	0	0	1	1.5
7	0.4	0.3	0.2	0	0.2	0.25
8	1	1	0.6	0.45	1.25	1.25
9	0.8	0.9	0.5	0.25	0.45	0.5
10	1.4	1.5	1.2	1	0.75	0.5
11	1.6	0.5	0.2	0.25	0.2	0
12	1.4	1.6	1	0	0	0
13	1.6	1.2	1	0.75	0	0.25
14	1	1.6	1.2	1	2	3.5
15	2	1.2	1.3	1.25	1	2.5
16	0	2.5	0.75	0.5	0.25	0.5
17	10.2	0	1.25	1	0.75	0.25
18	1.2	9.5	2	1.75	1	0.3
19	6.5	2	1	2.5	2.25	2
20	0.4	4.8	5.8	6	1	0.75
21	0.8	0.25	0.2	0.25	1.25	1.5
22	2	0.8	0.75	1	0.8	0.5
23	0.8	2.1	1.25	1	0.5	1
24	0.4	0.8	0.6	0.5	0.25	0.5
25	6	0	0	0.25	0.25	0.25
26	0.6	0.5	0.4	0.1	0.5	0.4
27	2	6.2	5.2	5	2	3.5
28	3	0.8	0.4	1	0	0.25
29	0.2	2.1	1.6	2.5	2	6.5
30	0.6	3.5	2.4	2	1.75	5.25
31	0	0.75	0.1	0	0	0.75
32	0	1	0.25	0	0.25	1.5
33	4	0	0.25	0	0	1
34	0.4	0	0	0	0	0
35	10.2	3.8	2.6	2	1.8	2.4
36	0	0.4	0.2	0.5	0.4	1
37	0.4	10	7.5	7	3	2.75
38	0.4	0	0	0.25	0.5	1
39	0.6	0.5	1.25	1	0.8	1.25
40	2	0.8	0.25	0	0	0
41	0.4	1	0.4	0.4	4	0.7

42	0	0	1.25	0.5	5.25	6.2
43	0	2.1	1.8	1	5.75	5.5
44	1.8	0.4	0.2	1.5	1	1.5
45	0	0	0	1	0.75	1
46	0	0	0.5	0.5	0.5	0.25
47	0.6	1.6	1.2	1	2.5	3
48	0	0	0	1.5	1	0
49	0.8	0	7.5	1	0.8	2.25
50	0.6	0.9	0.3	0.25	0.75	1
51	0	0	0	0	1.25	1
52	0.8	1	0.6	0.5	0.25	0
53	0.6	0.75	0.5	1.5	1	2
54	0	0	0	0	0.5	0.4
55	0	0	0	1.25	1	0.8
56	0	0	1	0.75	0.5	0.25
57	0	0	0.26	0.2	0.25	1.25
58	0.4	0.2	0.15	0.1	0	4
59	1	1.5	1	1.25	1	6.5
60	0.8	0.8	0.5	1	0.8	0.75
61	0	0	0	0	0	0.25
62	0.4	0	0.3	0.5	0.25	1
63	0	0.25	0	0	0.25	2.5
64	0	0	0.25	0.5	1.5	3
65	0.8	0.9	0.45	0.4	0.25	0
66	0.8	0.75	0.6	0.5	0.5	1
67	0	0	1.5	1	1.5	2
68	2.4	2.4	2	3.5	4.5	4
69	9.8	9.4	6.8	2.5	3	2.75
70	2.6	2.8	1.5	1	0.8	0.4
71	1	1.2	1	1.25	1	0.75
72	0.8	0.6	0.5	0.25	0.5	1.25
73	0.2	0.1	0.2	0	0	0.5
74	0	0	0	0	0.5	1.5
75	1	1.25	0.75	0.5	1.2	5.75
76	0	0	0.8	0.75	6.5	1
77	0.4	0.8	0.2	0	0.5	2
78	0	0	0	0.25	0.2	0.5
79	0.8	0.2	0.1	0.1	0	0
80	1	1.5	0.9	0.75	1.5	2.6
81	1.4	1.4	1.2	1	2.5	3
82	1	1.2	1	0.5	0.25	0.5
83	0.6	0.6	0.15	0.5	0.2	0.5
84	0.4	0.42	0.3	0.45	0.5	0.75
85	0	0	0	1.5	2	1.5
86	0.4	0.5	0.25	0	0	0
87	0	0	0	0	0	0.25

KASIM IBRAHIM LIBR.

88	1	0.75	0.8	0.5	0.8	0.75
89	0	0	0.25	0.2	0.4	0.25
90	1.4	1.3	1.2	1	0.75	1
91	0.2	0.4	0.1	0.25	0.1	0
92	0	0	0	0	2.5	3
93	1.6	1.6	1	0.75	1	2.25
94		0	0	0	2.6	1.6
95	0.6	0.8	0.5	0.25	1.8	0.8
96	0.4	0.2	0.25	0.15	0.5	0.75
97	0.6	0.6	0.5	0.25	0.4	1
98	1.4	1.5	1.25	1.25	0.2	0
99	0	0	0.75	0.8	0.25	0
100	3	2.9	2.75	2	1.75	2
101	0.8	0.8	0.45	0.5	0.25	1.5
102	0.6	0.5	0.35	1.5	0	0.75
103	0.3	0.9	0.6	0.5	0.2	0.5
104	1	1	0.85	0.75	0.5	0.5
105	0.6	0.4	0.25	0.2	1.25	2.5
106	0	0	0.2	0.6	0	0
107	0.6	0.2	1.2	2	1	0.8
108	0	0	0	0.2	0	0.2
109	0.4	0.2	0	0	0	0
110	1	0.4	1	0.8	2	1.8
111	0.8	1	1.2	2	0	0
112	1.6	1.4	2	2.4	0.4	0.2
113	1	0.6	0	0.4	0.2	0.2
114	0.4	0.2	0.4	1	0	1
115	5.6	6	4	4.8	2.4	2
116	2.2	2.4	2	2.6	1	0.8
117	0.2	1	0	0	0	0
118	0.6	0.8	0	0.2	0	0.6
119	0.4	0.4	0.2	0	0	0
120	0	0.2	0.4	0	0.2	0.8
121	0.2	0.2	0	0	0	0.2
122	0.6	0.8	0.4	1	2	1
123	0.4	0.4	0.6	0.4	0	0
124	2	0.8	2.4	3	1.8	1.4
125	0.2	1	2	2.6	1	1
126	0	0	0.8	1	0	2
127	0.6	0	0	0	0	0.2
128	1	1.6	1.2	2	1	1.8
129	1.25	1.4	1	1.8	1.4	2
130	0.8	0.2	0	0	0	0.2
131	0.4	1	0	0.2	0.2	0.6
132	0	0	0.8	1	0	0
133	0	0.2	0.4	0.8	1	1.6

134	0	0	0	0.25	0.5	0.25
135	0.6	0	0	0	0	0
136	0.2	0.4	0	0	0	0
137	0	0	0.25	0.5	0	0
138	0	0	0	0	0	0
139	0	0.2	1	0.75	0.5	0.75
140	0.2	0	0	0	0	0
141	0	0	0	0	0	0
142	0	0	0	0	0	0
143	0.4	0.8	0	0	0.25	0
144	0	0	0	0	0	0
145	0	0	0	0	0	0
146	0	0.2	0.75	0.25	1	0.5
147	0	0	0	0	0	0
148	0.2	0	0	0	0	0
149	0	0	0	0	0	0
150	0	0	0	0	0.25	0
MEAN	0.92	0.94	0.79	0.8	0.82	1.11
S.E.M	0.136	0.131	0.104	0.089	0.089	0.111

APPENDIX 1E

MIDDLE PIECE ABNORMALITIES

S/N	FS0	FS1	EN0	EN1	WS0	WS1
1	6.8	6.9	5	5.5	4.8	5
2	8.8	8.2	6.5	7.5	9.5	10.2
3	2.2	2	1.5	2.25	1.75	2.5
4	6	5.8	4.5	4	5.2	6.2
5	3.6	3.6	3	2.75	3	2.75
6	2	1.8	2.5	4	4.5	5.5
7	3.6	3.4	4	2.25	1	2
8	5.2	5	3.5	1.8	2	1.8
9	3	3	4.8	5	6.4	6.5
10	10.4	10.5	7.5	8	11.5	12.75
11	1.8	2.1	0.75	9.25	10.25	11.2
12	5.4	6.2	3.5	2.25	6.4	7.5
13	4	4	4.5	3.75	2.5	1.2
14	2	1.8	0.5	1	1.8	2
15	6	6.4	5.75	6	6	7.25
16	6.8	7.1	8.25	8	7.5	6.8
17	0.3	0.7	0.5	0.75	0	7.2
18	0.8	0.6	0.25	0.2	0	0
19	0.2	0.28	0	2	4.8	5.2
20	2.2	2.1	3.5	3	3	4.5
21	4	3.8	5.75	4.25	5.25	2.5
22	4.2	4.2	2.5	1.25	2.75	2
23	9.6	9.4	7.5	9	10.25	8.5
24	1.2	1.2	0.25	1	0	2.75
25	3	3	1.75	2.25	2.8	3
26	0.2	0.25	0	0.25	1	0.25
27	0.6	1	1.8	2	2.5	0.5
28	4	4.2	4	3	1.25	2
29	0.8	0.8	1	0.5	0	0.75
30	0.6	1	0.75	2.5	3.5	4
31	3.6	4.2	3.75	4.5	6.4	7.2
32	2.6	2.6	2	3.5	5.3	5.5
33	5.4	5	5	2.5	6.5	4.8
34	14.4	14.5	14.75	10.5	12.4	10.5
35	10.2	10.75	9.8	12	12.25	11.25
36	5	4.6	8.5	9.25	6.4	7.8
37	10	10	7.25	8.2	9.5	10.2
38	0	0	0.8	0	0.75	1.5
39	4.4	4.45	3.75	5.25	6.5	6.8
40	11.6	11.75	10	13.4	14.25	15
41	2.8	2.2	3.5	4.5	1.75	2

42	0.2	0.1	0.8	0.5	0.25	0.75
43	1.6	1.6	1	0.8	0	2
44	2.2	2.4	2.5	3.2	4.5	3
45	2.8	2.8	3	4.5	3.8	2.5
46	7.2	7	5.25	6.5	5.75	7.2
47	9	10.4	7.5	8.5	9.75	10.5
48	6.2	6.2	6	5.8	7.25	4.25
49	0	0	1	0.5	2.5	3.4
50	3.8	3.7	4	3.6	6.1	5.2
51	6.2	6	5.8	6.25	1.25	1.8
52	16.4	16.4	14.75	15.8	8.75	9.2
53	2.6	2.9	3	1.5	2.5	1.25
54	8.2	10.2	6.5	7.25	8.2	6.4
55	0	0	0	0.25	1	2.6
56	9.6	10.1	12.5	12	13.2	13
57	3.2	2.8	2.25	3.5	4.5	5.25
58	6.6	6.9	5.4	6.4	10.2	7
59	11.2	11	8.5	8	7.5	12
60	4.6	4.3	3.6	0	2.5	3
61	6.2	4.6	4	6.5	5.2	5
62	6.6	6.8	7.5	4.8	8.5	9.2
63	7.4	8.2	6.25	7.5	5.5	6.25
64	13.2	11.8	9.5	12	10.75	8.4
65	5.2	5.2	5	4.8	5.3	7.5
66	6	6	8.25	9.2	10.25	9.8
67	8	7.8	9.2	8	10	5.4
68	0	0	0.25	0	1.5	2
69	11.6	11.4	12.5	10.25	12.5	13
70	11.2	11.6	14.2	14	13.75	11.25
71	12	12	8.4	9.2	10.25	8.8
72	10.8	11.1	7.5	8	8.5	7.25
73	2.6	3	2	1.75	3.75	0.25
74	5.8	5.2	6.5	7.2	6.5	7.2
75	4.2	4.3	2.5	3	2.5	3
76	5.4	5.5	3.75	4.5	4	4.25
77	9.8	10.2	10.2	10	11.5	12
78	8.4	8.4	7.8	8.2	3.8	4
79	2.4	2.8	5.25	6	13.5	13
80	14.4	14	16.8	17.25	15	12.75
81	7	7	5.2	4.8	6.5	7
82	6.4	6	5.75	5	5.25	8.4
83	6.6	6.9	6	6.5	4.8	5.5
84	8.6	9.2	9.25	10.2	8.5	10
85	8.8	8.5	10.5	7.5	6.75	7.5
86	6.2	6.4	8.75	4.5	9.2	8.2
87	7.4	7	6.5	8.2	5.5	5

473756

88	11.2	11	8	9.5	10.2	11.75
89	5.8	5.9	4.25	5.25	5	4.75
90	7.6	7.8	8.4	10.5	7.75	9
91	6	6.2	6	7.25	8	6.5
92	11.2	15.4	17.8	15	13.2	9.25
93	13.6	14	14.25	13.5	12.8	12
94	10.8	10.8	8.6	7.8	11	14
95	5	5.5	4.8	5.25	6	5.25
96	2.6	3	1.8	2	1.5	3.25
97	5	4.8	3.75	4.25	5	7
98	9.4	10.4	8.25	6.4	6	5.4
99	10.6	10.6	10.4	10.5	11	10.2
100	7.2	7	7.5	8	9.2	8.75
101	10.8	11.2	13.2	13	10.5	11.8
102	6.4	16	14	15	13.6	14.25
103	9.4	9.2	10.2	11.75	14.25	10.2
104	9.4	10	11.5	11	9.5	6.5
105	4.2	3.6	4.75	5.25	3	4
106	10	8	11	11.75	12	11.25
107	8	9.2	10	10	11.25	13
108	2.2	4	5.2	6.25	5	6
109	5.2	5	4	5	4.75	4
110	10.4	11	9.8	10	12.25	12.5
111	2	1.8	1	0.75	1	1.25
112	0.4	0.2	1	1.5	2.5	4
113	0.8	0.4	2	2	1.75	2
114	4	5	6	7	5.25	4
115	9.6	9.4	7	8.25	8	8.75
116	3	3	2.8	3	4	5
117	1.2	1.6	2	2.5	3	3.75
118	0.2	1	2.6	3.75	3.25	4
119	0.5	0.2	0.8	1	1.75	2
120	4	5	6	5.75	6	7
121	3.6	4	3	4	2.25	3
122	8.8	9	8	9.25	10.5	8.75
123	6.8	8	10	10.5	12	11.25
124	5	3.2	4.8	5	5	4.75
125	10	7	11	13	12.75	14
126	11.6	13	12	10.5	14	14.25
127	1.6	1.2	1	3.25	6.25	7
128	2.8	3	4	4.5	5	3.25
129	5.2	4.2	6	6	7	8
130	9.6	7.6	10	11	8.25	8.25
131	3.6	4	2.8	2	1.5	4
132	6	4.2	5.8	4	3	3
133	10	8.8	9	8	11.25	12

134	8	7	11	10.75	11.25	11
135	11	12.2	12.25	12	13	12
136	9	10.4	13	14	8.25	8
137	12	13	15	14	12.75	13
138	12.8	12	10.25	9	11	10.25
139	10	10	13	12.5	12	11.25
140	12	11.8	12	12	8	9
141	8	10	13	15	16	16
142	10.2	11	11.75	12	11.25	10
143	12	7.8	9.25	10	13	13
144	12.8	12	10	10.25	11	12.25
145	7	7	8.75	9	10	11
146	8	8	9.5	7.25	8	9
147	14	15	16	12	13	13.5
148	3.8	2	3.5	2.75	4	4.5
149	7	8	7.5	7	6	5
150	9	6.8	6.75	8	9.25	12
MEAN	6.28	6.36	6.37	6.53	6.85	6.95
S.E.M	0.317	0.33	0.339	0.335	0.335	0.321

APPENDIX 1F

SPERM TAIL ABNORMALITIES

S/N	FS0	FS1	EN0	EN1	WS0	WS1
1	11.8	12	9.5	10.5	7	8.25
2	11	11	12.5	13	8	10
3	2.6	3	3	3.75	1.75	3.5
4	8.8	8.2	7.5	8.25	5.8	6.4
5	2.8	2.5	4.25	2.75	3	1.25
6	17	17	19.5	20	16.5	18.5
7	25	24.2	20.75	18.25	24	12.8
8	11.2	11.2	9.8	10	7.5	8.5
9	10.4	10	10	10.5	8.25	9.25
10	16.2	16	17.25	16.75	14.75	14.8
11	3	2.4	2.75	3	1.25	2
12	8.4	8.4	7.25	8.25	7	8.5
13	10.8	10.6	12.75	13	9.8	9
14	6.4	6.8	6.75	8.2	5.4	2.4
15	6.8	6.8	7.2	7	6	5.5
16	12	12	8.5	8	13.75	14.25
17	0.8	1	2	2.8	2	1.8
18	2.6	3	4.25	5.25	3	1.75
19	4	4.8	5	4.8	4	3.75
20	2.6	2.6	2.75	3	5.25	4.5
21	14	14.4	15.75	15	15	16.25
22	3	3	2.5	3	4.75	5
23	7	7.2	10.25	11	6.4	6
24	2	3.4	4.5	3.75	3.25	2
25	1.2	1.4	2	1.85	1	2.5
26	2.8	3	2	1.75	3	1.8
27	1.8	2	3.5	3.75	0	2.5
28	13.8	12.8	14.75	15	12.25	13
29	3.2	3.2	4.25	2.75	3	4.25
30	4.8	4.8	5	6.75	4	1.8
31	7	7.2	8	8.25	6.75	7
32	12.4	11.4	12	13	10.5	8.25
33	23.6	24	18.5	40	18.25	25
34	11.4	11	13.75	8.25	10.25	9.8
35	8.8	9	14.5	7.8	8	7.25
36	7.6	7.4	8	10.2	5.8	6
37	23.2	2.4	24.5	16.4	24	20.8
38	6.4	6.4	7.25	6.75	5	7.8
39	0	0	2	1.8	1.25	3.5
40	5.6	6	7	7.25	4.75	1.25
41	15	14	12.5	13	16	14

42	0	0	0	0.25	1.25	3
43	10.2	9.8	12.75	8.95	13.25	12
44	19.2	19.4	25.25	20	18	20.4
45	9.4	10.2	7.5	8	7.25	4.8
46	15.6	15.8	13.5	11.25	10.5	13.75
47	12.4	12	16.25	16	11	11.25
48	4	5	2.5	3	3.2	1.8
49	21.8	22	18.5	18	15.25	16.25
50	6.6	7.4	5	4.75	4	5.25
51	15	15.4	17.5	17.5	12.75	13
52	17.6	16	13.25	12	20.5	22.75
53	1.2	1.6	2	1.8	0	1.25
54	5.6	6	4.75	5.2	5	6.4
55	9.6	8.6	10.25	11.25	10	5.5
56	6.2	6.2	13.5	14	5.5	6.8
57	4.6	4.6	5.25	4.8	6	11.6
58	11.6	11.8	11	7.8	12.8	14.8
59	6.4	6.4	7	5.4	3.75	2
60	7.4	7.4	5	5.25	3	1.8
61	12.2	12.2	11.5	11	10.2	13.75
62	7.8	7.8	8	7.5	5.4	4.8
63	14	13.4	14.25	14	10.2	12.8
64	12.2	12.4	13	12.75	13.5	10
65	12	12	14.75	14	17.5	16.4
66	6.4	6.8	2.5	3	5.2	4.25
67	8.6	8.4	0.25	2.5	1.8	0
68	2.6	2.6	2.75	2	2.75	3.8
69	8.6	8.6	2	1.8	0	1.25
70	5	5.2	7	8.25	4.5	6.4
71	9.2	9	13.25	13.75	10.2	10
72	10.2	10	6.25	14.5	8.8	7.25
73	4.8	5	3.75	3.2	3.5	3
74	8	7.6	5	8	6.4	6.5
75	13.2	13.2	10.25	11.25	10.8	11.8
76	6.2	5.4	5	1.75	4.75	6.5
77	4.6	4.6	3.75	2.8	3.25	4.25
78	3.6	3.7	4	3.8	3	1.25
79	12.8	12.4	14	13.75	9.8	10.25
80	13.4	14	9.8	10	12.4	14
81	11.8	11.8	10.75	10.2	6.25	8
82	5.2	5	5.75	4.8	3.5	3
83	6.2	6.4	4.25	4	6	16.4
84	15	14.8	24	25.2	14.75	12
85	14.2	14	10.5	11.25	12.8	12.4
86	5.2	5.4	5	5.75	4.4	4
87	15	15.2	13.25	12	13	13.25

KASHIM IBRAHIM LIBRARY

88	17	18.2	16.4	14.25	18.75	14
89	16.4	16	15	15	10.25	6.75
90	16	16	13.5	12	14	10.5
91	7	7.8	5.5	10.5	1.8	2
92	7.4	7	7	9.5	5.4	6.25
93	8.2	8	8	12.4	4.8	5
94	13.2	13.4	14.5	6.5	10.25	11
95	14	14	10.25	9.8	12	13.8
96	5.6	5.8	6.5	6.25	4.25	5
97	7.2	7	5	5.75	6.8	4.2
98	8.4	8	4.75	5	3.25	1.8
99	9.4	10.2	3.25	4.25	6.4	6
100	6.4	6.8	4	10.8	2.75	1.8
101	17.2	17	18.2	19	12.8	12
102	7.8	7.8	8	7.25	4.75	5.75
103	7	7.4	5.25	3.75	2.8	1.25
104	8	8.5	8.8	7	5.75	6
105	2.8	2	1.25	4.8	1.8	10.25
106	6.4	4	6	6	7	7.25
107	5.8	6	6.25	7	8	7
108	11	12	13	13.25	12.5	12
109	2.6	3	4	6	6.75	7
110	8.8	5.6	6	5	8	9
111	17	20	18	21.25	23.5	21
112	10.4	9.2	11	13	14	13.5
113	8.2	3	4	4	2	4
114	12.5	12	12	11	14	10
115	4.8	5	4	6	7	7.75
116	2.6	2.2	3	3.5	2	3
117	3	3.4	4.75	5	4	4.25
118	7	8	9	8.75	9	11
119	1.8	2	3	2.25	3.5	4
120	3.4	4	5.25	6	6.5	5
121	7	7.6	8	9	10.5	11
122	6.4	6	7	9.25	9	8.25
123	10.8	11	12	13	13	11.5
124	16.2	18	20	19	21	24
125	10.4	12	9	7.5	7	9.25
126	23.6	21	22	23	24	20
127	8.8	7.2	8	9.25	11	12
128	6.4	6.6	9	10	10.25	11
129	8.8	8.8	10.25	9	8	9.25
130	25	26	24	23	24	21
131	12.6	12.8	13	12	13	14
132	2.8	3	4	5	4.25	5
133	7	9	6.75	5.25	6	7

134	8.8	1	20	20.5	22	21
135	15	1	13.75	14	15	15.5
136	13	1 .8	14	14	16	16.25
137	8.8	7	6.5	7	6	5
138	17	1	16.5	16.25	14.5	14
139	16	2	22	21	20	21.5
140	10	6 .8	7	7.5	8.25	9
141	3	3	5	6	7.75	7
142	4.8	5 4	4.25	3	4.25	4.25
143	8.8	8	11	10	11	11.5
144	15	1	15	14.75	16	15
145	6	7.2	7	6.25	5.75	6
146	4	4.8	5.25	5	6	8.25
147	5.4	5	4.75	6	6.25	7
148	6.8	6.8	8	9.25	10	8.5
149	6.2	6	7.5	8	7.5	6.5
150	10	1	13.25	14	15	13.75
MEAN	9.13	9.07	9.19	9.34	8.6	8.71
S.E.M	0.436	0.438	0.461	0.477	0.464	0.453

APPENDIX 1G

DETACHED NORMAL SPERM HEADS

S/N	FS0	FS1	EN0	EN1	WS0	WS1
1	4.6	5.2	5.75	8.25	1.75	3
2	18.8	18	12.2	7.4	8.5	6.25
3	5.4	5.4	3.75	3.75	3	1.25
4	1.8	2	0	0.8	0.25	2
5	2	2.5	4	4	1	3.75
6	1.2	2.1	3.5	6.2	3	4
7	0	0	1.8	3.25	0	1.25
8	2.6	3	2	1.8	1.8	2
9	20.2	20.2	11.25	10.75	14.5	16
10	8	8.2	6.3	11.8	4.8	5.25
11	2.4	2.6	3	4	2.5	6
12	3.8	3.4	12.75	14.5	4	2.25
13	1.6	1.6	3.8	6.4	2.5	3.5
14	1.2	1	0	2.5	1	1.25
15	1.2	2	3	1.2	3.5	5
16	2	3.1	5.5	7.8	1.5	2
17	0.5	0.6	1.75	3.5	0.75	0.25
18	0	0	2	0.8	0.25	1
19	0.5	0.4	1.2	0	1	0.75
20	0.75	0.8	3	3.8	3.4	3.25
21	2	3.1	1.8	2	1.8	2
22	5.1	5	7.8	7	3.5	4.5
23	4	4.8	2	1.25	2	1.25
24	3.4	3	1.8	1.75	3	0.75
25	2	2	3.4	3	0	0.25
26	0	0	12.5	12.2	10.8	10
27	0	0.3	1.8	5.8	4.6	4.25
28	0.9	1	2.4	3.75	1	2.25
29	0	0	6	2	3.75	4
30	0	0.8	1.25	11	0.25	0.5
31	1.8	2	3.4	0	0	0.5
32	2.2	2	0	3.8	1.75	2
33	0	0	0	2.75	0	0
34	4.2	4.2	7.2	7	3.9	6.5
35	0	0	0	0	0.25	0.5
36	2.6	3	1.5	0	3.4	4
37	0	0	0.25	3.5	2	2.5
38	0	0.8	0	4.25	3.5	4.75
39	0.4	0.4	1.8	0	0.8	1
40	0	0	0	0	2	3.5
41	0	0	0.75	1.25	3	4

42	8.8	8.8	3.5	4.75	5.75
43	0	0	0	0	0
44	5	5.1	0	3.2	2
45	7.8	7.9	6	6.5	6.4
46	4.4	4.4	3	1.25	2.5
47	11.4	10.2	8.2	0.75	0.5
48	0	0	0	0	2
49	0	0	1	4.8	3.4
50	5	5.4	2.5	3	4.6
51	2.6	2.6	1.8	2	2
52	0	0	0.8	7.5	0
53	2.8	3	1.7	0.25	1.8
54	0	0	0	0.75	0
55	0	0	0.8	0	4.25
56	0	0	0	1.25	0
57	6.8	7.8	3.5	4	5.2
58	0	0	0	10.5	0
59	1.8	2	1.8	3.4	1
60	0.8	0.4	0	4.75	0.8
61	9.6	9	6.5	5	10.5
62	3.2	3.4	0	8	4.8
63	0	0	0.25	0.8	0
64	0	0	0	0.25	0.75
65	8.8	10.2	13.4	11.75	9
66	4	4.8	1.75	0.25	3
67	0	0	0	3.75	3
68	0	0	1	2	1.75
69	7.8	7	10.2	11.5	6.5
70	0	0	0.25	3.4	0.25
71	2.2	3	2	8.2	6
72	7	6	6	11.45	8.4
73	0	0	0	7.2	2.6
74	2.4	2	1	0	0
75	1.4	1.5	13	11	4.5
76	8.8	8	5.4	6.4	7.5
77	5	5.2	2.5	3	2.5
78	0	0	11	12.8	9.5
79	3.6	4	5.8	2.4	4
80	3	2.8	0	1.8	2
81	0	0	0	2	0
82	2.2	2	6.75	6	0
83	9.4	10.2	13.5	12.75	10.75
84	3.6	4	1.8	2	2.4
85	4	4.8	3.4	4.75	2
86	0	0	2	0	0
87	9.8	8.8	0	0.25	7.5

134	2	1.8	2.2	3.5	4.5	4.5
135	0	0.4	0	1	2.5	3
136	4	2.6	3.75	2	3	2.75
137	0	0	0.25	0	1.75	2
138	3	1.8	2	3	4.25	4
139	0	0	0	0	0	0
140	0	0	0	0	0.75	0.5
141	1	2.4	1.75	0.75	1.5	1
142	0	0	0.25	0	0	0
143	0.8	1	0	0	2	2
144	0	0	0	0	0.5	0.5
145	6	4.8	5	4.75	6.25	6
146	2	3	2.5	3	2	2.75
147	0	0.8	1	0.5	1	0.5
148	0	0	0	0	0.75	1
149	5	3	6.25	6	8	8.25
150	2	5.2	3	3.25	2	3.25
MEAN	2.74	2.85	3.2	3.95	3.25	3.62
S.E.M	0.292	0.285	0.299	0.305	0.254	0.264

APPENDIX III

DETACHED ABNORMAL SPERM HEADS

S/N	FSO	FSI	ENO	EN1	WSO	WS1
1	9.6	9.6	11	13.5	15	16
2	6	6	12	10.75	10	10.25
3	7.6	7.8	3.5	4.5	2.75	3
4	2.2	2	1.75	2	1.25	1
5	3	3.4	4.75	4	1	1.25
6	4	4	5	4.75	4.25	4
7	1	1.2	0.25	1	2	2.75
8	4	4.3	0.25	0.5	1	3
9	2.6	3	6	3	3.5	3
10	14.2	14	13.5	12.75	13	12.75
11	3.6	3	6.25	6.25	5.75	6
12	2.4	2.2	4.5	4	4	2.75
13	5	5.4	7	8.25	8.5	8
14	4	4.2	1.25	0.75	1	1.25
15	5.2	5	5.25	5	5.25	6
16	4	4	6.5	6	3	9.25
17	0	0	0.25	1	0.25	1.75
18	0	0	0	0.5	0.5	1
19	1.5	2.4	4	4	4.25	4.25
20	1	1.8	3.5	3.75	4	3
21	2.2	2	6.25	6	4.25	4
22	9	9.2	10	13.5	13	14.75
23	0	0	0	0	0	2
24	0	0	0	0	0	0
25	2	1.8	3.5	4	6	7
26	0	0	0	0	1	1.25
27	0	0	0	0	0.25	1
28	3	3	1.75	2	1	0.75
29	0	0	0	0	0	0
30	0.75	0.8	0.5	1	3	2
31	3.2	3	4	2.75	4	5
32	0.8	0.8	1	1.25	3	3
33	0	0	0	0	0.25	0.5
34	4.6	4.6	5	6.25	7	6
35	0	0	0	0	0	0
36	5.6	5.9	6.25	4	5	7
37	5.3	6	7	8	9.25	10
38	0	0	0	0	0.25	2
39	1.2	2	2.5	3	6	4.25
40	0	0	0	0	0	1
41	0	0	0	0	1	3.5

APPENDIX VII

DETACHED, ABNORMAL SPERM HEADS

S/N	FSO	FSI	ENO	EN1	WSO	WS1
1	9.6	9.6	11	13.5	15	16
2	6	6	12	10.75	10	10.25
3	7.6	7.8	3.5	4.5	2.75	3
4	2.2	2	1.75	2	1.25	1
5	3	3.4	4.75	4	1	1.25
6	4	4	5	4.75	4.25	4
7	1	1.2	0.25	1	2	2.75
8	4	4.3	0.25	0.5	1	3
9	2.6	3	6	3	3.5	3
10	11.2	14	13.5	12.75	13	12.75
11	3.6	3	6.25	6.25	5.75	6
12	2.4	2.2	4.5	4	4	2.75
13	5	5.4	7	8.25	8.5	8
14	4	4.2	1.25	0.75	1	1.25
15	5.2	5	5.25	5	5.25	6
16	4	4	6.5	6	3	9.25
17	0	0	0.25	1	0.25	1.75
18	0	0	0	0.5	0.5	1
19	1.5	2.4	4	4	4.25	4.25
20	1	1.8	3.5	3.75	4	3
21	2.2	2	6.25	6	4.25	4
22	9	9.2	10	13.5	13	14.75
23	0	0	0	0	0	2
24	0	0	0	0	0	0
25	2	1.8	3.5	4	6	7
26	0	0	0	0	1	1.25
27	0	0	0	0	0.25	1
28	3	3	1.75	2	1	0.75
29	0	0	0	0	0	0
30	0.75	0.8	0.5	1	3	2
31	3.2	3	4	2.75	4	5
32	0.8	0.8	1	1.25	3	3
33	0	0	0	0	0.25	0.5
34	4.6	4.6	5	6.25	7	6
35	0	0	0	0	0	0
36	5.6	5.9	6.25	4	5	7
37	5.3	6	7	8	9.25	10
38	0	0	0	0	0.25	2
39	1.2	2	2.5	3	6	4.25
40	0	0	0	0	0	1
41	0	0	0	0	1	3.5

42	6.6	7	8	8	7	7.25
43	0	0	0	0	0	0
44	9.6	10.2	8.25	9	11.25	11
45	10.2	10	7.5	8.25	12	10
46	8.6	8.6	4.25	4	2.5	6
47	16	16	20.5	17.5	15	13.75
48	6.6	7.2	9	9.25	10	13
49	0	0	0	0.75	2	1.5
50	9.6	8.6	11.25	11	13.25	13.75
51	4.6	4.4	6.25	7	6	6
52	7	7.3	8.25	6.5	7.75	8
53	6.2	6.2	4.5	3.5	4	3
54	0	0	0	0	2.25	2
55	0	0	2.5	3	1	1.75
56	6	6	8	9	3.25	6
57	9	10.2	11.25	7	9	4
58	0	0	0	0.25	2	3.5
59	6	5.8	7.25	9	11	13
60	7	7.2	8.75	6	7	5.75
61	18.8	19.2	20	13.75	14.25	14
62	4	4	5.5	4	7	8
63	0	0	0	0	0.25	1.75
64	0	0	0	1	2	1
65	14	15.2	13	14	12	13
66	7.4	8	12	10	7.5	8
67	0	0	0.5	1	3.25	4
68	2.4	3.4	5.25	6.75	4	2
69	9.8	10.2	11	11.25	8.8	6.25
70	2.6	3.6	5.25	6	3	1.75
71	11.8	11.8	10	8	8.75	9
72	9.8	10.2	7	11.25	2	1
73	1.4	1.5	0.75	1.5	0	0.25
74	9.8	9	10.25	11	10	11.25
75	2.4	2.5	3	1.25	3.5	2.75
76	8.6	8.4	9	8.5	10.25	10.5
77	2.8	3	2	0.5	4.5	5
78	2.6	3.2	1.5	2	1.75	2
79	11.4	11.4	8	6.75	9	11
80	8.4	8.4	6.5	6	11.25	13
81	1.4	1.5	2	2.25	4	2.75
82	3.2	2.9	4.25	5	4.25	5
83	10.8	10.4	11.25	13	9	11
84	5	5.2	10	9.25	3.25	2
85	10	10.4	11.25	12	13	11.25
86	10.6	10.8	8.5	8	10	8.75
87	14	15.2	11	12	13	14

88	6	4.8	7.25	5.75	7	6
89	0	0	1.5	1	2.25	2
90	6.4	6.4	4.75	8	3.5	3
91	3	3.2	3	1.5	2.25	3.75
92	7.6	7.2	8	7.25	8.5	9
93	7.8	8.4	19	17	13.25	10
94	8.8	8.8	7	6.25	8.25	6.25
95	4.6	4	4.75	4.75	6	4
96	6.2	6.8	4.25	4	5	6
97	4.6	4.6	1.75	2	4	1
98	3.2	3	6	8.75	10.75	9
99	0	0	0	0.25	3.75	4.5
100	10	10	13.75	13	11	8.25
101	8.4	8	13	13	10	13
102	3.4	3.7	7.5	5.25	6.5	15.25
103	6.8	6.8	4.25	4	4.75	3
104	4.2	4	11	11.25	13	11.75
105	7.6	7.2	6.5	5	2	7
106	11	10	12.5	12	11.25	13
107	7	7.2	8	9	10	12.5
108	9.6	9	8.75	10	9	7
109	7.6	6.8	7.25	5.5	9.25	11.75
110	3	2.2	3.5	3	2.75	3
111	4	4	4.5	4	5	4.25
112	1	0.8	2	1.75	0.5	1
113	0	0.2	0	0	0.75	4.5
114	5	6	6.25	7.25	8	7.75
115	0	0.2	0.5	10	0	1
116	0	0.6	0	0	0	0.25
117	9.6	11	12	13.25	10	11.5
118	2	1.8	2.75	2	4	5
119	0	2	1.5	2	1.75	2.5
120	0	1.8	0	0	0.25	0
121	0	0	0	0.5	0	0
122	4.6	5	6	7	7.75	8.25
123	5.6	4.8	5	4	6	5.75
124	6.3	6	7.75	6.25	5	4
125	9.6	9.4	10	11	12.5	14
126	7	7	5	5	4.75	6
127	4	4.2	3.5	4.25	3	4
128	0	0	0.75	1	0	0
129	9.6	10.6	10	8.75	4.25	5
130	3	2.8	3	3.25	4	4.75
131	14.2	14	16	15	18	17
132	3.6	4	3	2.75	5	4.3
133	2.2	2.8	2	4	6	3

473756

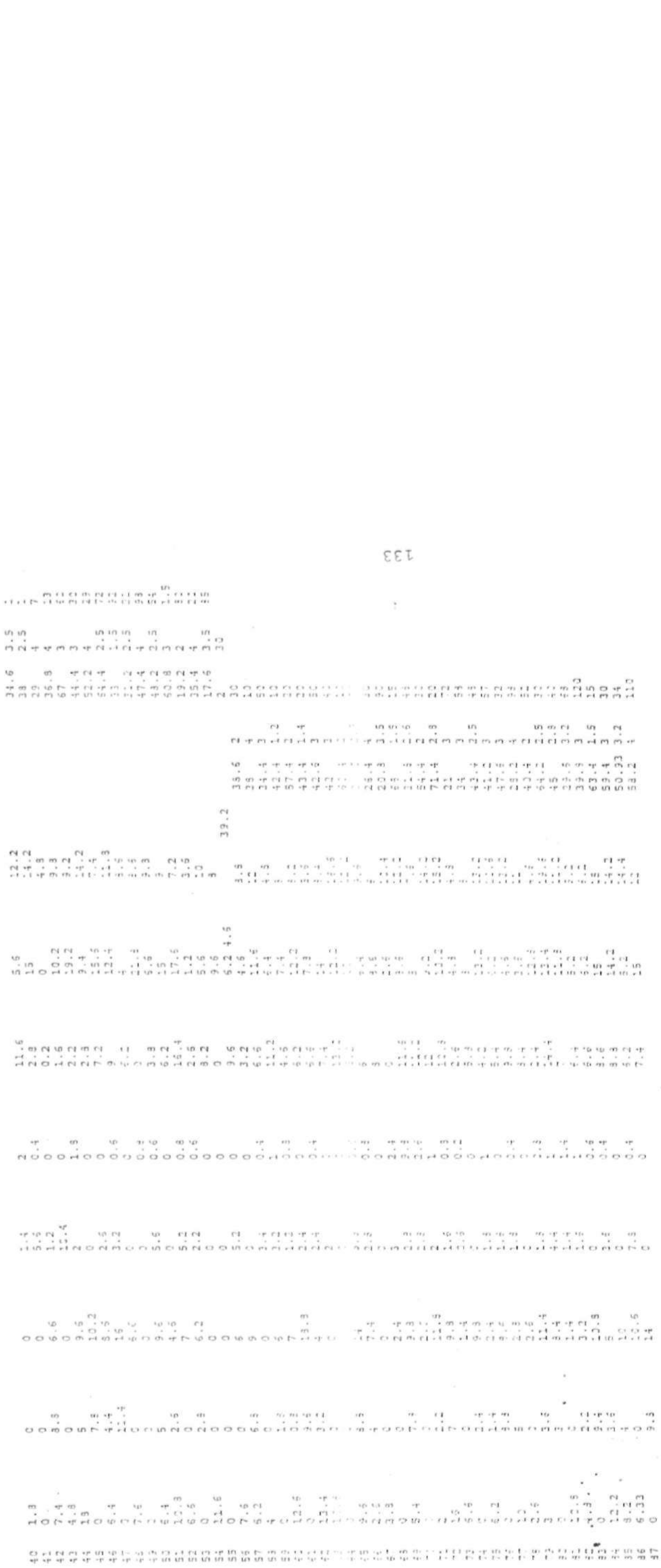
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135	7	7	8	8	10.25	11
136	6	5.8	4.5	5	6	5.25
137	0	0.2	0	0	1	0
138	2	1.8	3.5	3	4.25	4
139	6	6	6.25	6	6.5	6.25
140	7	7.4	8	8.75	9	7.25
141	4	3	4.25	6	4.75	5
142	0	0	0	0	0	0
143	5	6	4	3.75	4	4
144	0	0	0	0	0	0
145	10	11	13.25	15	14	15
146	3	1.8	1.5	2	1.5	1
147	0	0	0	0	0.25	0
148	0	0.4	2	1.5	1.75	2
149	2	1.8	1.25	2	3	2.5
150	6	7.2	9	7.25	8.25	8
MEAN	4.7	4.82	5.27	5.3	5.4	5.62
S.E.M	0.332	0.336	0.369	0.357	0.347	0.358

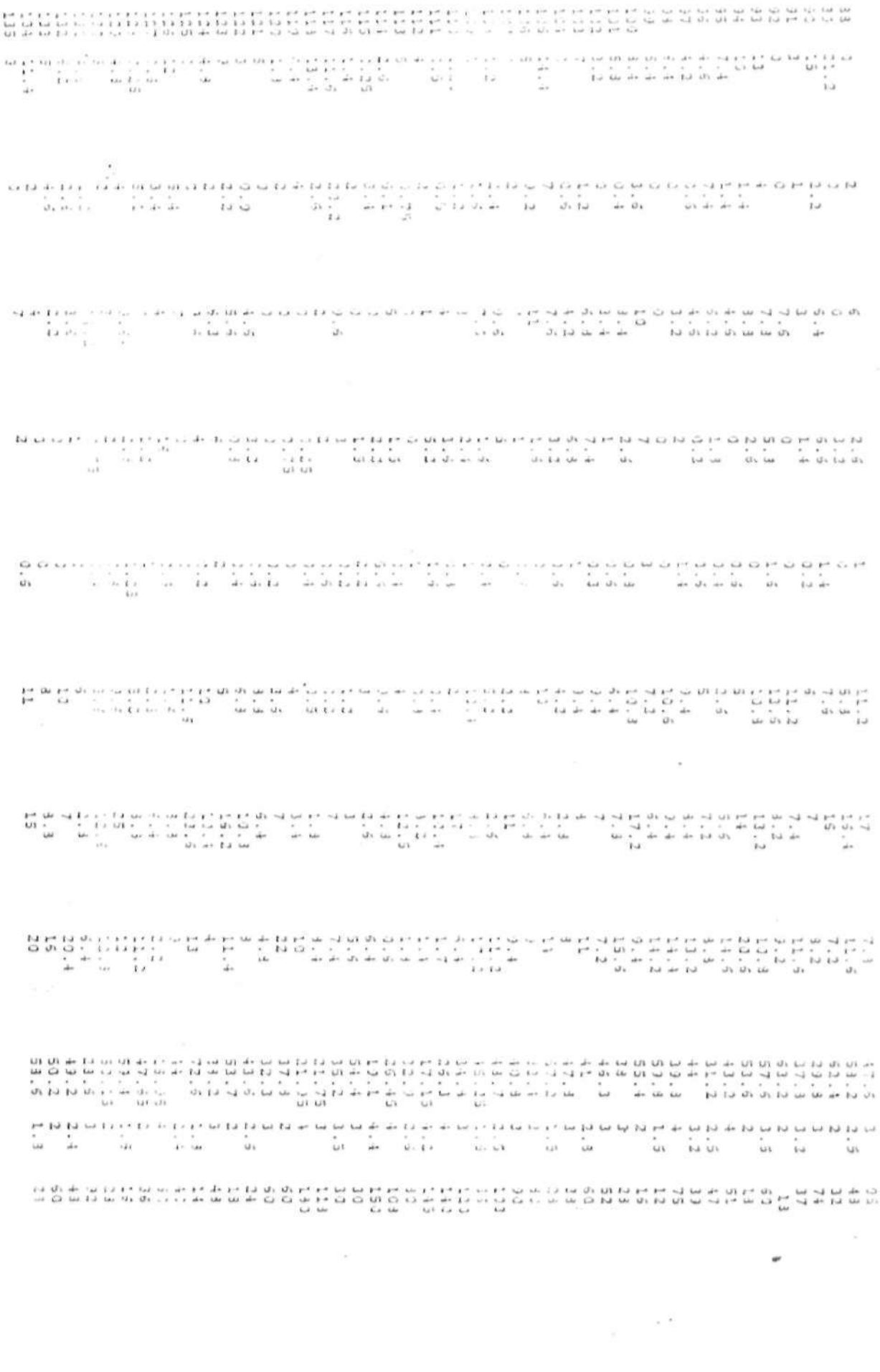
APPENDIX FOR MORPHOLOGICAL STUDIES (VALUES FOR TOTAL MORPHOLOGICAL ABNORMALITIES, SEMEN VOLUME SPERM CONCENTRATION)

APPENDIX II

VALUES FOR TOTAL MORPHOLOGICAL ABNORMALITIES, SEMEN VOLUME, SPERM CONCENTRATION

S/N	Aspects	Normal	Abnormal	Proximal	Distal	Mid-piece	Tail	Head	Total	Vol	Conc/ml
1	3.4	4.6	9.6	4.2	3.6	5.8	11.8	22.6	63.6	3.8	10
2	13.8	13.8	6	5	1.6	3.8	11	9.4	7.4	1.8	69
3	6.2	5.4	7.6	1	1.4	2.12	2.6	11.2	36.6	0.6	1
4	7	1.8	2.2	1.6	0.47	6	3.8	16.4	44.3	3.6	40
5	4.8	2	3	2.8	1.4	1.6	2.8	6.4	25.8	2	36
6	4.6	1.2	4	0.4	0.2	2	17	17.2	46.6	4	30
7	3	0	1	1.8	0.4	3.6	25	25	58.8	2	80
8	7.8	2.6	4	1.4	1	5.2	11.2	11.2	44.2	4	60
9	6	22.2	2.6	2	0.8	2	15.4	10.4	56.4	6	84
10	0.4	4	14.2	4.4	1.6	1.8	13.2	16.2	77	4.6	27
11	6	3.4	3.6	1.2	1.6	1.8	3	22.6	33.2	4	26
12	11	3.8	2.4	1.8	1.4	5.4	3.4	17.6	51.8	3.6	34
13	24.6	1.6	8	14	1.6	4	12.8	12	73.4	1.8	13
14	3	1.6	4	7.8	1	1	9.4	21.2	62.6	2	1
15	3.4	0.8	0.8	0.8	0.8	1.2	11.6	25.6	53	2	1
16	1.6	0.8	0	14.76	1.2	2.6	11.4	2.6	43.25	6	29
17	0.6	0.8	0	1.76	0.8	1.2	1.6	13.4	23.1	1	16
18	0.6	1.6	0	1.2	0.8	1.2	3.6	6.4	15.35	2.5	23
19	0.8	0.8	0	1.3	0.4	1.2	3	17	46.3	12	12
20	3.4	0.4	0	2.3	0.8	1.2	1.2	4.9	33.7	6	91
21	0.4	0	0	3.4	0.4	1.2	1.2	12.3	42.6	2	9
22	0.4	0	0	1.6	0.4	1.2	1.2	10	21.6	2.2	60
23	0.8	0	0	0.8	0.8	1.2	1.2	0.4	13.6	1.6	67
24	0.8	0	0	0.8	0.8	1.2	1.2	0.4	4.65	2	41
25	0.8	0	0	4	0.8	1.2	1.2	1.6	3.8	2.6	40
26	0.8	0	0	21	0.8	1.2	1.2	13.8	73.7	4.6	3
27	0.8	0	0	1	0.8	1.2	1.2	2.4	9.25	3	1
28	0.8	0	0	0.8	0.8	1.2	1.2	1.6	13.35	2.5	1
29	0.8	0	0	0.8	0.8	1.2	1.2	11.6			
30	0.8	0	0	0.8	0.8	1.2	1.2	1.6	13		
31	0.8	0	0	0.8	0.8	1.2	1.2	1.6	27.8		
32	0.8	0	0	0.8	0.8	1.2	1.2	1.6	78.8		
33	0.8	0	0	0.8	0.8	1.2	1.2	1.6	33.6	2	3
34	0.8	0	0	0.8	0.8	1.2	1.2	1.6	42.6	2	3
35	0.8	0	0	0.8	0.8	1.2	1.2	1.6	73.1	4	3
36	0.8	0	0	0.8	0.8	1.2	1.2	1.6	30	2.5	4
37	0	0	0	0.8	0.8	1.2	1.2	1.6	26	2.5	3
38	0	2	5.3	14.6	0.4	2	6.4	22.2			
39	5	2.4	1.2	2.8	0.6	4.4	0	11.6			





APPENDIX III
MONTHLY MEANS OF SPERM MORPHOLOGICAL ABNORMALITIES IN 1995

APPENDIX III

Monthly Means of sperm morphological abnormalities in 1995

% Mean Abnormalities									
Month	Acro-some	Det. Nor Head	Det. Abnor Head	Prox .Cyto Drop-lets	Dist. Cyto Drop-lets	Mid-piece	Tail	Head	Total
Jan.	7.32	3.37	6.93	3.43	0.38	8.63	11.53	10.25	55.02
Feb.	5.67	1.51	5.47	2.85	0.79	7.87	9.47	12.71	46.35
Mar	4.79	3.2	5.07	2.48	1.09	5.15	8.21	7.33	37.34
Apr.	6.2	5.33	4.32	2.2	0.6	7.33	8.38	11.7	44.85
May	7.59	2.72	4.11	3.18	1.19	6.01	8.8	13.35	46.95
Jun.	5.63	1.42	2.19	2.13	0.68	5.52	6.8	10.44	34.80
Jul	7.53	1.45	2.3	4.13	1.71	6.15	11.51	13.55	48.32
Aug	6.2	3.53	6.93	2.76	0.51	5.51	13.0	9.71	48.15
Sept	5.25	0.53	4.28	1.90	0.35	5.75	6.58	7.48	33.10
Oct	4.56	3.34	5.90	2.40	1.68	7.54	8.34	9.68	44.04
Nov.	5.83	3.33	6.66	1.34	0.43	7.23	8.03	11.31	44.70
Dec	5.2	3.2	6.6	1.6	0.65	7.83	10.28	10.88	46.25

Z-Test - Means not significantly different ($P < 0.05$)
SEM = Standard Error of Mean.

473756

137

APPENDIX FOR STORAGE AND STAINING SOLUTIONS