KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

COLLEGE OF HEALTH SCIENCES

SCHOOL OF MEDICAL SCIENCES

DEPARTMENT OF ANATOMY

An Evaluation of the Effect of Ethanolic Root Extract of *Zingiber Officinale* (Ginger) on the Morphology of the Reproductive System of Male Wistar Rats.

BY

YAW OCTHERE DONKOR

SEPTEMBER, 2016

AN EVALUATION OF THE EFFECT OF ETHANOLIC ROOT EXTRACT OF *ZINGIBER OFFICINALE* (GINGER) ON THE MORPHOLOGY OF THE

REPRODUCTIVE SYSTEM OF MALE WISTAR RATS

A THESIS SUBMITTED IN PARTIAL

FULFILMENT OF THE

REQUIREMENTS FOR THE DEGREE OF

MASTER OF PHILOSOPHY IN HUMAN

ANATOMY AND CELL BIOLOGY

IN THE

DEPARTMENT OF ANATOMY, SCHOOL OF MEDICAL SCIENCES,
COLLEGE OF HEALTH SCIENCES

BY

YAW OTCHERE DONKOR

KWAME NKRUMAH UNIVERSITY OF SCIENCE & TECHNOLOGY,
KUMASI SEPTEMBER, 2016

AN EVALUATION OF THE EFFECT OF ETHANOLIC ROOT EXTRACT OF *ZINGIBER OFFICINALE* (GINGER) ON THE MORPHOLOGY OF THE REPRODUCTIVE SYSTEM OF MALE WISTAR RATS

A THESIS SUBMITTED IN PARTIAL

FULFILMENT OF THE

REQUIREMENTS FOR THE DEGREE OF

MASTER OF PHILOSOPHY IN HUMAN

ANATOMY AND CELL BIOLOGY

IN THE

DEPARTMENT OF ANATOMY, SCHOOL OF MEDICAL SCIENCES,
COLLEGE OF HEALTH SCIENCES

BY

YAW OTCHERE DONKOR

KWAME NKRUMAH UNIVERSITY OF SCIENCE & TECHNOLOGY, KUMASI SEPTEMBER, 2016

DECLARATION

The experimental work described in this thesis was carried out at the Department of Anatomy, School of Medical Sciences, Kwame Nkrumah University of Science and Technology. This work has not been submitted for any other degree.

Yaw Otchere Donkor …… ……

(Student) Signature Date

Certified By:

Prof. Chrissie Stansie Abaidoo …… ……

(Supervisor) Signature Date

Prof. Chrissie Stansie Abaidoo ……. ……..

(Head of Department) Signature Date

ACKNOWLEDGMENTS

I gratefully acknowledge the advice, patience, guidance, support, encouragement and constructive criticisms of my supervisor, Prof. Chrissie Stansie Abaidoo**,** who fostered my interest in Human Anatomy and the fascinating field of Reproductive Anatomy.

I sincerely thank all the Staff of the Anatomy Department, especially, Miss Nancy Darkoa Darko, for sparing much time within her tight schedule for me. This was done with good humour and infinite patience. I would like to thank my colleagues, Mr. Ohene-Djan Atuahene, Mr Atta Kusi Appiah, Mr. Joshua Tetteh and Mr Thomas Diby for their friendship and moral support throughout the project. I heartily thank Mr. Djan and all the Staff of the Department of Pharmacology, KNUST for their technical support. I am especially grateful to Mr. Eric Acheampong and all the Staff of the Department of Theoretical and Applied Biology for their help in many ways.

I would like to thank my dear parents Mr. K. A. Donkor and Mrs. Faustina Donkor for their love, financial support, encouragement and constant prayers. To my siblings Sandra, Henrietta and Caleb, God bless you all for your support, love and understanding.

Above all, my gratitude goes to **ALMIGHTY GOD** for His divine protection and strength and by Whose infinite love and mercy has made everything possible.

ABSTRACT

Ginger has been shown to have a positive effect on the weight of the male reproductive organs, and to possess antidiabetic, antimicrobial, antioxidant, anticholestereolemic, anticancerous, antiemetic, anti-rhinoviral, anti-inflammatory and anti-insecticidal properties. As a result, dietary patterns in Africa especially Ghana are characterized by a high consumption of ginger. However there is very little information on the effects of ginger on the morphology of the male reproductive system. Therefore the present study was designed to investigate the effects of ginger on the structure of the testes, epididymis and semen parameters of the male rat using quantitative and qualitative methods. Forty-eight male wistar rats were divided into four groups designated as control, A, B and C and administered daily by gavage with 1 ml distilled water, 100 mg/kg, 300 mg/kg and 500 mg/kg of ethanolic ginger extract respectively for 30 days. Results of the present study show that there was an increase in body weight, testicular and epididymal weight in the extract-treated rats in a dose dependent manner which was not statistically significant. Both the control and extract-treated male rats showed normal morphology of the testes. However, in the extract-treated animals, there was a considerable increase in the proportion of interstitial tissues and seminiferous tubules. There was a strong positive correlation between volume fraction of the seminiferous tubules and sperm count. In addition, there was a strong positive correlation between sperm count, body weight, epididymal and testicular weight in the extract-treated animals. Ethanolic ginger extract caused a significant ($p = 0.001$) increase in sperm count, motility, viability and morphology. Therefore, ginger extract may be potentially useful in the management of male infertility especially those with low sperm count.

TABLE OF CONTENTS

CHAPTER FOUR

38 **RESULTS**

38

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

ABTS	Radical 2, 2´-azinobis-(3 ethylbenzothiazoline-6-sulphonic acid)
AMH	Anti-Mullerian Hormone
ANOVA	Analysis Of Variance
AR	Androgen receptor
ATP	Adenosine Triphosphate
BTB	Blood – Testis Barrier
CAE	Crude Aqueous Extract
CFA	Complete Freund's Adjuvant
CG	Control Group
Cm	Mean Count
CP	Crude powder
DHT	Dihydrotestosterone
DNA	Deoxyribonucleic Acid
DPX	Distyrene Plasticizer Xylene
F	Dilution Factor
FSH	Follicle-Stimulating Hormone
GHRF	Growth Hormone – Releasing Factor
GnRH	Gonadotropin-Releasing-Hormone
H_2O_2	Hydrogen Peroxide
HPG	Hypothalamic-Pituitary-Gonadal axis
HSD	High Significant Difference

ICSH	Interstitial- Cell Stimulating Hormone
KNUST	Kwame Nkrumah University of Science and Technology
LES	Lower Oesophageal Sphincter
LH	Luteinizing Hormone
LSD	Least Significant Difference
NaCl	Sodium Chloride
$NaHCO_3$	Sodium Bicarbonate
NAPRALERT	Natural Products Alert
NDA	New Drug Application
NF-κB	Nuclear Factor Kappa B
PPS	Preputial Separation
ROS	Reactive Oxygen Species
SMS	School of Medical Sciences
St	Seminiferous Tubules
T	Testosterone
Tt	Testicular Tissue
V	Volume of Counting Chamber
V/V	Volume Fraction
WHO	World Health Organization µL Microliter

CHAPTER ONE

INTRODUCTION

For centuries humans have been dependant on plants for all their basic needs in terms of shelter, clothing, food and medicine (Gurib-Fakim, 2006). Plants play a vital role in health due to their active biological components (Devasagayam *et al*., 2004). Several plant species have been reported of having medicinal and nutritive importance for prevention and treatment of diseases (Srivastava *et al*., 1996). Preparations from medicinal plants are useful as antibiotics, antioxidant and anticancer agents **(**Farnsworth and Soejarto, 1991**)**. Most of these medicinal plants come from the developing countries. Available evidence suggests that thirty per cent of all modern drugs are produced from plants (Burns, 2000).

The World Health Organization (WHO) reported that 80% of most Asian and African countries use herbal medicine for primary health care (McKay and Blumberg, 2007; WHO, 1999). Patient and/or consumer satisfaction of the effectiveness and inexpensiveness of herbal preparations has caused increasing demand for herbal medicines (Ebadi, 2006). Biological compounds derived from plants as well as those produced by chemists have been used as drugs in humans and veterinary medicine for a long time (Farnsworth, 2008). The application of plants as herbal medicines was first reported and documented in ancient China and India in the year 600 B.C. (Detlef *et al*., 1999).

Inappropriate preparation and use of herbal medicines with potent pharmacological properties may lead to adverse reactions that are sometimes life threatening (Ernst, 2007). Although many consumers believe that herbal medicines are safe because they are "natural" products, herbal medicines and synthetic drugs may cause some toxic response in the patient (Srivastava *et al*., 1996; Desai *et al*., 2009). Natural products and their derivatives represent more than 50% of all the drugs for clinical use in the world (Gurib-Fakim, 2006). Higher plants contribute approximately 25% of the total

medicinal plants (Gurib-Fakim, 2006). In addition, recent studies have shown that certain herbal preparations can be toxic at different doses (Chan, 2003). It has been hypothesized that all substances are poisonous in nature, but the dosage lowers the toxicity of the poison (Hann and Keserü, 2012). Vickers (2007) suggested that proper clinical trials are required to determine the safety and efficacy of each plant before it can be used. Most adults use plant preparations as dietary supplement and herbal medications without recommendation from a general medical practitioner and/or knowledge of the usefulness of these herbal medicines to their health (Cohen *et al*., 2002).

Herbal preparations may contain potent biochemical properties, hence its side effects should be considered (Ernst, 2007). Besides, most of the traditional medicines taken as treatment require scientific data validating their possible effects and responses (Engdal *et al*., 2009). Herbal medicine is extensively used in Africa regardless of the limited information available on their safety and efficacy (Johnson *et al*., 2007). Also, some of the available evidence concerning the efficacy and safety of these herbal preparations might be deceptive (Ernst and Schmidt, 2002).

More than 60% of certified plants in the new drug application (NDA) of natural preparations contain biological factors such as vaccines and monoclonal antibodies (Demain, 1999; Srivastava *et al*., 1996). The herbal medicines may be in the form of powder, liquid, ointment and liniment (Onyeagba *et al*., 2004). It has been documented that medicinal plants like *Zingiber officinale* (ginger), *Allium sativum* (garlic), *Xylopia aethiopica* (black pepper), *Alchornea cordifolia* (Christmas bush), *Strophanthus hispidus* (Arrow poison), *Terminalia ivorensis* (Ivory Coast almond), and *Sphenocentrum jollyanum Pierre* (moonseed family) are used in the management of male infertility, boosting sperm quantity and quality production (Burns, 2000). Infertility is a major health issue worldwide and has been estimated that 30%

of infertility cases may be due to a male factor (Isidori *et al.,* 2006). There is increasing evidence to suggest that sperm counts have declined over the last 50 years resulting in a consistent increase in male infertility (Carlsen *et al.*, 1992). Many factors such as drug and toxicants, nutritional imbalance, and life style may cause infertility. Reduction of sperm quality and counts are as a result of interference in the process of spermatogenesis due to factors such as toxins, drug treatment, radiations and chemotherapy (Amann and Berndtson, 1986).

Several studies have documented the effects of reactive oxygen species (ROS) on spermatogenesis. ROS has been shown to be responsible for boosting sperm parameters and activation, however higher levels of ROS can destroy sperm morphology, reduce motility and damage its DNA (Agarwal and Said, 2005; Ford, 2004). Studies have also indicated that more than 40% of men with infertility have increased levels of ROS in their seminal fluid (Zini *et al*., 1993). Hence, research on the male reproductive system has concentrated on antioxidant compounds in an attempt to prevent impairment of sperm viability, motility and morphology thereby increasing significantly their fertilizing ability.

1.2 THE PRESENT STUDY

The use of plants and herbs as medicines and boosters of sperm production has increased significantly over the last few years (Rates, 2001). Plant-based traditional medicines are still of vast significance to people living in the developed and developing countries to prevent and cure diseases (Dattner, 2003; Ternes, 1998). Studies have

indicated that about 80% of Africans depend on herbal healthcare medication and conventional plants for their routine health management (Abebe and Hagos, 1991; Johnson *et al*., 2007; Sharma *et al*., 2007). This is because herbal medicine offers the advantages of easy accessibility, availability and affordability as compared to the conventional drugs. One of the medicinal plants commonly used for herbal preparations in Africa is ginger. Ginger *(Zingiber officiale)* is widely consumed in Ghana as ginger tea and also as spices in food and drinks.

Ginger has been demonstrated to have beneficial therapeutic effect in the treatment and management of several diseases such as male infertility, cancer, ulcer, common cold, headaches, flu-like symptoms, rheumatism, menstrual pains, toothache, osteoarthritis, orchiditis, bronchitis and diarrhoea (Saad, 2015). Ginger is known to have antidiabetic, antimicrobial, antioxidant, anticholesteremic, anticancerous, antiemetic, anti-rhinoviral, anti-inflammatory and anti-insecticidal properties (Karangiya *et al*., 2016). The active components in ginger has been reported to significantly increase muscular activity of the gastrointestinal tract resulting in the stimulation of digestion, absorption, relieving constipation and flatulence (Banerjee *et al*., 2011). It improves blood circulation through the body, increasing cellular metabolic activity and relieving cramps (Banerjee *et al.*, 2011). In addition, ginger supplements are recognized for their strong stimulating effect on the immune and digestive systems, production of saliva and also act as an aphrodisiac (Bhowmik *et al*., 2010).

Dietary pattern in Ghana are mostly characterised by high amount of fat and carbohydrates which are known to expose people to numerous disease such as hypertension and atherosclerosis (Vicker, 2016). Ginger and other natural preparations for dietary therapy have been shown to reduce high cholesterol level and heart related diseases (Craig, 1999). Several studies have also speculated that ethanolic ginger extract can increase

testicular weight and also influence the growth and function of the accessory reproductive organs (Ansari *et al*., 2006). In addition, other investigators have reported that ginger causes an increase in sperm count, sperm motility and have an excitatory response on the level of testosterone (Lee *et al*., 2001; Park *et al*., 2007; Arash *et al*., 2009). Furthermore, ginger has been shown to affect spermatogenic activity in the male rat however, there is very little information on the effect of ginger on the structural organisation of the testes and other male reproductive organs.

Based on the broad usage of ginger in the local dishes, beverages and herbal preparations in Ghana, the present study was designed to investigate the possible effects of ginger (*Zingiber officinale*) on the structure of the reproductive system of male rats (*Rattus norvegicus*).

1.3 AIM AND OBJECTIVES

1.3.1 AIM

To investigate the effects of ginger (*Zingiber officinale*) on the structure of the testes, epididymis and semen parameters of the male rat using quantitative and qualitative methods.

1.3.2 OBJECTIVES

Specific objectives include:

- To determine the body weight, testicular and epididymal weights of male rats after administering ethanolic ginger extract.
- To determine possible structural changes in the testes of the male rats after administering ethanolic ginger extracts using qualitative and quantitative methods.

- To determine the relationship between the volume fraction of seminiferous tubule, testicular weight, epididymal weight, body weight and sperm count in the male rats.
- To determine the effects of ethanolic ginger extracts on the sperm morphology, motility, viability and sperm count in the male rats.

CHAPTER TWO

LITERATURE REVIEW

2.1 MEDICINAL PLANTS

Medicinal plants are widely used in treating, curing, preventing and managing several diseases and ailments, and therefore play an essential role in health care delivery (Srivastava *et al*., 1996). University of Illinois database of the Natural Products Alert (NAPRALERT) indicated that 9,000 plant species such as pteridophytes, dicotyledons, monocotyledons, gymnosperms, lichens and bryophytes have medicinal properties (Farnsworth and Soejarto, 1991). Medicinal properties of these plants may be in the form of leaf, stem, flower, root, bark, seed or fruit.

In developed countries, health care practitioners have limited their dependency on medicinal plants for health delivery, however most of the developing countries still depend on plant preparations as medicines for treatment (Calixto, 2000). Compared to orthodox drugs these

herbal preparations are readily available and affordable. In most developing countries, herbal medicines are preferred because they are natural products obtained from the environment. World Health Organisation (WHO) reported that approximately 80% of people in developing countries rely on herbal preparation in health care delivery (Bannerman, 1982). Research on the preparations and application of herbal medicine has significantly increased in the developed countries using modern scientific technologies (Grunwald, 1994; Srivastava *et al*., 1996).

The use of medicinal plants as herbal remedies has been demonstrated in most aspects of medicine regardless of the fundamental logical evidence. In Ghana, individuals living in the rural communities are closer to nature hence have easy access to most of these medicinal plants and apply them often without much scientific information (Runganga *et al.*, 1992). Traditional healers in Ghana use medicinal plants for treatment and management of diseases especially infertility (Maregesi *et al*., 2007). In addition, most common laxatives, cold and cough remedies, are produced from plants. In North America and Europe for instance, the usage of plant or herbal medication is being driven by increase in consumer demands for medications that are considered as natural products (Lewington, 1993). In these countries, most of these plants and herbs have been used to address issues of erectile dysfunction and low sperm count in males. Some of the medicinal plants currently in use are ginger (*Zingiber officinale*), black pepper (*Xylopia aethiopica*), christmas bush (*Alchornea cordifolia*), arrow poison (*Strophanthus hispidus*), Ivory Coast almond (*Terminalia ivorensis*), and river bush willow (*Sphenocentrum jollyanum Pierre*) (Burns, 2000).

In Africa and Asia, most adults indulge in the usage of medicinal plants as dietary supplements and herbal medicine with or without guidance from a medical practitioner and on the assumption that these extracts will have no adverse effect on them (Cohen *et al*., 2002). On the contrary, high doses of these preparations when taken might be life

threatening (O'Brien, 1997). Although data concerning the efficacy and safety of these remedies have been documented since ancient times, most of the information available might be deceptive (Ernst and Schmidt, 2002). In Ghana, it has been reported that, black pepper (*Xylopia Aethiopica*), garlic (*Allium Sativum*) and river bush willow (*Spenocentrum Jollyanum*) have some effects on the male reproductive system (Abaidoo *et al*., 2011). Therefore there is the need to scientifically authenticate some of these plants and their extracts for effective usage in the treatment of diseases (Owiredu *et al*., 2007).

2.2 DESCRIPTION AND USES OF GINGER

Zingiber officinale commonly called ginger is in the order Zingiberale and family Zingiberaceae, which comprises of approximately 1300 species and 50 genera (GarnerWizard *et al*., 2006). Ginger belongs to both tropical and subtropical climates. It was first discovered in the South East of Asia, mostly indigenous of India and Arabia as a herbal traditional plant since ancient times (Shukla and Singh, 2007). It is harvested in larger quantities especially in India, Nigeria and Ghana (Kirtikar and Basu, 1993). In Ghana, *Z. officinale* is commercially produced on a large scale in a community called Jankobaa in the Atwima Nwabiagya district of the Ashanti region. Ginger is a herbaceous perennial plant that is grown annually for commercial use (Nishina *et al*., 1992). Ginger is an erect plant with leaves arranged alternately on the stem. Its flowers are blooming green or pale yellow and cone shaped on the stem as shown in figure 1. The edible rhizome is covered with a brownish skin (Figure 1). Ginger plants do not produce seed, however the rhizome is its vegetative reproductive organ (Fnimh, 2001).

A B C

Figure 1. Photographs showing ginger plant with flower (A), foliage (B) and rhizome bud (C), (Source: Crop Protection Compendium CABI, 2012).

Commercially *Zinigiber officinale* is referred to as root ginger, stem ginger, pink ginger, green ginger, spring ginger, true ginger or garden ginger (Nishina *et al*., 1992). Ginger (*Zingiber officinale Roscoe*) is mostly used as a dietary spice condiment and natural medicine in the world (Surh *et al*., 1999; Sekiwa *et al*., 2000). It is a pungent, sweet, heating appetizer and has stayed in use as traditional oriental medicine for years. In Ghana, the roots are used in the preparation of almost all staple foods especially in spicing fresh meat and in preparing ‘‘Hausa Koko’’, a local porridge, ‘‘Soobolo’’, a local drink, and ‘‘Amuduro’’ also a local drink. In Nigeria, ginger is used commonly in traditional medicine preparation, and used as spice in most of their local delicacy including tea, and serves as a key ingredient in “Zobo” a local drink. Ginger and garlic mixed together are used for treating heart diseases, rheumatism, dysentery, eye related problems and body pains (Rahmatullah *et al*., 2012). Ginger is well known to help relieve the body of headaches, common cold, menstrual pains and flu-like symptoms (Grontved *et al*., 1988). Ginger has a 'carminative' effect, which is used to treat flatulence (Lohsiriwat *et al*., 2010). Taking in food prepared with ginger can decrease the sensation of hunger to some extent and also provide calories with no effect on satiety (Mansour *et al*., 2012). Ginger has been historically proven to eliminate nausea and sea

sickness (Schmid *et al*., 1994). Although ginger has been widely speculated to be safe and healthy there is limited information concerning its pharmacokinetics (Kaul and Joshi, 2001). Wilkinson (2000a) therefore recommends caution in its therapeutic application.

2.3 CHEMICAL COMPOSITION OF GINGER

Chemically, ginger contains several classes of compounds including: fibres 5%, proteins 10%, starch 40-60%, fats 10%, essential oil (oleoresin) 1- 4%, residual moisture 10% and inorganic material 6% (Shamsi and Tajuddin, 2010). Major chemical components of ginger include gingerols, gingerdiol, zingibrene, zingerone and shaogals (Zancan *et al*., 2000). Zingibrene, a volatile oil component of ginger, is a major factor which gives it a distinct aroma and taste. Non-volatile oil (oleoresin) constituents including gingerols, shaogals, paradols and zingerone contribute to the pungent odour that generate a ‘‘hot’’ sensation in the mouth. Gingerol is the most active constituent in fresh rhizome. It is straw in colour with sticky and fragrancefree fluid of greatly pungent taste (Goto *et al*., 1990). Shaogal is a new homologous sequence and the dried up state of gingerols which is the dominant pungent component in dehydrated ginger. Additionally, paradol is analogous to gingerol and it is produced on hydrogenation of shaogal (Gao, 2015). Oleoresins extract of ginger consists of waxes, fats, carbohydrates, minerals and vitamins. Also ginger rhizomes have an effective proteolytic enzyme known as Zingibain (Shukla and Singh, 2007).

2.4 PHARMACOLOGICAL ACTIVITY OF GINGER

The pharmacological effects of fresh and dried rhizome of ginger include anti-platelet, antitumour, anti-rhino viral, anti-hepatotoxic, anti-arthritic, antipyretic, analgesic and antihypersensitivity (Ali *et al*., 2008). Studies have shown that ginger contains biological properties which includes anticancer, antioxidation, anti-inflammation and antimicrobial effects (Ghayur and Gilani, 2005; Natarajan *et al*., 2006). Gingerol is the main constituent

with essential anticarcinogenic effects. Findings from the study by Rhode *et al*. (2007) pointed out that ginger also displays an anti-neoplastic impact via the restriction of nuclear factor kappa B (NF-κB) (Rhode *et al*., 2007). They concluded that 6-shaogal is made up of an essential anti-inflammatory agent that is beneficial to health care delivery (Jana, 1999). Aqueous extract from ginger and powdered ginger were administered to sheep infected with parasitic helminths. It was deduced that application of ginger extracts triggered an antihelminthic response in sheep infested with parasitic worm (Iqbal *et al*., 2006). Ginger extracts of ethyl acetate and methanol have been observed to induce a potentially advantageous response in obese individual. Reduction in lipid levels, insulin and serum glucose significantly leads to loss of body weight (Goyal and Kadnur, 2006). Based on the findings of Goyal and Kadnur, ginger extract may be used as part of herbal remedies to manage obesity in individuals. On the contrary, an *in vitro* study conducted to assess the immune modulatory responses of the volatile oil of ginger showed that, the volatile oil of ginger functionally blocked T lymphocyte production (Zhou *et al*., 2006). Reduction in the number of T lymphocytes led to a significant decrease in helper T cells. However, there was a rise in T suppressor cells (Zhou *et al*., 2006).

2.5 EFFECTS OF GINGER ON THE TESTIS

Ginger is believed to have a lot of biological properties, such as antioxidant and androgenic effects (Devasagayam *et al*., 2004). Currently, a lot of ongoing plant research are concentrated on the effect of ginger on the male reproductive system (Sikka, 2001). It has been reported that administration of 200 mg/kg body weight of ginger extract on short and long term basis in rats causes weight gain in the reproductive organs as a result of its potent androgenic and antioxidant activities (Memudu *et al*., 2012). Saeid *et al*. (2011) recently conducted a study on the effect of aqueous extract of ginger (*Zingiber Officinale*) on seminal parameters and androgen hormonal factors using male breeder broilers. After 30 days of

administration of the extract in their drinking water, they observed significant increases in testicular weight, sperm count, ejaculate volume, motility and significant decrease in abnormal sperm motility. These findings showed that the use of *Zingiber officinale* significantly increases sperm count and motility (Saeid *et al*., 2011; Arash *et al*., 2009; Zancan *et al.*, 2002). The antioxidant activity of ginger have been reported to enhance sperm quality and improve male fertility (Yang *et al.*, 2006). Also Amr and Hamza (2006) demonstrated that ginger has a defensive effect against oxidative stress in rats. In a related study on the effects of ginger on the male reproductive system of rats, it was observed that ginger reduced DNA damage caused by Hydrogen Peroxide (H_2O_2) and improved healthy seminal parameters in

rats (Khaki *et al.*, 2009a).

Administration of 100 mg/kg ginger extracts to male rats led to a significant rise in sperm viability and motility (Arash *et al*., 2009). Extracts of aqueous concentration of ginger of 600 mg/kg body weight have been established to increase weight of testes, serum testosterone and testicle cholesterol content in adult male rats (Kamtchouing *et al*., 2002). Qureshi *et al*. (1989) also observed that ginger (*Zingiber officinale*) significantly raised sperm counts and sperm motility in the testes without causing spermatozoa damage. Ginger has been shown to decrease cellular apoptosis caused by metiram, testicular damage and germ cell apoptosis (Amin *et al*., 2008). Morakinyo *et al*. (2010) demonstrated that administration of aqueous ginger extract with Arsenite in rats protected the reproductive organs against adverse effects and weight loss. From the result it was speculated that ginger increased sperm functions, raised the level of reproduction along with an increase in antioxidant activities and a decrease in peroxidation.

2.6 EMBRYONIC DEVELOPMENT OF THE TESTES

In humans, sex is determined at fertilization by the presence of an XX or XY sex chromosome (Koopman, 1991). The initial stages of foetal gonadal and genital development are not sex specific because the gonadal anlagen is identical in XX and XY embryos (Byskov, 1986). The development of the testis begins at the 7th week of gestation. Somatic cells form sex cords and sertoli cells begin to secrete anti-mullerian hormone (AMH) regulated by the testes determining factor SRY (Sajjad, 2010). AMH induces the regression of mullerian ducts, the anlagen of the uterus, fallopian tubes and upper vagina, upon binding to membrane receptor in the mesenchymal cells (Josso *et al*., 2005). At the 8th week, Leydig cells appear in differentiating testes and begin to secrete testosterone (Huhtaniemi and Pelliniemi, 1992).

Testosterone induces the differentiation of the wolffian ducts into epididymis, vas deferens, seminal vesicle, urethra and ejaculatory duct. Testosterone is also converted to dihydrotestosterone, which is responsible for masculinization of the urogenital sinus and external genitalia during the first 2 months (Siiteri and Wilson, 1974). The anogenital distance lengthens, and labioscrotal swellings gradually fuse to form the scrotum (Rao and Burnett, 2013). The rims of the urethral groove fuse to form the penis whilst the external urethral orifice forms in the glans (Van der Putte, 2005). Mesenchymal cells develop into penile corpus cavernosa and corpus spongiosum, in the process of masculinization, which ends in week 14 (Baskin, 2000).

Gubernaculum is a mesenchymal structure which before migration of the testes, moves mainly within the abdomen in a peritoneal fold that extends from the testes across the mesonephros to the inguinal region (Wensing, 1988). The developed testes remain close to the inguinal area by the gubernaculum while the cranial suspensory ligament holds the urogenital tract (Hutson, 2012). Descent of the testes is relative. During the first phase of movement the gubernaculum thickens and shortens, leaving the testes at the inguinal ring as the abdomen grows in the craniocaudal axis (Kogan and Hafez, 2012). During the second

phase of testicular descent, the testis passes through the inguinal canal to reach the scrotum in about 97% of normal new-borns (Huston *et al*., 1997). The significance of the gubernaculum in testicular descent has been shown in previous studies in transgenic mice with targeted disruption of the Hoxa l0 gene. The mouse gubernaculum normally expresses high levels of Hoxa l0 and abnormal gubernacular development causing cryptorchidism in the transgenic males (Pryor, 2000). The testis reaches the internal inguinal ring by week 24 in the foetus. Various factors play a role in testicular descent, abdominal pressure, migration of the gubernaculum across the pubic region to the scrotum and hormones (Hughes and Acerini, 2008). In the absence of AMH and dihydrotestosterone, the internal and external genitalia primordia differentiate following the female pathway even in the absence of ovaries (Rey and Grinspson, 2011). Cryptorchidism and micropenis in males result from the effects of reduced numbers of Leydig cells and low testosterone production (Bay *et al*., 2011).

2.7 MORPHOLOGY AND FUNCTIONS OF THE MALE REPRODUCTIVE SYSTEM

The male reproductive system is composed of the paired testes which are located outside the abdominal cavity, a duct system consisting of the epididymis, ductus deferens, ejaculatory ducts and urethra, a set of accessory sex glands (seminal vesicles, prostate, and bulbourethral glands) and the external genitalia (Figure 2).

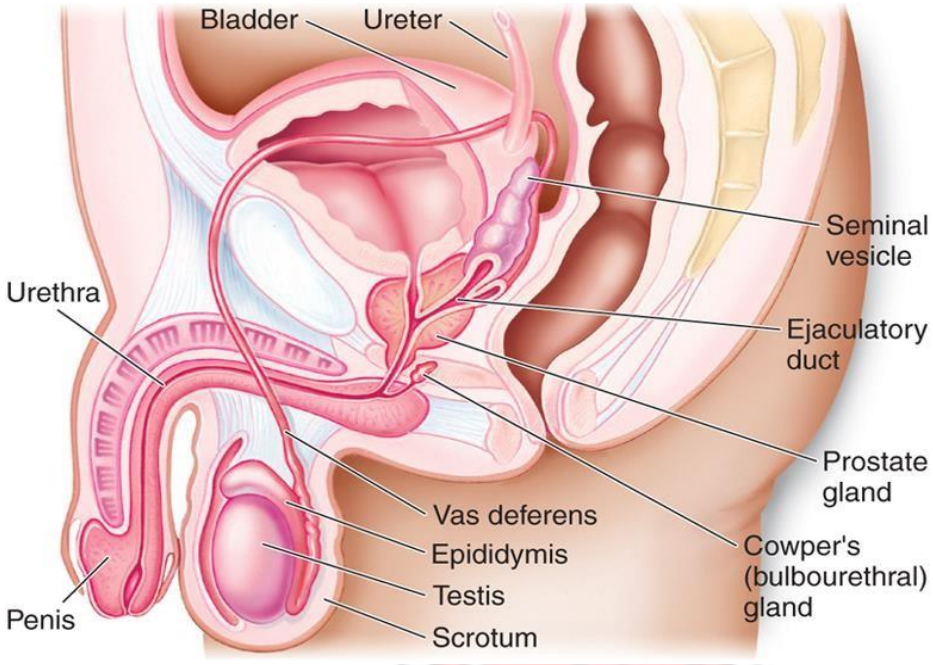


Figure 2: A diagram showing sagittal section of organs of the male reproductive system (Mader, 2004).

The testes are oval organs, enclosed in a fleshy sac called the scrotum (Figure 2). The location of the scrotum is essential for providing a temperature of 1.5 to 3°C below normal body temperature for spermatogenesis. The scrotum is composed of the skin, dartus muscle and cremasteric muscle which suspends the testes from the abdominal wall (Setchell *et al.*, 1994). Also the abdominal peritoneum which surrounds the anterolateral surface of the testes and carry with them blood vessels, lymphatic vessels and autonomic nerves is the tunica vaginalis. All these layers protect the testes from mechanical injury. The testes receive blood from the testicular arteries, which are branches of the abdominal aorta. These are highly convoluted and surround the pampiniform venous plexus which carries blood from the testes to the abdominal veins. This arrangement allows heat exchange between the blood vessels to maintain a lower temperature in the testes (Mitchell and Myers, 1968).

Deep into the tunica vaginalis, the testes are enveloped with a tough fibrous connective tissue capsule known as tunica albuginea (Figure 3). The tunica albuginea is made of fibroblast, collagen bundles and smooth muscle cells (Leeson and Cookson, 1974). The inner

aspect of the tunica albuginea consists of loose connective tissue which is highly vascularized and is termed as tunica vasculosa. At the posterior margin of the testes, the tunica albuginea thickens and projects into the interior of the gland as the mediastinum testes (De Kratser and Kerr, 1988). From the mediastinum, delicate septa of connective tissue divides the testis into incomplete lobules called testicular lobules. Each testicular lobule contains 1- 4 highly coiled sperm producing tubules known as seminiferous tubules (Clermont, 1972; Shokri *et al*., 2012).

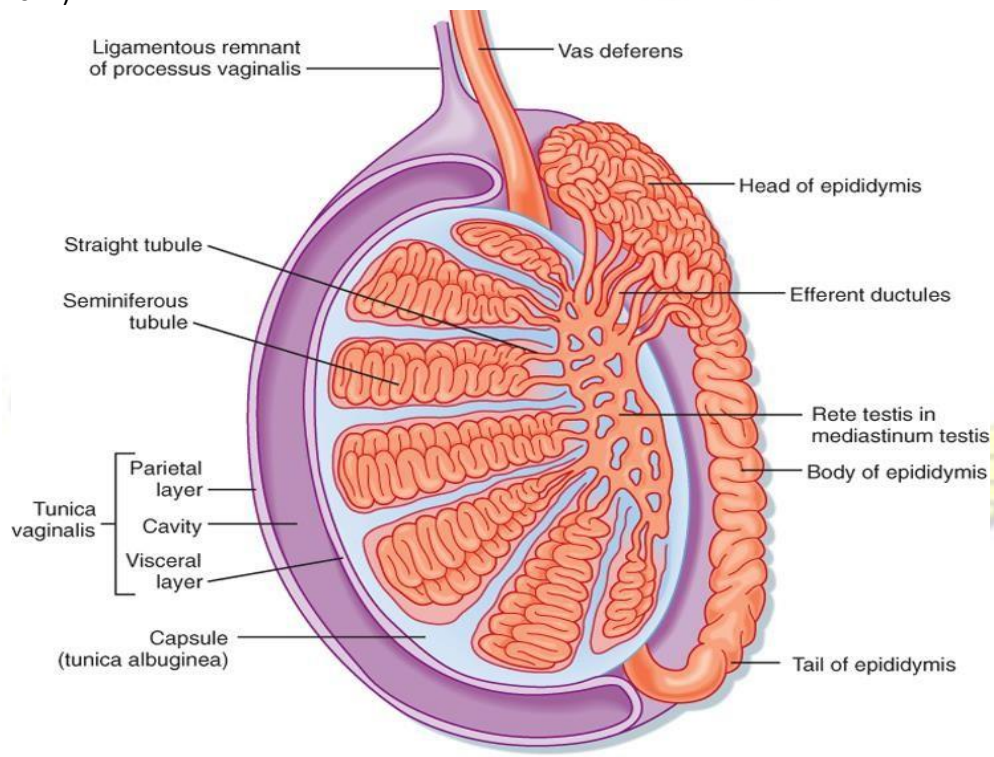


Figure 3: A diagram showing the Testes (Koppen and Berne, 2008).

The seminiferous tubules within a given testis lobules are arranged mainly in a form of coiled loops. Each end of the seminiferous tubule is uncoiled to form a straight tubule called the tubuli recti which joins a series of interconnected epithelial-lined channels within the mediastinum testes called the rete testis. Each of the uncoiled seminiferous tubules can measure up to 80 cm in length and 200-250 µm in diameter (Steinberger and Steinberger, 1971). The seminiferous tubules are lined by a highly complex and specialised stratified epithelium called the seminiferous or germinal epithelium. In the adult, the epithelium is composed of two populations of cells; a highly proliferative population known as the

spermatogenic cells (germ cell) and a non-replicating supporting population known as the sertoli or sustentacular cells (Figure 4). The straight tubules and rete testes are lined with simple columnar epithelium and simple cuboidal epithelium with a single cilium and few apical microvilli respectively (Wing and Christensen, 1982; Rheubert *et al*., 2010). Surrounding the seminiferous epithelium are collagen and elastic fibrils and multiple layers of flattened peritubular contractile cells called myoid cells which may be involved in the propulsive movement of sperms and testicular fluid from the seminiferous tubules (Clermont, 1958; Dym, 1994). Sertoli cells are non-dividing elongated columnar cells which extend from the base of the seminiferous epithelium to the tubule lumen (Elftman, 1963; Wang *et al*., 2008). The sertoli cells consist of; a narrow portion resting on the basal lamina, an intermediate portion that provides lateral processes to form the sertoli-sertoli junctional complex around which the spermatocytes and spermatids are arranged and apical processes that enclose the late spermatids just before they are released into the tubule lumen. Sertoli cells possess basally located irregular nuclei, abundant profiles of smooth endoplasmic reticulum and cytoskeletal contractile elements (De Kratser and Kerr, 1988). Sertoli cells play important functions: support, protect and nourish the spermatogenic cells, secrete a fructose-rich fluid into the lumen to provide energy and facilitate the transport of spermatozoa, and synthesize Androgen-Binding Protein (ABP) under the influence of Follicle Stimulating Hormone (FSH). ABP is a glycoprotein which binds to testosterone and maintains it to a high level to regulate spermatogenesis in the seminiferous tubule. In addition, sertoli cells secrete inhibin that inhibits the synthesis and release of FSH and activin which boosts FSH release from the anterior pituitary. During foetal development they synthesize and release antimullerian hormone for determination of foetal sex (Murphy, 2014; De Kratser and Kerr, 1988).

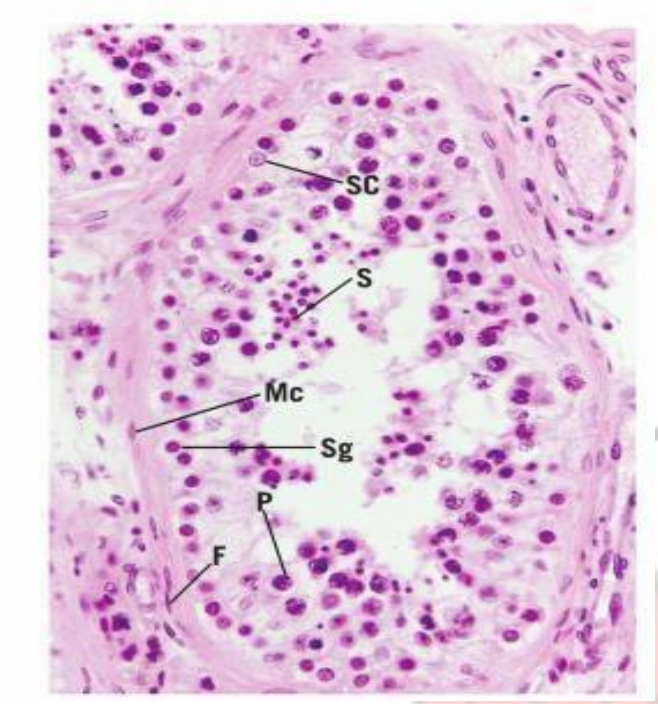


Figure 4. A light micrograph showing the transverse section of the seminiferous tubule: Myoid cells (Mc), fibroblasts (F), Spermatogonia (Sg) Sertoli cells (Sc), primary spermatocytes (P) and spermatids (S), (Gatner, 1998).

In between the seminiferous tubules are loose connective tissues containing blood vessels, macrophages, monocytes, mast cells, lymphocytes and a special cell type called Interstitial Cells of Leydig (Fawcett, 1973; Nistal *et al*., 1984). Leydig cells are responsible for the production of testosterone (Setchell and Breed, 2006). The testosterone plays important roles in maintaining spermatogenesis, secondary sexual characteristics and growth and function of the accessory sex organs. Leydig cells are closely associated with numerous blood vessels to promote the transport of steroid hormones produced (Fawcett *et al*., 1973). Rete testis empty into the proximal portion of the efferent ductules which ends the intratesticular duct system.

The extratesticular duct system begin from the distal part of the efferent ductules. Efferent ductules connect the channels of rete testis to the epididymis and are lined with pseudostratified columnar epithelium with cilia and a brush border. The epithelium contains alternating tall and short columnar cells which give the luminal surface a saw tooth

appearance. Most of the fluid secreted in the seminiferous tubules are reabsorbed in the efferent ductules. In adults, the efferent ductules is made up of approximately 10 to 20 tubules (Ilio and Hess, 1994). The wall of the efferent ductules contains smooth muscle layer. Interspersed among these muscle cells are elastic fibres. Transport of sperm in the efferent ductules is by both the ciliary action and muscular contraction (Nistal *et al*., 2015).

Located at the posterior and superior surfaces of the testis is the epididymis. The epididymis is an organ measuring about 7.5 cm in length and divided into head, body and tail. The duct of the epididymis is a highly coiled tube known as the ductus epididymis. The duct of epididymis is also lined with pseudostratified columnar epithelium with stereocilia composed of tall principal cells and short basal cells. The main roles of the epididymis are sperm maturation, sperm storage prior to ejaculation, secretion of fluid contributing to seminal fluid, spermatozoa incapacitation, recognition and removal of damaged spermatozoa (Sutovsky *et al.,* 2001). The smooth muscle coat of the duct of the epididymis gradually increases in thickness to become three-layers in the tail (Ir, 2013). Muscle layer in the head and body of the epididymis is responsible for spontaneous rhythmic peristaltic contractions to move the sperm along the duct whereas muscle layers in the tail of the epididymis cause few peristaltic contractions and serve as the principal reservoir for matured sperm (Carlson, 2013).

Ductus deferens into which the ductus epididymis empties is the longest part of the excurrent duct system. It has pseudostratified columnar epithelium with stereocilia and three layers of muscle arrangement (Razi, 2010). It ascends along the posterior border of the testis and enters the abdomen as a component of the spermatic cord, by passing through the inguinal canal. After leaving the spermatic cord, the ductus deferens descends into the pelvis to the level of the urinary bladder, where its distal end enlarges to form the ampulla of ductus deferens (Clulow *et al*., 1998). The ampulla is joined by the duct of the seminal vesicle and continues through the prostate gland to the urethra as the ejaculatory duct (Senger, 2003).

The seminal vesicles are paired diverticula and continue from the ampulla of the ductus deferens. They have pseudostratified columnar epithelium and highly branched mucosal folds which give them a honeycomb appearance (Hafez, 1980). The seminal vesicles are located posterior to the urinary bladder. They produce a viscous, light-yellow nutrient material with pH of 7.29 that contains mucoproteins, prostaglandins and fructose which provides energy for the spermatozoa (Govardhan Naik *et al*., 2014). The prostate gland is a walnut-like organ that surrounds the prostatic urethra at the base of the urinary bladder. It is the largest accessory gland in the male genital system with patches of simple columnar, simple cuboidal or simple squamous, pseudostratified columnar epithelium. Prostate gland consists of 30-50 compound tubuloalveolar glands with seminal colliculus through which the secretions are discharged directly into the urethra. The gland is arranged in three concentric layers; an inner mucosal layer, intermediate submucosal layer and peripherial layer and covered by smooth muscle fibroelastic capsule. It secretes a clear, slightly acidic (pH 6.5) fluid that contributes to the formation of seminal fluid to provide nutrients and lubricants for the spermatozoa (Ludwig *et al*., 2000). Bulbourethral glands are pea-like structures located in the urogenital diaphragm. They are lined with simple columnar epithelium and surrounded by a fibroelastic capsule containing skeletal muscle. They secrete viscous, slippery preseminal fluid which contains considerable amounts of galactose and galactosamine, galacturonic acid, sialic acid and methylpentose. Sexual stimulation causes release of these secretions into the lumen of the membranous urethra to lubricate and wash urine remnants from the penile urethra. The penis is composed of a root, body or shaft and a glans. The penis has three erectile tissues; two dorsal corpora cavernosa and a ventral corpus spongiosum in which the spongy urethra is located (Mi *et al*., 2008). These erectile tissues are surrounded by thick tunica albuginea. The erectile tissues have numerous vascular spaces which are filled with blood from the helicine arteries during erection. Anatomically, the glans penis is similar to the glans of the clitoris. The sensory receptors of

the glans penis are basically for induction of ejaculation (Senger, 2003). Malfunction of organs of the male reproductive system can alter seminal parameters and hence infertility (Woode *et al*., 2012).

2.7.1 SPERMATOGENESIS

Just before puberty, a rise in gonadotrophins initiate spermatogenesis (Brehm *et al*., 2007). Spermatogenesis is a series of changes that proceeds in the development of spermatozoa from spermatogonia. Spermatogenesis can be grouped into three stages: germ cell regeneration through the course of mitosis, the reduction of the chromosome number by meiosis to form haploid cells and spermiogenesis (Kerr *et al.*, 2006). Spermatogonia are diploid germ cells which constitute pale the type A spermatogonia (mitotically active), the dark type A spermatogonia (mitotically inactive) and the type B spermatogonia. Some of the Type B spermatogonia cells divide to form primary spermatocytes. Primary spermatocytes go through meiosis I (reductional division) to produce secondary spermatocyte (Hess, 1990; Cheng and Mruk, 2010). Then secondary spermatocytes begin the second stage of meiotic division to produce haploid spherical spermatids. The transformation of spherical spermatids into the complete elongated spermatozoa form is obtained, through a process known as spermiogenesis (O'Donnell *et al.*, 2001). Spermiogenesis involves the production and growth of the acrosome and flagellum, concentration of chromatin, remodelling and longitudinal expansion of the nucleus, and elimination of the cytoplasm (Leblond and Clermont, 1952). The acrosome contains hydrolytic enzymes such as acid phosphatase, neuraminidase, hyaluronidase and proteases that aid sperm penetration through the corona radiata and zona pellucida of the oocyte. Spermatozoa are released into the lumen of the seminiferous tubule by a process called spermiation (Figure 5). A spermatozoon has a head, neck and tail piece. The head is flattened and pointed with acrosomal cap covering anterior two thirds of the nucleus. The neck contains centrioles for developing the flagellum. The tail

constitutes the middle piece, principal piece and the end piece. Middle piece contains sheath of mitochondria and extends from the neck to the annulus. The principal piece extends from the annulus to the end piece and contains the axoneme with its surrounding fibers which are encircled by circumferential ribs known as fibrous sheath. The endpiece consists of the axoneme and the surrounding plasma membrane.

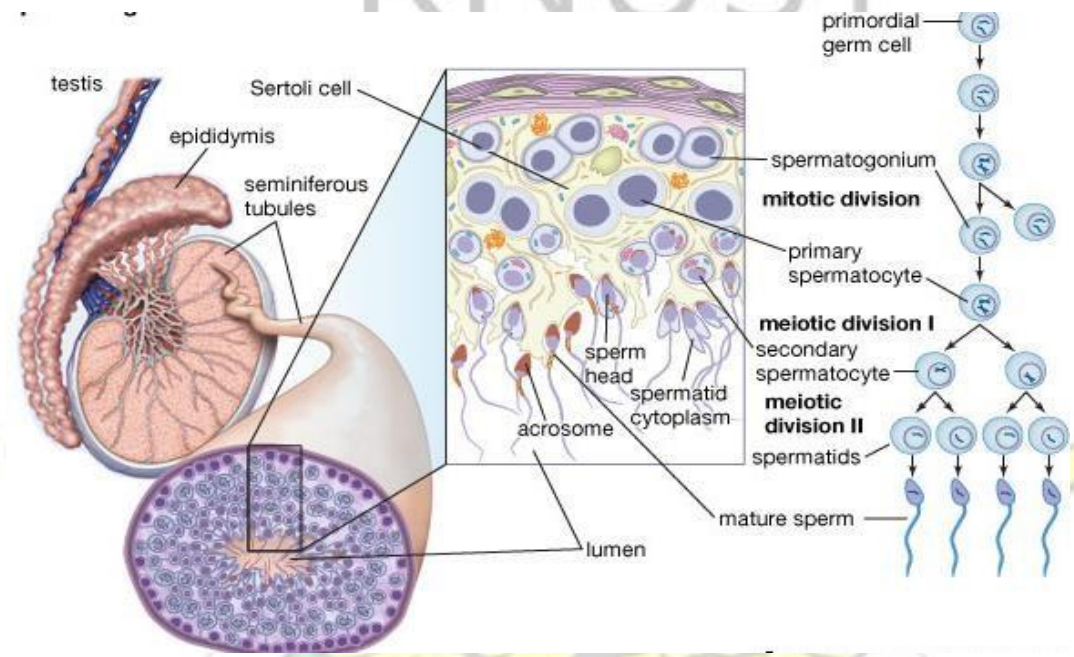


Figure 5: A diagrammatic representation of spermatogenesis (Clermont, 1972).

2.7.2 HORMONAL REGULATION OF THE TESTIS

Hormones are essential factors in the regulation of spermatogenesis. The hypothalamus releases gonadotropin-releasing hormone (GnRH) which stimulates the anterior pituitary gland to secrete interstitial cell-stimulating hormone (ICSH) and Follicle stimulating hormone (FSH). ICSH stimulates Leydig cells to secrete testosterone whereas FSH stimulates sertoli cells to release inhibin (McLachlan *et al.*, 2002). Higher levels of Inhibin and testosterone inhibit the release of FSH and ICSH from the anterior pituitary (Figure 6). Sertoli cells contain receptors for ICSH and testosterone which are the major hormones that control spermatogenesis (Holdcraft and Braun, 2004). High concentration of testosterone in the

seminiferous tubules plays a key role in the production of spermatozoa (Fritz *et al.*, 1975; Bardin and Paulsen, 1981).

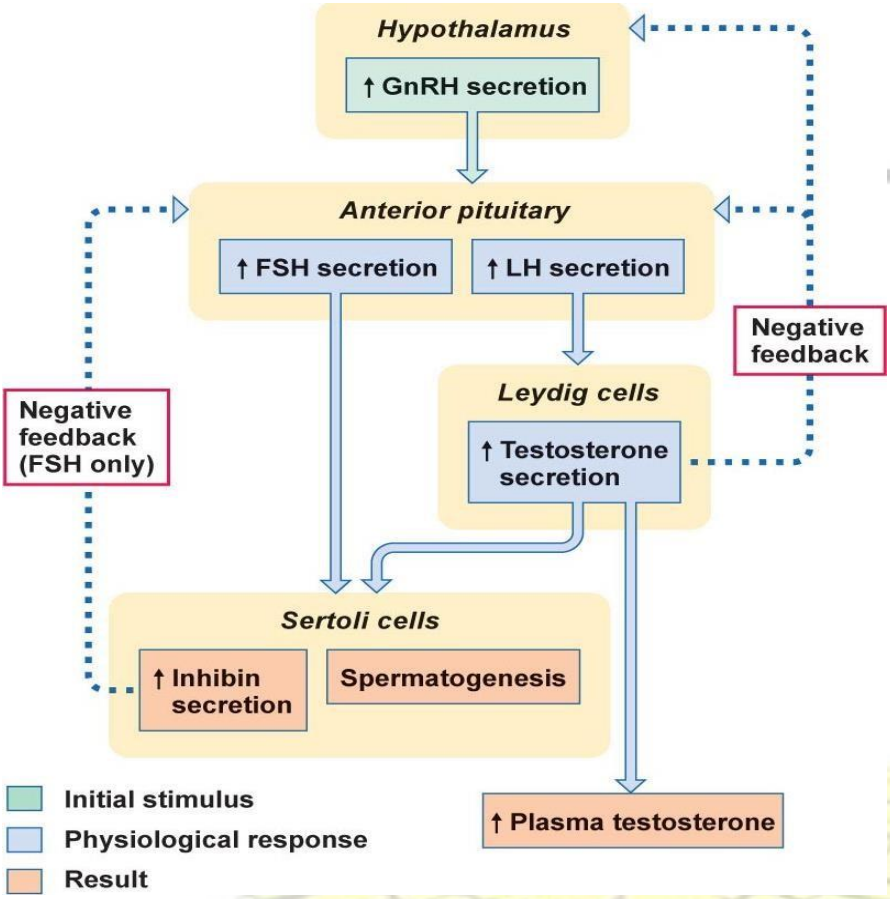


Figure 6: Feedback regulation of testosterone production by the Leydig cells via the hypothalamic-pituitary-gonadal axis (Holdcraft and Braun, 2004).

2.8 EFFECTS OF HERBAL PREPARATIONS AND ORTHODOX DRUGS ON THE MALE REPRODUCTIVE SYSTEM

The male reproductive system is extremely sensitive to various environmental factors such as herbal preparations, conventional drugs and pollution which induce structural and functional alterations (Kavlock *et al*., 1996). There is evidence in literature to suggest a decline in sperm density in humans over the past 20 years (Carlsen *et al*., 1992; Giwercman *et al*., 1993; Kidd *et al*., 2001). A variety of substances have been shown to induce negative effects on spermatogenesis in several mammalian species (Fody and Walker, 1985). There

are several reports to suggest the use of herbal medicines to enhance male fertility (Rowland and Tai, 2003; Makker *et al*., 2009). Administration of aluminium to rats cause tessticular damage, reduce sperm counts and viability. However administration of onion (*Allium Cepa*) extracts demonstrated that onion has antitoxic and antioxidant effects on the male reproductive organs. In addition, it was observed that there was an improvement in sperm quality and reduction in aluminium levels in the rats (Pandey and Jain, 2013). Alhassan (2012) reported on the effect of ethanolic extract of black pepper (*Xylopia Athiopica*) on the male rat reproductive function. The study observed a significant increase in body weight, testicular weight, epididymal weight and sperm count.

Studies were conducted on the effect of some 14 chemotherapeutic drugs on spermatogenesis in male mouse. It was established that there was structural, testicular cell and genetic damage. All the chemotherapeutic drugs tested damaged differentiated spermatogonia (Meistrich *et al*., 1982). Drugs used for chemotherapy have been discovered to contain harmful effects on reproductive function (Meirow and Nugent, 2001). Besides, several groups of anticancer medications have been shown to influence the testis negatively in their development and function (Chapman, 1983; Dumontet and Sikic, 1999). According to Merlin (1972), Colchicine and its derivatives have been reported to halt mitosis in the metaphase which result in reduction of sperm counts. Higher levels of oestrogen have been observed to retard testicular functions to a much higher degree than androgens due to the reported inhibition of gonadotropin releasing hormones (Boas and Ludwig, 1950). Antiandrogen substance Cyproterone acetate, has been indicated to inhibit spermatogenesis in men in a dose dependent manner within a month (Neumann *et al.*, 1970). Authentic antiandrogen flutamide with no progestational properties imparted stimulatory effects on testicular functions (Mathur and Chattopadhyay, 1982). A histamine inhibitor, Cimetidine, has been shown to reduce testosterone levels, weight of prostate and seminal vesicles and

sperm counts, in men and rats (Winters *et al*., 1979). Furthermore, the Phenolic compound Gossypol, has been indicated to suppress testosterone production and sperm counts (Qian and Wang, 1984).

2.9 MALE INFERTILITY

Infertility is known as the inability to conceive after 12 months of consistent, unprotected sexual intercourse on the average of three times a week or the failure to carry a pregnancy to live birth (Metzger, 1998). The experience of infertility is an undesirable condition to those who believe that parenting is a key individual success in life. Child bearing is the main expectation in the lives of couples especially in Africa (Sundby, 1997). Infertility may result from either the female, the male, or both couples (Davajan and Israel, 1991). Social stigmatization, emotional stress and economic impact of infertility make couples reluctant to seek for medical treatments. They therefore resort to herbal preparations regardless of its adverse effects on their health (Ikechebelu *et al*., 2003; Bunting and Boivin, 2007). Studies on fertility centers in Africa have shown that mostly women visit these centers to seek medical help (Dyer *et al*., 2004).

There is yet to be identified a condition more demoralizing to man's personality than infertility. It over powers his very essence of masculinity (Owiredu *et al*., 2007). Furthermore, next to thirst, starvation and sleep, sexual desire is the greatest biological drive in men (Tharakan and Manyam, 2005). Among the causes of male infertility are abnormal sperm motility (asthenospermia), antisperm antibodies, hormonal abnormalities, retrograde ejaculation, erectile dysfunction, oligozoospermia and lack of sperm (azoospermia) (Davajan and Isreal, 1991). However, oligozoospermia is one of the major factors of male infertility (Tortora and Grabowski, 1996). There are a number of causes responsible for this condition, such as overheating of testicles and low levels of zinc in diet. It is also observed that moderate exposure to lead and cadmium can significantly reduce sperm quality (Telisman *et*

al., 2000). Reduction of sperm quality and counts are as a result of interference in the process of spermatogenesis and these are caused by factors such as toxins, drug treatment, environmental factors and chemotherapy (Amann and Berndtson, 1986). There is evidence in the literature to suggest that the prevalence of male infertility has increased considerably with a decline in sperm count (Carlsen *et al.*, 1992). Due to the high cost of orthodox infertility treatment in Africa most people rely on traditional medicinal plants which are less expensive, locally accessible and easily prepared for consumption (Surveswaran *et al*., 2007). Most men with infertility cases feel shy and ashamed of themselves, they therefore stay away from seeking conventional medical treatment and resort to personal treatment (Wang, 1994).

2.10 DESCRIPTION OF WISTAR STRAIN ALBINO RATS (*RATTUS NORVEGICUS*)

Laboratory rats have been used in research studies to broaden our knowledge on diseases, genetics, health, medicines and the effects of drugs. Albino rats are mostly used for research because they tend to have similar anatomical and functional features with humans (Krinke, 2000). There are several strains of albino rats such as the Sprague Dawley rats, Wistar rats, Long Evans rats and Zucker rats amongst others. Presently, the Wistar strain is one of the most commonly used rats strain for scientific laboratory studies (Clause, 1993). The Wistar rat has long ears, wide head and a long tail that is constantly less than its body length. Wistar rats are extra lively and breed faster as compared to other strains like Sprague Dawley rats (Clause, 1993). Bernett and Vickery (1970) reported that matured albino rats have body weight of 120 g to

300 g and body length of 148 mm – 150 mm.

The male reproductive system of the Wistar rat consists of the paired testes, epididymis and vas deferens, the urethra, penis and accessory glands. The testis is responsible for the

production of testosterone and spermatozoa. The structures and functions of the male reproductive system in the Wistar rat is similar to the human male reproductive system (Sharpe and Skakkebaek, 1993).

CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY DESIGN

The study was conducted from February 2015 to May 2016 on Wistar male rats procured from the animal house at the Department of Theoretical and Applied Biology, KNUST. Ethical approval was obtained from the Committee on Human Research, Publication and Ethics (CHRPE) Kwame Nkrumah University of Science and Technology, School of Medical Sciences (SMS) and Komfo Anokye Teaching Hospital, (KATH).

3.2 PLANT COLLLECTION

Samples of *Zingiber officinale* (ginger) were purchased from Jankobaa, Nwabiagya district in the Ashanti region of Ghana. Samples were classified and authenticated in the herbarium at the Theoretical and Applied Biology Department, KNUST. A voucher specimen was kept at the Department's herbarium with a voucher reference number 15/001.

3.2.1 GINGER EXTRACT PREPARATION

Fresh ginger rhizomes weighing 4.5 kg were washed, peeled, cut into approximate dimensions and sun-dried for two weeks. The dried rhizome pieces were ground into powder using an electrical grinder (Hamilton Beach 58148A Power Elite Blender, USA). The powdered ginger (243.3g) was dissolved completely in 1500 ml of 70% ethanol and kept at room temperature for 48 hours. The mixture was then filtered twice using a small pore stainless sieve (Stainless steel Colander, EU) and filter paper (Whatman No. 1). The extract was concentrated using a rotary vacuum water vapour (Rotavapor R-215, BUCHI Laboortechnik AG, Fawil, Switzerland) at a temperature of 50ºC. A syrup mass was collected in a porcelain bowl and kept in an electric hot air oven (DSO-DF Series, US) for five (5) days to ensure complete dryness at 40 ºC. The solid mass (20.0g: 8.22%) extract of ginger was used to prepare three different aqueous extracts (100 mg, 300 mg and 500 mg) of ethanolic ginger extract.

These concentrations were prepared by dissolving the solid extract with distilled water: 2.5 g/10ml, 7.5 g/ml and 10 g/ml respectively and kept in a refrigerator at 4-8ºC.

3.3 EXPERIMENTAL ANIMALS

A total of 48 healthy 12-week old adult male Wistar rats (*Rattus norveigicus*) with an average weight of 254 ± 31.05g (200g - 300g) were procured from the animal house at the Department of Theoretical and Applied Biology, KNUST for the experiment. The rats were randomly divided into 4 groups with 12 rats per group. The experimental groups were designated as control, A, B and C. They were acclimatized for one week prior to the study. Their cages were covered with a welded mesh to allow proper ventilation, the floor was lined with sand and wood dust which helped to prevent sores on the feet of the rats and also served as bedding for the animals.

Male rats were accommodated in temperature regulated housings (25°C) with continuous humidity (40 to 70%) and 12 hour light and dark cycle preceding to research practices at the animal house. All rats were fed with standard diet and water. All albino rats were treated according to the Principles of National Institute of Health Guidelines for Care and Use of Laboratory animals. The weights of the rats were recorded before and after the treatment using a weighing scale (Calory scale, UK).

3.4 ADMINISTRATION OF GINGER EXTRACT

The control group (CG) for the experiment were given 1ml of distilled water. Treatment groups A, B and C received 100 mg/kg, 300 mg/kg and 500 mg/kg body weight of ethanolic ginger extract respectively. The animals were given a single oral daily dose of 0.6 ml/200g body weight by gavage for thirty (30) consecutive days. To determine the volume of ginger extract to be given to each animal, the weight of each animal was taken using an electric

balance (Precision Electronic balance RS232, UK). The dosage was determined using the formula by Boxenbaum and DiLea (1995):

In humans 200 ml: 70,000 g

Therefore in rats, 200 g body weight = $\frac{200 \text{ ml} \times 200 \text{ g}}{70000 \text{ g}}$

= 0.6 ml

3.5 SEMEN COLLECTION AND PREPARATION

Normal saline solution (0.9g of NaCl in 10 ml of distilled water) was first prepared before anaesthetizing the animals. After the three-week period, all the animals in groups Control, A, B and C were anaesthetized using chloroform and dissected to remove the epididymis after which the animals were euthanized. The covering fat, blood vessels and connective tissues were removed and the organ from each animal was placed in a beaker containing normal saline solution.

The dissected epididymis and the testes were weighed using an electric balance (Precision Electronic balance RS232, UK). The epididymis was then transferred onto a Petri dish and the sperms were ejected by making a longitudinal incision of the cauda epididymis with a pair of fine-pointed scissors and squeezing with forceps to release the sperms from the duct of the epididymis. The semen was diluted with 10 µL of diluent. The diluent was prepared with 100 ml of distilled water, 50 g of sodium bicarbonate ($NaHCO_3$) and 10 ml of 35% (v/v) formalin. Using a Pasteur pipette, 5 µL of the homogenate was diluted with 95 µL of diluent to give a 1 in 20 dilution.

3.5.1 SPERM COUNT

Sperm cell count was carried out using the Bearden and Fuquay method (Bearden and Fuquay, 1980). Haemocytometer (Weber Scientific International Ltd., England) with improved double Neubauer ruling was used for the evaluation of spermatozoa under a light microscope (Leica DMD 500, Germany). An aliquot of the final solution was then dropped onto the improved Neubauer Haemocytometer. To ease counting, the loaded haemocytometer was positioned in a humid place for about 5 minutes so that the sperms head may relax to the same focal plane. The quantity of sperm heads were enumerated with the help of a light microscope (Leica DM E Basic, Germany). The average counts for 4 haemocytometer chambers were recorded. The sperm count of each cauda epididymis was calculated using the formula: Sperm number = Cm× F×V

Where Cm = mean count

F= dilution factor

V = Volume of counting chamber

The data were expressed as the $x10^6$ spermatozoa per ml ($x10^6$ cell/ml) (Momeni *et al.*, 2009). The results were recorded.

3.5.2 SPERM MORPHOLOGY

The liquefied well mixed semen was smeared on a glass slide and air dried overnight at room temperature. The slide was fixed in 4% formalin to clear any mucus detected, stained with Aniline Blue (pH 3.5) and Eosin (Wong *et al.*, 2008), and then counted under the light microscope using a magnification of X40. The preparation was then examined for abnormal and normal spermatozoa. Under the light microscope the spermatozoa were classified as normal and abnormal. Normal spermatozoa had an oval-shaped head, a short middle piece, and a long thin tail whereas the abnormal spermatozoa had either no head, middle piece, and/or tail.

3.5.3 SPERM MOTILITY

One drop of a suspension of epididymal semen was fixed on a glass slide (ASI™ Frosted Glass Microscope Slides, USA) and covered with a cover slip (MS-SLIDCV, USA). Using x40 objective lens of a light microscope (Leica DMD 500, Germany). Several fields were scanned for sperm motility. A total of 200 spermatozoa were counted and the percentage; sperm motility was reported as either linear progressive motility, sluggish progressive motility or immotile sperm (WHO, 1999).

3.5.4 SPERM VIABILITY

A drop of diluted semen was stained with 0.5% eosin-nigrosin using the Barth and Oko method (Barth and Oko, 1994). The seminal smears were evaluated for sperm viability by determining the relative proportion of live and dead sperms. The proportion of live sperms were expressed as a percentage. Viable spermatozoa were unstained whereas non-viable spermatozoa stained red (Amman, 1982).

3.6 TISSUE PROCESSING FOR LIGHT MICROSCOPY

Each testis was cut into smaller pieces in a standard manner and immediately fixed in 3% glutaraldehyde in 0.1M phosphate buffer for 24 hours. The tissue was then washed in phosphate buffer solution and dehydrated through a graded series of ethanol (70%, 80% and 90%) for one and half hours each. It was then passed through two changes of absolute ethanol, one hour each change. The dehydrated specimens were cleared with chloroform (BPH Laboratory Supplies, Poole, England) and left overnight.

The cleared specimens were moved into two changes of molten paraffin wax for an hour each to infiltrate and replace the xylene. The specimens were removed from the plastic cassettes and placed into moulds filled with molten paraffin wax. They were then placed on a cold plate to solidify.

The paraffin wax blocks were trimmed and sectioned at approximately 5µm using the Rotary type microtome (Leica RM2125RT, Biosystems Peterborough Ltd., UK). The sections were picked with a pair of fine forceps and gently placed onto the surface of water in a float-out water bath (Leica HI 1210, Biosystems Peterborough Ltd, UK). The sections floated, expanded gently and became flatter. The sections were then placed onto grease-free glass slides (Chance Propper Limited, Warley, England). The slides were placed on racks and kept in an oven (Digit heat, JB selecta, sa, Barcelona, Spain) at 60°C for 20 minutes. The sections were passed through two changes of xylene for 2 minutes each. They were then transferred through decreasing concentrations of ethanol (absolute ethanol, 90%, and 70%) for two minutes each, rinsed in distilled water for 2 minutes and stained with Haematoxylin (Sigma Chemicals Company, UK) for about 20 minutes. The sections were washed in gentle running tap water for 2-3 minutes, differentiated in 1% Hydrochloric acid (Sigma Chemicals Company, UK) and 70% ethanol and then washed in running tap water for 5 minutes. They were stained in 1% aqueous eosin (Sigma Chemicals Company, UK) for 3 minutes, washed in tap water and dehydrated through an increasing concentration of ethanol. The sections were cleared in xylene, mounted with DPX (BDH Laboratory Supplies, Poole, England) and coverslips (Chance Propper Limited, Warley, England) and examined with a Leica DMD 108 Microscope (Leica Instruments, Germany).

3.7 MORPHOMETRY

The volume fraction (V/v) of the seminiferous tubules was estimated using a point counting technique (William, 1977) and a 5 mm square lattice (separation of point on the tissue is 6.2 µm with aid of drawing tube attached to Leica Microscope (Leica DMD 108, Germany) (Figure 7). The total number of intersections on the lattice square grid was 945. At least five nonoverlapping systematically random sampled fields of view were examined for each slide of tissue. The square lattice were superimposed in the image of tissue and the number of

point falling on the seminiferous tubules and the total points falling on the entire testes were recorded with the aid of hand tally counter (William, 1977; Weibel, 1979). Calculation of the volume fraction (Vv) of the seminiferous tubules to the testis (Weibel, 1979);

VV = St /Tt

Where St = total number of points of intersection falling on nuclei of seminiferous tubules Tt = total number of points of intersection falling on the entire tissue of the testis.

Figure 7: Photograph showing Leica DMD 108 Microscope (× 0.16).

3.8 STATISTICAL ANALYSIS

The data were analysed with Microsoft excel version 2013. Individual values were pooled within groups and their means and standard deviations were calculated for each group. The differences between groups were determined using a one-way analysis of variance (ANOVA). Further comparison between groups was accomplished using Least Significant Difference (LSD) and Tukey High Significant Difference (HSD) test by means of the Tukey-Kramer Multiple Comparisons Test using Genestat Eleventh Edition software. Pearson's correlation co-efficient was used to determine relationship between parameters using Graph Pad prism 5.02. All differences were measured at a significant level of 95%.

CHAPTER FOUR

RESULTS

4.1 EFFECT OF ETHANOLIC GINGER EXTRACTS ON BODY WEIGHT

The mean differences in body weights were 11 ± 6.63 g, 12 ± 7.48 g, 16 ± 3.74 g and 20 ± 3.16 g for the control CG, and treatment groups A, B and C respectively. Ethanolic ginger extract caused no significant difference in the body weights of the treatment groups compared with the control ($p = 0.19$). However, there was a dose dependent increase in the body weight of the treatment groups (Table 1).

4.2 EFFECT OF ETHANOLIC GINGER EXTRACTS ON TESTICULAR WEIGHT

The mean testicular weights recorded in the male rats were 2.85 ± 0.36 g, 2.81 ± 0.21 g, 2.98 ± 0.16 g and 3.11 ± 0.32 g for the control and treatment groups A, B, and C respectively.

There was no statistically significant difference in the testicular weights of the treatment groups as compared to the control group ($p = 0.41$). However, the weight of the testes showed

a decline in treatment group A, but increased in treated groups B and C compared with the control animals as shown in Table 1.

4.3 EFFECT OF ETHANOLIC GINGER EXTRACTS ON EPIDIDYMAL WEIGHT

From table 1, the mean epididymal weights of the male rats were 1.19 ± 0.30 g, 1.10 ± 0.14 g, 1.25 ± 0.12 g and 1.34 ± 0.122g for the control and treatment groups A, B, C respectively. Epididymal weights of the male rats showed no statistically significant difference in the treatment and the control groups (p = 0.36). However, there was a decrease in the epididymal weight of treatment group A and a numerical increase in treatment groups B and C compared with the control animals.

Table 1: Statistical comparison of body, testicular and epididymal weights of rats given ethanolic extracts of *Z. officinale* for 30days

PARAMETER (grams)	CONTROL GROUP	GROUP A (100mg/kg)	GROUP B (300mg/kg)	GROUP C (500mg/kg)	P value
BW	11.00 ± 6.63	12.00 ± 7.48	16.00 ± 3.74	20.00 ± 3.16	0.19[c]
TW	2.85 ± 0.36	2.81 ± 0.21	2.98 ± 0.16	3.11 ± 0.32	0.41
EW	1.19 ± 0.30	1.10 ± 0.14	1.25 ± 0.12	1.34 ± 0.12	0.36

BW:Body weight, TW: Testicular weight, EW: Epididymal weight. Data are presented as group means (± SD) Experimental groups significantly different from control: *P<0.05 (One-way ANOVA followed by Tukey-Kramer Multiple Comparisons Test) c = control versus group C. There were 12 animals in each group.

4.4 EFFECT OF ETHANOLIC GINGER EXTRACTS ON SPERM MORPHOLOGY

For each group, the morphology of two hundred sperms were counted and classified as either normal sperm morphology or abnormal sperm morphology based on the presence or absence of a head, middle piece and tail. The mean number of sperms with normal

morphology observed in the male rats were 65 ± 4.47, 71 ± 6.63, 75 ± 6.32 and 80 ± 6.32 for the control and treatment groups A, B, C respectively. Administration of ginger extract significantly increased normal sperm morphology (p = 0.02) in a dose-dependent manner in the treated rats compared with the control group (Table 2).

The mean number of sperms with abnormal morphology observed in the male rats were 35 ± 4.47, 29 ± 6.63, 25 ± 6.32 and 20 ± 6.32 for the control and treatment groups A, B, C respectively (Table 2). The treated animals also demonstrated a significant decrease in abnormal sperm morphology (p = 0.02) compared with the control group in a dosedependent manner.

4.5 EFFECT OF ETHANOLIC GINGER EXTRACTS ON SPERM COUNTS

In table 2, administration of the ginger extract significantly increased epididymal sperm count (p = 0.001) in a dose-dependent manner in treatment rats B and C but decreased in treatment group A compared with the control group.

4.6 EFFECT OF ETHANOLIC GINGER EXTRACTS ON SPERM VIABILITY

Out of the two hundred sperms counted, viability was classified as either viable sperm or non-viable sperm based on the presence of their ability to pick the eosin-nigrosin stain or vice versa (viable remain colourless whereas non-viable are stained). The mean viable sperms in the rats were 65 ± 5.48, 68 ± 7.48, 74 ± 8.60 and 83 ± 6.00 for the control and treatment groups A, B, C respectively. Administration of the ginger extract significantly increased viable sperm count (p = 0.01) in a dose-dependent manner in the treated rats compared with the control group (Table 2).

With respect to non-viable sperms, the mean observed in the male rats were 35 ± 5.48, 32 ± 7.48, 26 ± 8.60 and 17 ± 6.00 for the control and treatment groups A, B, C respectively. The treated animals also showed a significant decrease in the number of non-viable sperms ($p = 0.01$) compared with the control group (Table 2).

4.7 EFFECT OF ETHANOLIC GINGER EXTRACTS ON SPERM MOTILITY

Out of the two hundred sperms counted for each group, sperm motility was classified as linear progressive motility, sluggish progressive motility and non-motile sperm. As shown in Table 2, the extracts significantly increased ($p = 0.01$) linear progressive motility in a dosedependent manner as compared to the control group.

In contrast, the extract-treated animals exhibited no significant decrease in sluggish motility ($P > 0.05$) compared to the control group (Table 2). The mean number of non–motile sperms in the male rats were 16 ± 8.00, 21 ± 4.90, 13 ± 4.00 and 9 ± 2.00 for control and treatment groups A, B, C respectively. Compared to the controls, there was an increase in the number of non-motile sperms in treatment group A and a significant decrease ($p = 0.03$) in treatment groups B and C (Table 2).

Table 2: Summary of statistical comparisons of sperm characteristics in four groups of rats given ethanolic extracts of *Z. officinale* for 30 days.

CONCENTRATION OF *Z. OFFICINALE*	CONTROL 0 mg/kg	GROUP A (100 mg/kg)	GROUP B (300 mg/kg)	GROUP C (500 mg/kg)	P VALUE
SPERM COUNT (×10$_7$ cell/ml)	9.04 ± 1.95	8.54 ± 0.78	10.02 ±1.24	13.4 ± 1.34	0.001$_{c,e,f}$
SPERM VIABILITY (%)					
Viable	65 ± 5.48	68 ± 7.48	74 ± 8.60	83 ± 6.00	0.01$_{c,e}$
Non-Viable	35 ± 5.48	32 ± 7.48	26 ± 8.60	17 ± 6.00	0.01$_{c,e}$
SPERM MORPHOLOGY (%)					
Normal Morphology	65 ± 4.47	71 ± 6.63	75 ± 6.23	80 ± 6.32	0.02$_{b,c,e}$
Abnormal Morphology	35 ± 4.47	29 ± 6.63	25 ± 6.32	20 ± 6.32	0.02$_{b,c,e}$
SPERM MOTILITY (%)					
Linear Progressive Motility	59 ± 6.63	64 ± 7.35	69 ± 6.63	76 ± 5.83	0.01$_{b,c,e}$
Sluggish Motility	25 ± 4.47	15 ± 4.47	18 ± 7.48	15 ± 4.47	0.06$_{a,c}$
Non-Motile	16 ± 8.00	21 ± 4.90	13 ± 4.00	9 ± 2.00	0.03$_{d,e}$

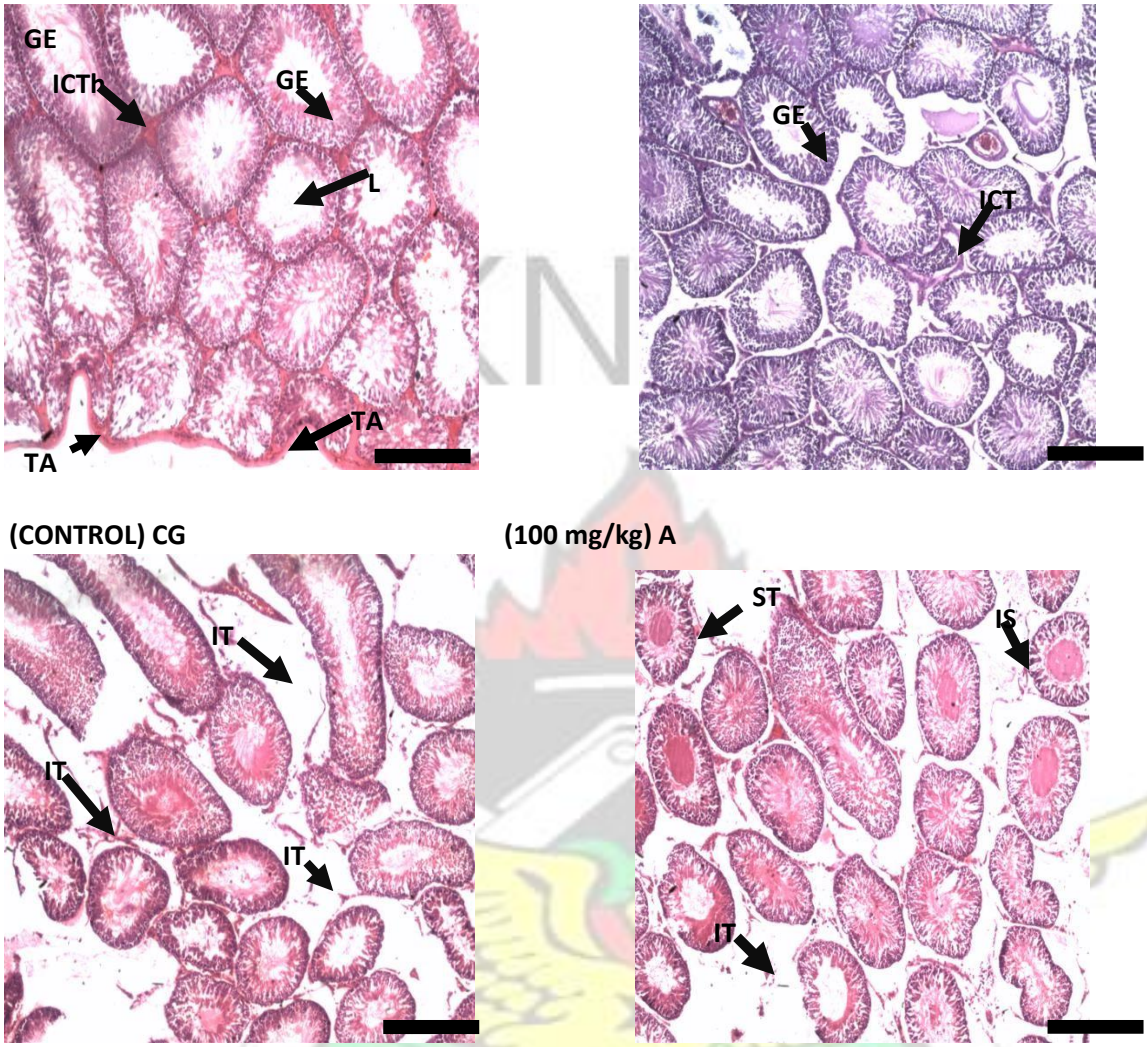
Data are presented as group means ± SD (standard deviation) Experimental groups significantly different from control: *P < 0.05, (One-way ANOVA followed by Tukey-Kramer Multiple Comparisons Test) a = control versus A; b= control versus B; c = control versus C; d=A versus B; e= A versus C; f= B versus C. **There were 12 animals in each group.**

4.8 VOLUME FRACTION ESTIMATES

The mean volume fractions of the seminiferous tubules in the male rats were 0.51 ± 0.09, 0.49 ± 0.05, 0.56 ± 0.07 and 0.75 ± 0.20 for the control and treatment groups A, B, C respectively. Administration of ginger extracts significantly ($p = 0.026$) increased the volume fraction of the seminiferous tubules in treatment groups B and C compared to the control animals.

4.9 MORHOLOGICAL CHARACTERISTICS OF THE TESTIS OF THE CONTROL AND EXTRACT-TREATED RATS

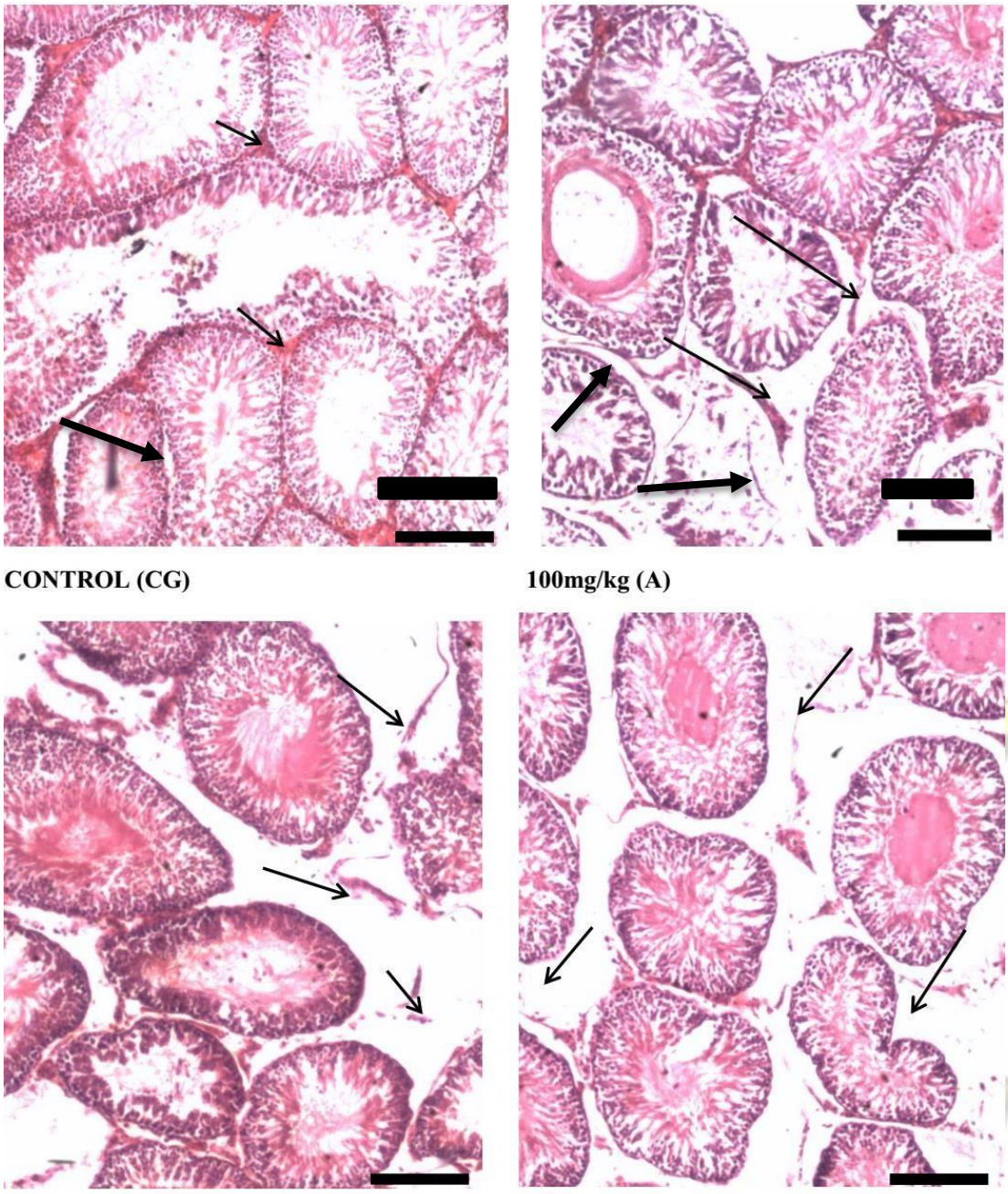
The testes of the control and extract-treated rats were covered by an outer fibrous connective tissue sheath, tunica albuginea. The inner aspect of the tunica albuginea was highly vascularized loose connective tissue. The loose connective tissue extended in between the profiles of seminiferous tubules separating them from one another (Figure 8). More interstitial tissues were seen between the seminiferous tubules of the extract-treated rats compared to the controls (Figure 9a). The epithelial lining of the seminiferous tubules consisted of complex stratified epithelium which were sertoli and spermatogenic cells at various stages of development (Figure 9b). Surrounding the seminiferous epithelium are flattened peritubular myoid cells (Figure 9b). The seminiferous tubules of the control group showed uniform regular profiles whereas in the extract-treated groups the tubules varied in size and shape (Figure 8).



(CONTROL) CG (100 mg/kg) A

(300 mg/kg) B (500 mg/kg) C

Figure 8: Light micrographs of the testes showing the tunica albuginea (TA), seminiferous tubules (ST), germinal epithelium (GE) and interstitial tissue (IT) lumen (L); Sections taken from control group CG, and treatment groups A, B, C (Staining: Haematoxylin and eosin. Bar represents 10 µm).



CONTROL (CG)

100mg/kg (A)

300 mg/kg (B)

500 mg/kg (C)

Figure 9: High power light micrograph of the testes black arrows showing increasing interstitial tissue between the seminiferous tubules in treated groups in a dose dependant manner, (Staining: Haematoxylin and eosin. Bar represents 20 µm).

4.10 CORRELATION BETWEEN VOLUME FRACTION OF THE SEMINIFEROUS TUBULE AND THE TESTICULAR WEIGHT OF THE RATS

Pearson's correlation coefficient showed weak association between volume fraction and weight of the testes in male rats for both control and treatment groups A and C. The correlation coefficients between volume fraction and control group, volume fraction and treatment group A and volume fraction and treatment group C were 0.13 ($p = 0.64$), 0.10 ($p = 0.74$) and 0.20 ($p = 0.47$) respectively (Figure 10). However, a moderate statistically significant correlation ($r = 0.52$, $p = 0.04$) was observed between volume fraction and weight of the testes in treatment group B.

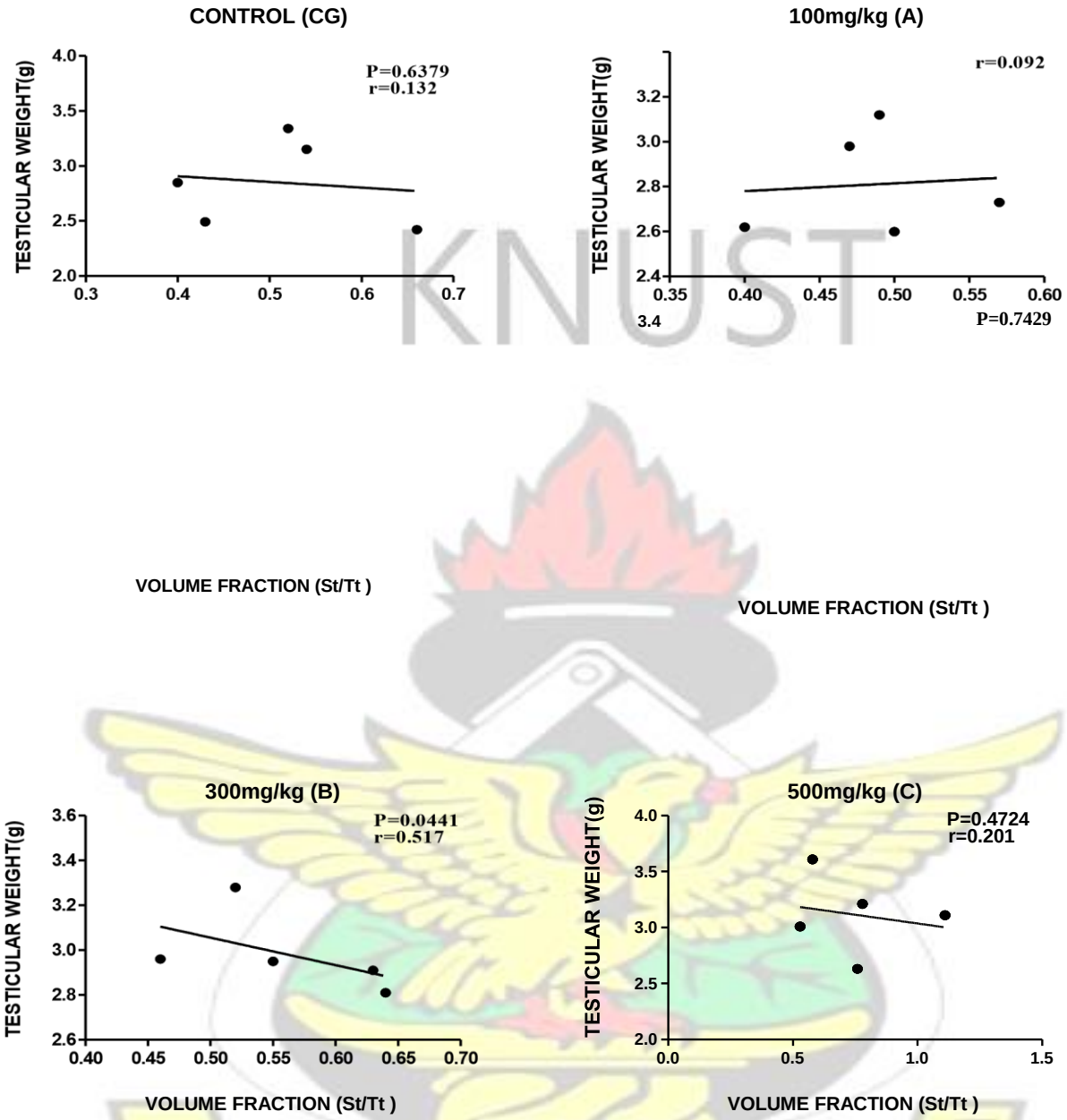


Figure 10: Correlation graph of testicular weight against volume fraction of the testes of control group and treatment groups. ***P= statistically significant at (P<0.05)***

4.11 CORRELATION BETWEEN VOLUME FRACTION OF THE SEMINIFEROUS TUBULES AND SPERM COUNT IN RATS

Pearson's correlation between volume fraction of the seminiferous tubules and sperm counts in male rats were statistically significant in treatment groups A (p = 0.01) and B (p = 0.04) with a

strong correlation co-efficient of 0.40 and 0.53 respectively (Figure 11). On the contrary, correlations between volume fraction of the seminiferous tubules and sperm counts in the control group (r = 0.03, p = 0.91) and treatment group C (r = 0.53, p = 0.18) were not statistically significant (Figure 11).

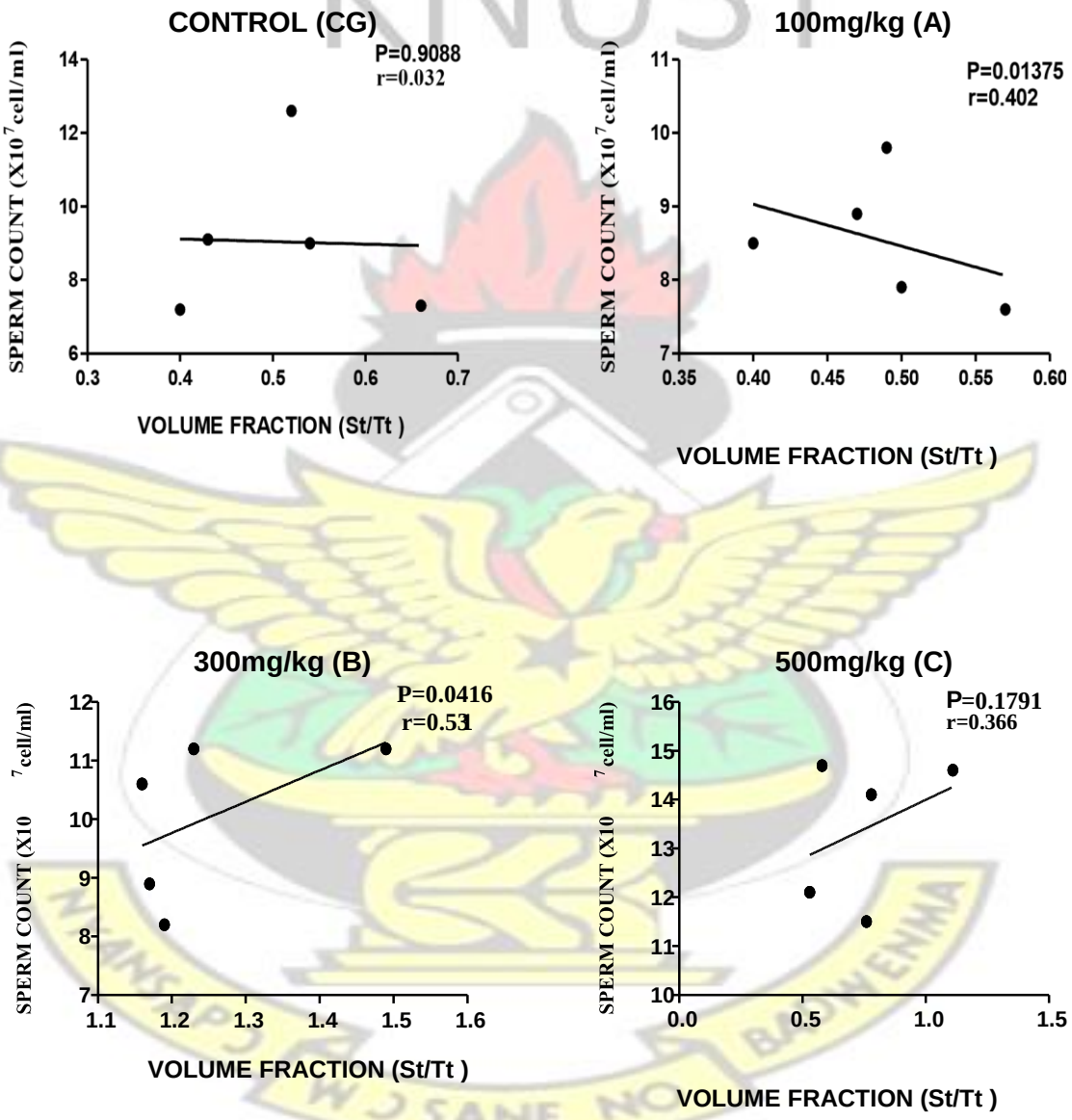


Figure 11: Correlation graph of sperm count against volume fraction testes of control group and treatment groups. *P= statistically significant at P(<0.05)*

4.12 CORRELATION BETWEEN EPIDIDYMAL WEIGHT AND SPERM COUNT IN RATS

According to Pearson's correlation, a statistically significant association was observed between epididymal weight and sperm count in male rats for control group (r = 0.70, p = 0.003), treatment group B (r = 0.28, p = 0.04) and treatment group C (r = 0.66, p = 0.01) (Figure 12). However, correlation between epididymal weight and sperm count in treatment group A was not statistically significant (p = 0.07) although a moderate correlation coefficient of 0.48 was observed (Figure 12).

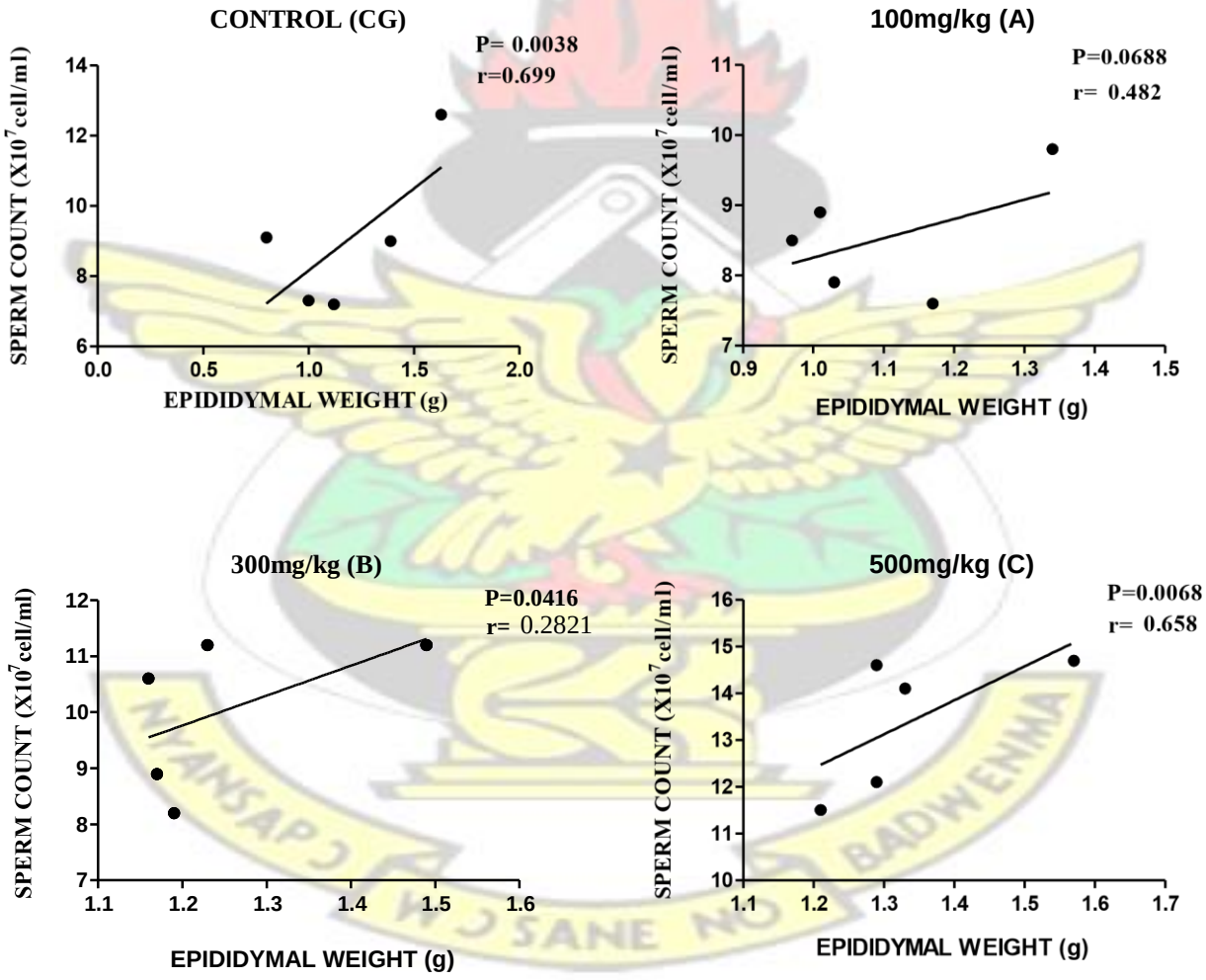


Figure 12: Correlation graph of epididymal weight against sperm count of control group and treatment groups. *P= statistically significant at (P<0.05)*

4.13 CORRELATION BETWEEN SPERM COUNT AND TESTICULAR WEIGHT OF RATS

There was a strong association between sperm count and weight of the testes in male rats for control group (r = 0.70, p = 0.003), treatment group A (r = 0.84, p < 0.0001) and treatment group C (r = 0.83, p = 0.0001) (Figure 13). However, there was a weak correlation between sperm count and weight of the testes in male rats in treatment group B with correlation co-efficient of 0.40. This was not statistically significant (p = 0.14) (Figure 13).

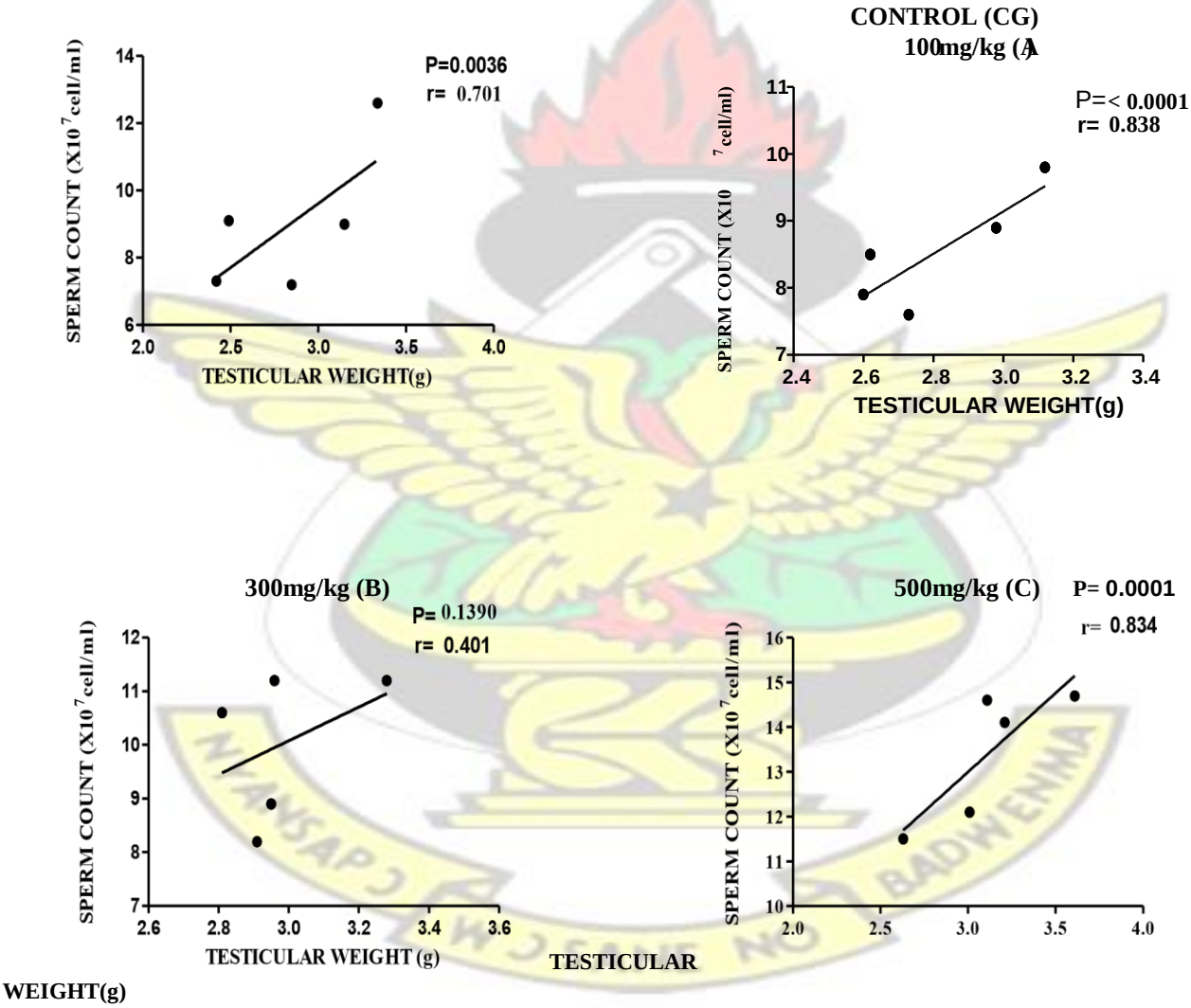


Figure 13: Correlation graph of sperm count against testicular weight of control group and treatment groups. *P= statistically significant at (P<0.05)*

4.14 CORRELATION BETWEEN BODY WEIGHT AND TESTICULAR WEIGHT OF THE RATS

Using Pearson's correlation, there was a strong statistically significant association between body weight and testicular weight of the control and treatment groups with the following correlation coefficients; control group ($r = 0.97$, $p < 0.0001$), treatment group A ($r = 0.88$, $p < 0.0001$), treatment group B ($r = 0.95$, $p < 0.0001$) and treatment group C ($r = 0.97$, $p < 0.0001$) (Figure 14).

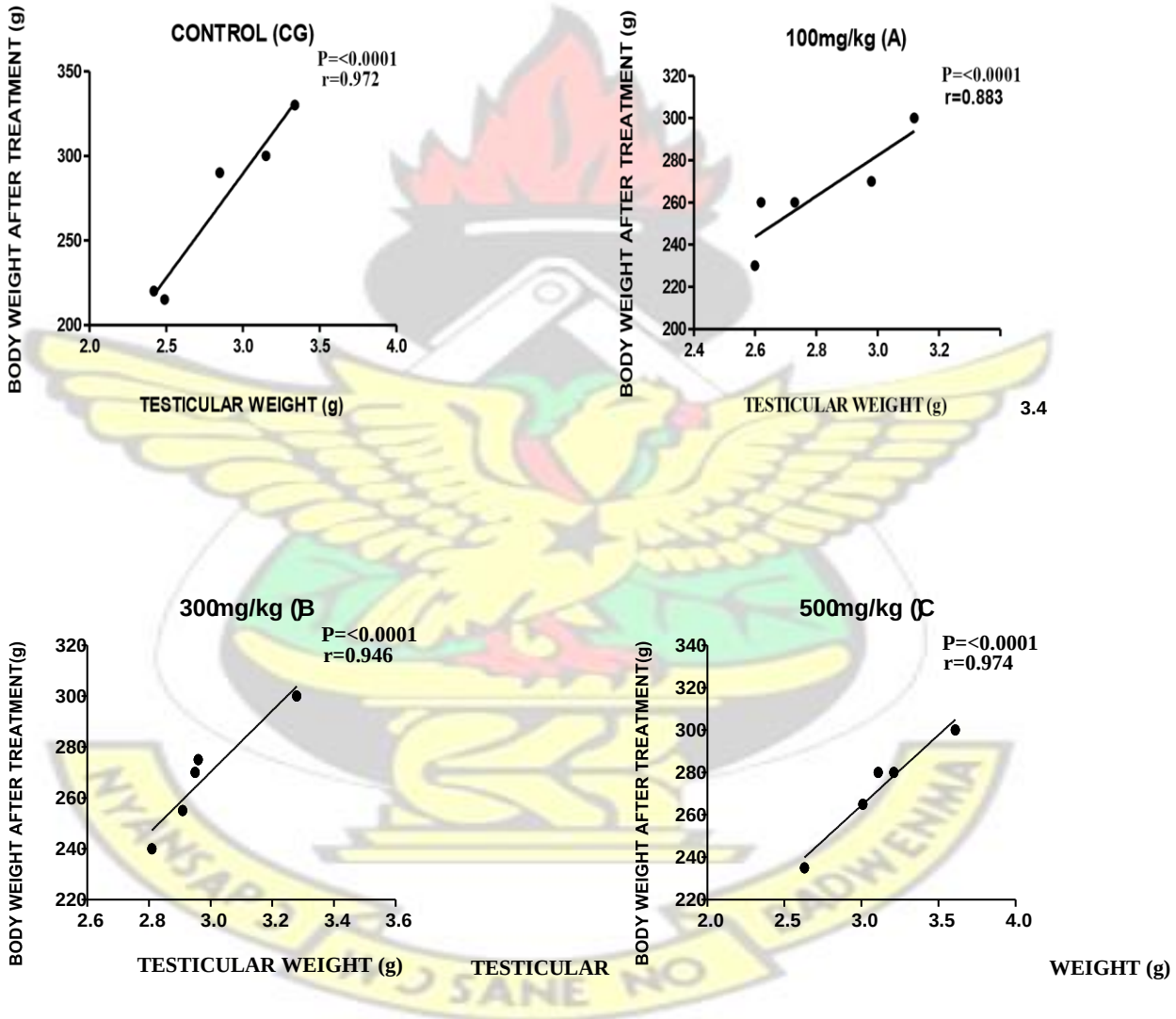


Figure 14: Correlation graph of body weight against testicular weight of control group and treatment groups. *P= statistically significant at (P<0.05)*

4.15 CORRELATION BETWEEN BODY WEIGHT AND EPIDIDYMAL WEIGHT OF THE RATS

Pearson's correlation showed a statistically significant ($p < 0.0001$) strong association between body weight and epididymal weight in male rats for the control ($r = 0.93$) and treatment group A ($r = 0.70$), treatment group B ($r = 0.86$) and treatment group C ($r = 0.84$) (Figure 15).

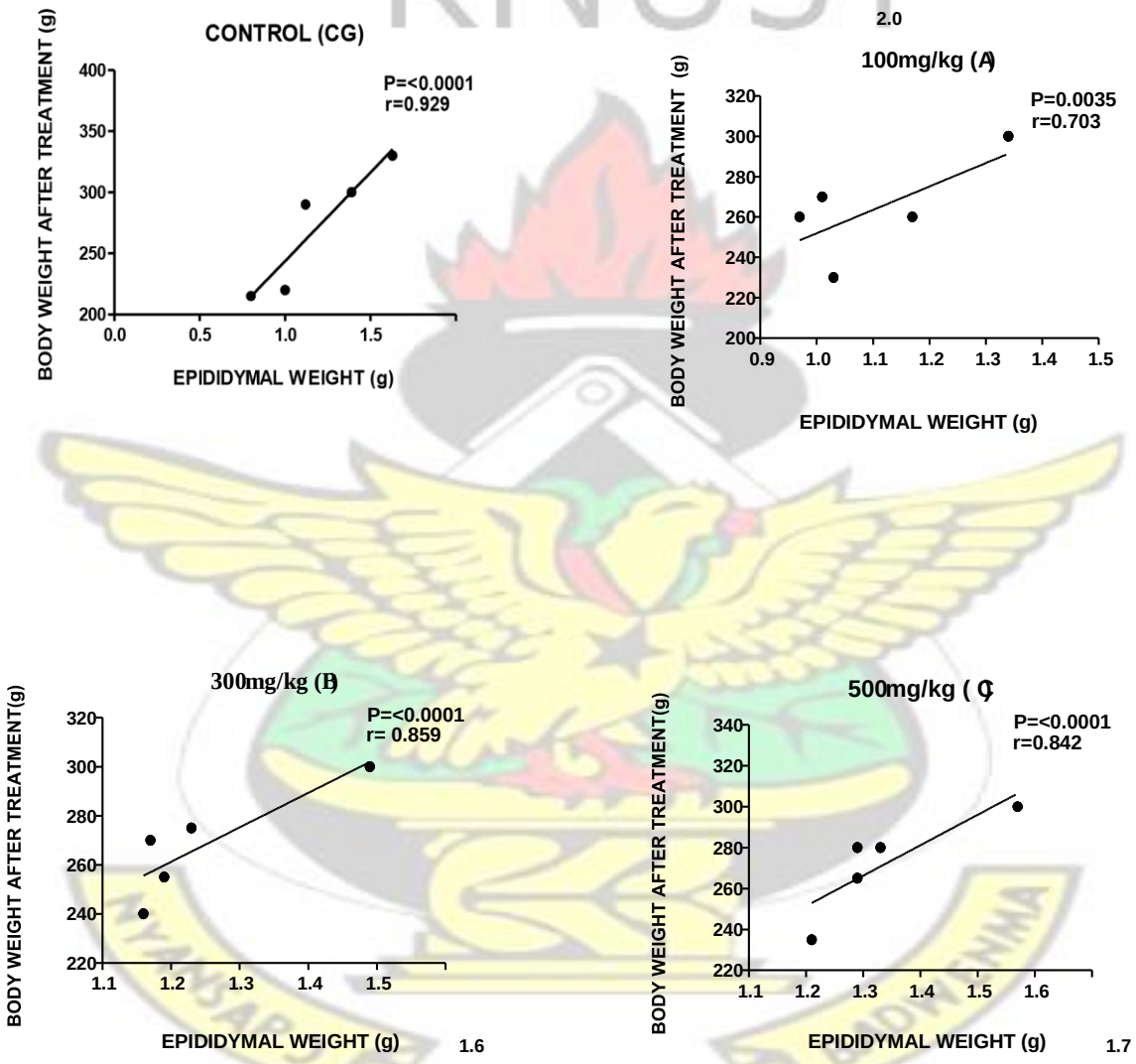


Figure 15: Correlation graph of body weight against epididymal weight of control group and treatment groups. *P= statistically significant at (P<0.05)*

CHAPTER FIVE

DISCUSSION

5.1 EFFECTS OF ETHANOLIC ROOT EXTRACTS OF *Z. OFFICINALE* ON BODY WEIGHT AND REPRODUCTIVE ORGAN WEIGHT IN THE MALE RAT.

Administration of ethanolic extract of *Z. officinale* (ginger) at 100 mg/kg, 300 mg/kg and 500 mg/kg body weight for thirty consecutive days to male albino rats resulted in a numerical increase in body weight, testicular and epididymal weights in a dose-dependent manner. The increase in the weight of the reproductive organs could be due to the effect of *Z. officinale* on androgenic activity in the rats. This finding is consistent with the reports of other studies which have demonstrated the androgenic effects of ethanolic ginger extract in male rats (Kamtchoving *et al*., 2002; Amr and Hamza 2006). Also it has been reported that weight and secretory functions of reproductive organs are mainly regulated by androgens (Agrawal *et al*., 1986).

The hypothalamus secretes gonadotropin releasing hormones (GnRH) which stimulate the anterior pituitary gland to release interstitial cell stimulating hormone (ICSH) which in turn stimulates the Leydig cells of the testes to produce testosterone. Higher levels of testosterone initiate spermatogenesis (Hackney *et al*., 2005). Increase in testicular weight may be largely due to increase in the mass of differentiated spermatogenic cells (Abarikwu, 2010). Therefore, higher levels of testosterone might lead to more differentiated spermatogenic cells, leading to higher sperm counts and an increase in testicular, epididymal and body weights (Yamamoto *et al.,* 1998; Ochsenkühn and De Kretser, 2003). Schrade (2003) also observed weight gain of the accessory sex organs upon administration of *Z. officinale* and concluded that the increase in weight of the accessory organs corresponds to the level of testosterone. This suggests that changes in the levels of testosterone could alter the weight of the reproductive organs. This was also confirmed

by several authors (Arash *et al*., 2009; Dalia, 2010; Saeid *et al.,* 2011). Dalia (2010) observed an increment in testicular weight upon oral administration of ginger extract at 250 mg/kg and 500 mg/kg to diabetic male rats for 65 days. Increase in epididymal weight may result from an increased number of maturing sperms and/or testicular fluid in the tail of the epididymis. Furthermore, a study by Obeten and his colleagues (2014) demostrated that administration of ginger extract in male rats results in an increase in body weight in a dose-dependent manner. This might be due to the effects of testosterone on the growth and development of other parts of the body in addition to the testis. Increased body weight in the extract-treated rats in the present study could also be due to the stimulatory effects of the extract on the digestive enzymes and its overall improvement on digestion and absorption. This is in line with the findings of several studies which showed that the use of ginger in the diet of broilers caused body weight gain as compared to the control birds (Behne *et al*., 1996; Sadeghi *et al*., 2011; Mohamed *et al*., 2012; Arshad *et al*., 2012 and Karangiya *et al*., 2016). Also body weight gain could be due to the antibacterial effect of ginger extract which stimulates lactic acid bacteria causing a decrease in pathogenic bacteria such as mesophilic aerobic, coliform and *Escherichia coli* and thus improves absorption of nutrients (Tekeli *et al*., 2011).

5.2 EFFECT OF ETHANOLIC GINGER EXTRACT ON SPERM CHARACTERISTICS OF THE RATS The results of the present study showed a statistically significant increase in sperm count, motility, viability and normal morphology of the sperm in the extract-treated animals compared to the control group. These might be due to improved spermatogenesis by the ginger extract which is believed to have biologically active agents including androgenic, antioxidant, anticancer, anti-

inflammatory and antimicrobial effects (Ghayur and Gilani, 2005). It has also been reported that ginger exhibits protective effects against oxidative stress in rats (Amr and Hamza, 2006).

A positive feedback effect results in a higher release of GnRH by the hypothalamus, and a subsequent release of LH by the anterior pituitary (Clayton *et al*., 1980). The resultant effects cause an increase in testosterone levels and secretion by the gonads resulting in a rise in these sperm count (Lee *et al*., 2001). However, Ochsenkühn and De Kretser (2003) reported that lower levels of testosterone may result in a decrease in sperm count. In addition, high levels of testosterone stimulate myoid cells to increase peristaltic movement resulting in the release of spermatozoa (Zhang *et al*., 2006). Even though the blood serum testosterone levels were not estimated in this study, it has been well documented that an increase in blood testosterone level results in an increase in spermatogenesis (Hackney *et al*., 2005).

The significant increase in sperm count in the treated groups observed in the present study is in agreement with the findings of previous studies (Sharma and Agarwal, 1996; Abdel-Aziz *et al.,* 1997; Yamamoto *et al.,* 1998; Dalia, 2010). Waleed and Wisam (2012) reported that there was a significant increase in sperm count by 16.2% in infertile men after treatment with ginger. Similarly in the present study, sperm count increased from 12.8% to 48.2% when 300 mg/kg and 500 mg/kg of ginger extract were given. It is possible that the ginger extract regulates the production of Reactive Oxygen Species (ROS) and improves sperm parameters such as sperm morphology, viability, motility and thereby increase reproductive efficiency of the rats.

A significant increase ($p = 0.018$) in normal sperm morphology was observed in the treated male rats. This finding is in line with the observations of previous studies which reported a significant ($p < 0.01$) rise of 18.4% in normal sperm morphology of infertile men after treatment with ginger (Khaki *et al*., 2009a; Waleed and Wisam, 2012). With respect to abnormal sperm morphology, the present study observed a significant decrease in the treated groups in a dose-dependent

manner. Abnormal sperm morphology could be due to oxidative stress imposed by higher levels of free radicals of ROS in the absence of ginger. This observation is also in line with the findings of Dalia (2010) who reported a significant decrease in abnormal sperm morphology in gingertreated rats.

The increase in progressive sperm motility of treated groups observed in the present study could be due to the counter oxidative stress of ginger on sperm production. This is in agreement with the findings of earlier studies (Aitken *et al*., 1995; Amr and Hamza, 2006; Arash *et al*. 2009; Dalia, 2010). Sperm motility is as a result of ATP, produced by breakdown of fructose secreted by the seminal vesicle (Mann, 1974). Ginger might play a role in the normal functioning of the seminal vesicle in increasing ATP production (Brem *et al*., 2002). Sperm motility also depends on the coordinated propagation of flagella wave under acetylcholinesterase control during sperm maturation (Nelson, 1972; Egbunike, 1980). In addition, increase in sperm motility may be due to the positive feedback from the functions of the seminal vesicle, epididymis and sperm acetylcholinesterase activity by the ginger extract (Sathiyaji *et al*., 2010). Arash *et al*. (2009), on the administration of ginger extract exhibited a significant increase in sperm motility in male rats. According to a study by Dalia (2010), oral administration of ginger extract at 250 mg/kg and 500 mg/kg body weight to diabetic induced rats for 65 days increased sperm motility suggesting that ginger may have antidiabetic activity. Amr and Hamza (2006) also established that *Z. officinale* treatment caused an increase in the activities of testicular antioxidant enzymes and restored sperm motility of cisplatin-treated rats.

In the present study, there was a significant increase in sperm viability in the extract-treated male rats. This finding is consistent with the reports of previous studies in which administration of ethanolic ginger extract to normal and diabetic rats for 20 consecutive days caused an increase in sperm viability (Arash *et al*., 2009; Dalia, 2010; Nassiri *et al*., 2009; Waleed and Wisam, 2012).

In humans, it has been reported that low sperm count, malformed spermatozoa, and/or reduced or deficient motility are the main causes of the increase in male infertility (Davajan and Israel, 1991; Chemes and Rawe, 2003). Therefore, the use of ethanolic ginger extract may offer an accessible affordable alternative for the treatment and management of infertility.

5.3 EFFECT OF GINGER EXTRACT ON THE MORPHOLOGY OF THE TESTIS

In the present study, qualitative examination of the testis in both control and extract-treated rats showed seminiferous tubules with spermatogenic cells at different stages of development, accumulations of germ cells in the lumina of seminiferous tubules and prominent peritubular myoid cells. This finding is in agreement with the reports by Arash *et al*. (2009) and Nashwa *et al*. (2011) who demonstrated normal microscopic features of the testes of male rats after administration of 50 mg/kg and 100 mg/kg ginger extract for 20 days.

In the present study, there was an increase in the number of seminiferous tubules and interstitial tissue in the treated animals and this was confirmed quantitatively using point counting methods. The large areas of interstitial tissue contained a higher population of Leydig cells which probably led to an increase in the production of testosterone and a corresponding increase in spermatogenesis and the overall sperm count in the extract-treated animals. This might explain the significant increase in sperm counts observed in treatment groups B (300 mg/kg) and C (500 mg/kg). Quantitatively, there was a significant increase in the total volume fraction of the seminiferous tubule in treated rats with 100 mg/kg, 300 mg/kg and 500 mg/kg. This could be due to an increase in the number of seminiferous tubules and spermatogenic cells which in turn increased testicular weight in the extract-treated rats. Similarly, Hossein *et al.* (2013) upon oral administration of ginger extract of 50 mg/kg and 100 mg/kg to busulfan-induce male rats lead to a significant increase in the total volume of the testis. It has been documented that zingirbrene,

gingerdiole, zingerone, gingerls and shaogals in ginger prevent DNA damage induced by hydrogen peroxide and reduce apoptosis in spermatogenesis (Khaki *et al.*, 2009a; Sakr and Gamal, 2011).

Several studies have speculated that ginger has a potent androgenic activity and may be responsible for high levels of testosterone and increased sperm count in male rats (Yamamoto *et al.,* 1998; Ochsenkühn and De Kretser, 2003; Amr and Hamza, 2006). Also, Oyewo *et al*. (2012) reported thickness of cell membrane and fibrous nature of the myoid cells after administration of 0.2 ml/kg ginger extract to male rats for 21 days. Contrary to the findings of the present study, Oyewo *et al*. (2012) reported fusion and distortions of seminiferous tubules and damage to the epithelial lining when the dosage of ginger extract was increased from 0.4ml/kg to 0.8ml/kg. Nashwa *et al.* (2011) identified deterioration, desquamation and clear necrosis of the spermatogonia following the administration of Ciprofloxacin, however subsequent treatment with ginger exhibited normal seminiferous tubules with normal spermatogenic cells.

5.4 CORRELATION BETWEEN VOLUME FRACTION, TESTICULAR WEIGHT AND SPERM COUNT IN THE RATS

The results of the present study showed a weak correlation between weight of the testes and volume fraction of the seminiferous tubules in both the extract-treated (100 mg/kg and 500 mg/kg) and the control groups. Although change in the volume fraction of the seminiferous tubules might be used to determine testicular weight, this association was not statistically significant ($P > 0.05$) to predict the weight of the testes. It has been shown that change in testicular weight could be due to a rise in the mass of the differentiated spermatogenic cells, large interstitial connective tissue, inhibition of spermatogenesis and steroidogenic enzyme activity (Chapin *et al*., 1997; Takahashi and Oishi, 2001).

In this study, a strong positive correlation was observed between volume fraction of the seminiferous tubules and sperm count in both treated and controlled groups. Thus an increase in volume fraction of the seminiferous tubules may lead to increase in sperm count. The increase in sperm count and volume fraction of the testes could be as a result of the androgenic and spermatogenic effect of ginger treatment.

The results of the present study is in line with the finding of Sakr and Gamal (2011) who reported a significant increase in the volume fraction of the seminiferous tubules and the spermatogenic cells after treatment with *Z. officinale*. In addition, ginger has been shown to cause an increase in the population of spermatozoa and the volume of seminiferous tubules by preventing apoptosis during spermatogenesis (Sakr and Gamal, 2011).

5.5 CORRELATION BETWEEN EPIDIDYMAL WEIGHT, TESTICULAR WEIGHT, BODY WEIGHT AND SPERM COUNT IN THE RATS

The present study observed a strong correlation between epididymal weight, testicular weight and sperm count in the male rats. This could be explained by the androgenic property of ginger increasing the rate of spermatogenesis in the testes, movement and deposition of male germ cells in the epididymis and a corresponding increase in epididymal weight. The increased sperm counts as well as increased epididymal weight may suggest an improvement in the fertility of the male rats as a result of the ginger extract treatment**.** These results suggest that the weight of the epididymis could be used to predict sperm count. The strong association between testicular weight and sperm count in the present study, shows that an increase in testicular weight will result in a corresponding increase in sperm count in the treated male rats. In the present study, a strong positive correlation between body weight and testicular weight in male rats was shown.

It can be deduced that testicular weight is a key factor in determining body weight of the male rats. There was however a weak correlation between body weight and epididymal weight hence epididymal weight cannot be used to predict body weight.

CHAPTER SIX

CONCLUSION

In this study, administration of ethanolic ginger extract for 30 consecutive days to male albino rats resulted in an increase in body weight, testicular and epididymal weight in a dose dependant manner.

Both the control and extract-treated male rats showed normal morphology of the testes. However, in the extract-treated animals, there was a considerable increase in the proportion of interstitial tissues and seminiferous tubules.

There was a strong positive correlation between volume fraction of the seminiferous tubules and sperm count. Also, there was a strong positive correlation between sperm count, body weight, epididymal and testicular weight in the extract-treated animals.

Ethanolic ginger extract caused a significant increase in sperm count, motility, viability and morphology. Therefore, ginger extract may be potentially useful in the management of male infertility especially those with low sperm count.

6.2 FUTURE WORK

- Further work should involve detailed light and electron microscopical and morphometric analysis of the testes of the extract-treated rats.
- Further work should include haematological, hormonal and biochemical analysis of the extract-treated rats to confirm the hormonal levels and effects of the ginger extract on the liver.
- Further studies should be conducted to determine the chemical composition of the ginger extract.
- Future studies should consider oral administration of ginger in male human participants to ascertain the possible effects of ginger on the hormonal levels

REFERENCES

Abaidoo, C. S., Woode, E. and Alhassan, A. (2011). An evaluation of the effect of ethanolic fruit extracts of *Xylopia aethiopica* on haematological and biochemical parameters in male rats. *Der Pharmacia Sinica*, **2**: 39-45.

Abarikwu, S. O., Adesiyan, A. C., Oyeloja, T. O., Oyeyemi, M. O. and Farombi, E. O. (2010). Changes in sperm characteristics and induction of oxidative stress in the testis and epididymis of experimental rats by a herbicide, Atrazine. *Archives of Environmental Contamination and Toxicology*, **58**(3): 874-882.

Abdel Aziz, A. H., Shouman, S. A., Attia, A. S. and Saad, S. F. (1997). A study on the reproductive toxicity of erythrosine in male mice. *Pharmacological Research,* **35**(5): 457-462.

Abebe, D. and Hagos, E. (1991). In the traditional health practices of Ethiopia. *Plant Genetic Resources of Ethiopia*, 101.

Agarwal, A. and Said, T. M. (2005). Oxidative stress, DNA damage and apoptosis in male infertility: a clinical approach. *British Journal of Urologist International,* **95**: 503-507.

Agrawal, S., Chauhan, S. and Mathur, R. (1986). Antifertility effects of embelin in male rats. *Andrologia,* **18**: 125-131.

Aitken, R. J., Buckingham, D. W., Brindle, J., Gomez, E., Baker, H. W. and Irvine, D. S. (1995). Analysis of sperm movement in relation to the oxidative stress created by leukocytes in washed sperm preparations and seminal plasma. *Human Reproduction,* **10**: 2061-2071.

Alhassan, A. (2012). Effect of ethanolic fruit extract of *Xylopia aethiopica* (dunal) a Rich (annonaceae) and Xylopic acid on reproductive function in male rats. PhD thesis Kwame Nkrumah University of Science and Technology, Ghana.

Ali, B. H., Blunden, G., Tanira, M. O. and Nemmar, A. (2008). Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale Roscoe*): a review of recent research. *Food and Chemical Toxicology*, **46**(2): 409-420.

Amann, R. P. (1982). Use of animal models for detecting specific alteration in reproduction. *Fundamental and Applied Toxicology,* **2**: 13-36.

Amann, R. P. and Berndtson, W. E. (1986). Assessment of procedures for screening agents for effects on male reproduction: effects of dibromochloropropane (DBCP) on the rat. *Fundamental and Applied Toxicology,* **7**: 244-255.

Amin, A., Hamza, A., Kambal, A. and Daoud, S. (2008). Herbal extracts counteract cisplatinmediated cell death in rat testis. *Asian Journal of Andrology,* **10**: 291-297.

Amr, A. and Hamza, A. E. A. (2006). Effects of Roselle and Ginger on cisplatin-induced reproductive toxicity in rats. *Asian Journal of Andrology,* **8**: 607-612.

Ansari, M. N., Bhandari, U. and Pillai, K. K. (2006). Ethanolic *zingiber officinale* Extract pretreatment alleviates isoproternol- induced oxidative myocardial necrosis in rats. *Indian Journal of Experimental Biology,* **44**: 892-897.

Arash, K., Fatemeh, F., Mohammad, N., Amir, A. K., Chelar, C. O., Marefat, G. and Mohammad, H. (2009). The effects of Ginger on spermatogenesis and sperm parameters. *Iranian Journal of Reproductive Medicine,* **7**(1): 7-12.

Arshad, M., Kakar, A. H., Durrani, F. R., Akhtar, A., and Niamatullah, M. (2012). Economical and immunological impact of ginger (*Z. Officinale*) extract on broiler chicks. *Pakistan Journal of Science*, **64**(1).

Banerjee, S., Mullick, H. I., Banerjee, J. and Ghosh, A. (2011). *Zingiber officinale*: a natural gold. *International Journal of Pharmaceutical and BiologicalScience*, **2**: 283-294.

Bannerman, R. H. (1982). Traditional medicine in modern health care. *World Health Forum,* **3**(1): 813.

Bardin, C. W. and Paulsen, C. A. (1981). Testis. In textbook of Endocrinology, 6th Edition (R. H. Williams). *Walters Burns Saunders, Philadelphia,* 239-354.

Barth, A. D. and Oko, R. J. (1994). Abnormal Morphology of Bovine Spermatozo. *Iowa State University Press. Ames, Iowa, USA*, 8-12.

Baskin, L. S. (2000). Hypospadias and urethral development. *The Journal of Urology*, **163**(3), 951956.

Bay, K., Main, K. M., Toppari, J. and Skakkebæk, N. E. (2011). Testicular descent: INSL3, testosterone, genes and the intrauterine milieu. *Nature Reviews Urology*, **8**(4): 187-196.

Bearden, H. J. and Fuquay, J. W. (1980). Applied Animal Reproduction. *Reston Publishing Company, Incoporation. Reston, Virginia*, 157-165.

Behne, D., Weiler, H. and Kyriakopoulos, A. (1996). Effects of selenium deficiency on testicular morphology and function in rats. *Journal of Reproduction and Fertility*, **106**(2), 291-297.

Bennett, J. P. and Vickery, B. H. (1970). Reproduction and Breeding Techniques for Laboratory Animals, Rats and Mice. *In Hafez Edition Philadelphia: Lea and Febiger. Los Angeles,* 107-122.

Bhowmik, D., Tripathi, K. K., Chandira, M. R. and Kumar, K. P. (2010). *Zingiber officinale* the Herbal and Traditional Medicine and Its Therapeutically Importance. *Research Journal of Pharmacognosy and Phytochemistry*, **2**(2): 102-110.

Boas, N. F. and Ludwig, A. W. (1950). The mechanism of estrogen inhibition of comb growth in the cockerel, with histologic observations. *Endocrinology,* **46**(3):

298-306.

Boxenbaum, H. and DiLea, C. (1995). First_time_ _in human dose selection: Allometric thoughts and perspectives. *The Journal of Clinical Pharmacology*, **35**(10): 957-966.

Brehm, R., Zeiler, M., Rüttinger, C., Herde, K., Kibschull, M., Winterhager, E. and Konrad, L. (2007). A sertoli cell-specific knockout of connexin43 prevents initiation of spermatogenesis. *The American Journal of Pathology*, **171**(1): 19-31.

Brem, B., Seger, C., Pacher, T., Hofer, O., Vajrodaya, S. and Greger, H. (2002). Feeding deterrence and contact toxicity of Stemona alkaloid-source of potent natural insecticides. *Journal of Agricultural and Food Chemistry,* **50**: 6383-6388.

Bunting, L. and Boivin, J. (2007). Decision-making about seeking medical advice in an internet sample of women trying to get pregnant. *Human Reproduction,* **22**(6): 1662-1668.

Burns, M. M. (2000). Alternative medicine: Herbal preparation. *Clinical Pediatric Emergency Medecine,* **1**: 186-190.

Byskov, A. G. (1986). Differentiation of mammalian embryonic gonad. *Physiological Reviews*, **66**(1): 71-117.

CABI Crop Protection Compendium. (2012). *Zingiber officinale* datasheet. Available at: http://www.cabi.org/cpc/datasheet/57537. (Accessed 2014 December 15 at 3:30 GMT).

Calixto, J. B. (2000). Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). *Brazilian Journal of Medical and Biological Research*, **33**(2): 179-189.

Carlsen, E., Giwercman, A., Keiding, N. and Skakkebaek, N. E. (1992). Evidence for decreasing quality of semen during past 50 years. *British Medical Journal,* **305**: 609-613.

Carlson, B. M. (2013). *Human Embryology and Developmental Biology*. Elsevier Health Sciences, Amsterdan. pp 506.

Chan, K. (2003). Some aspects of toxic contaminants in herbal medicines. *Chemosphere*, **52**(9): 1361-1371.

Chapin, R. E., Harris, M., W., Davis, B. J., Ward, S. M., Wilson, R. E. and Mauney, M. A. (1997). The effects of perinatal/juvenile methoxychlor on adult rat nervous, immune and reproductive system function. *Fundamental and Applied Toxicology,* **40**: 138–57.

Chapman, R. M. (1983). Gonadal injury resulting from chemotherapy. *American Journal Industrial Medicine,* **4**(1-2): 149-161.

Chemes, H. E. and Rawe, V. Y. (2003). Sperm pathology: a step beyond descriptive morphology.

Origin, characterization and fertility potential of abnormal sperm phenotypes in infertile men. *Human Reproduction Update*, **9**(5): 405-428.

Cheng, C. Y. and Mruk, D. D. (2010). The biology of spermatogenesis: the past, present and future. *Philosophical Transaction of The Royal Society London B: Biological Science,* **365**(1546): 1459-1463.

Clause, B. T. (1993). The Wister rat as a right choice: establishing mammalian standards and the ideal of a standardized mammal. *Journal of the History of Biology,* **26**: 329-349.

Clayton, R. N., Solano, A. R., Garcia-vela, A., Dufau, M. L. and Catt, K. J. (1980). Regulation of pituitary receptors for gonadotropin-releasing hormone during the rat estrous cycle. *Endocrinology*, **107**(3): 699-706.

Clermont, Y. (1958). Structure of the epithelium of the seminiferous tubules and the mechanism of regeneration of the spermatogonia in the duck. *Archives D'Anatomie Pathologique (Paris),* **47**(1): 47-66.

Clermont, Y. (1972). Kinetics of spermatogenesis in mammals: seminiferous epithelium cycle and spermatogonial renewal. *Physiological Reviews,* **52**(1): 198-236.

Clulow, J., Jones, R. C., Hansen, L. A. and Man, S. Y. (1998). Fluid and electrolyte reabsorption in the ductuli efferentes testis. *Journal of Reproduction and Fertility-Supplement,* 1-14.

Cohen, R. J., Ek, K. and Pan, C. X. (2002). Complementary and alternative medicine (CAM) use by older adults: A comparison of self-report and physician chart documentation. *Journals of Gerontology Series A Biological Sciences and Medical Science,* **57**(4): 223– 227.

Craig, W. J. (1999). Health-promoting properties of common herbs. *The American Journal of Clinical Nutrition*, **70**(3): 491-499.

Dalia, A. H. (2010). Effect of Extracts of Ginger Goots and Cinnamon Bark on Fertility of Male Diabetic Rats. *Journal of American Science,* **6**(10): 940-947.

Dattner, A. M. (2003). From medical herbalism to phytotherapy in dermatology: back to the future. *Dermatology and Therapy,* **16**: 106-113.

Davajan, V. and Israel, R. (1991). Diagnosis and medical treatment of infertility. In A.L. Stanton C. Dunkel-Schetter Edition, *Infertility: Perspectives from Stress and Coping Research,* New York, NY: Plenum Press. (Accessed 2015 February 20 at 9:00 GMT).

De Kratser, O. M. and Kerr, J. B. (1988). The cytology of the testis. In: The Physiology of reproduction. Editions, E Knobil and JD Neil. Raven Press, New York, 837-932.

Demain, A. L. (1999). Pharmaceutically active secondary metabolites of microorganisms. *Applied Microbiology and Biotechnology,* **52**(4): 455-463.

Desai, N., Sharma, R., Makker, K. and Sabannegh, E. A. A. (2009). Physiologic and pathologic levels of reactive oxygen species in neat semen of infertile men. *Fertility and Sterility,* **92**: 16261631.

Detlef, S. J., Jia, Benno, B. and Eckhart, G. H. (1999). Herbal Products for Liver Diseases: A Therapeutic Challenge for the New Millennium. *Hepatology*, **30**(4): 1099-1104.

Devasagayam, T. P. A., Tilak, J. C., Boloor, K. K., Sane, K. S., Ghaskadbi, S. S. and Lele, R. D. (2004). Free radicals and antioxidants in human health: current status and future prospects. *Japi*, **52**(794804): 4.

Dumontet, C. and Sikic, B. I. (1999). Mechanisms of action of and resistance to antitubulin agents: microtubule dynamics, drug transport, and cell death. *Journal of Clinical Oncology*, **17**(3): 1061-1061.

Dyer, S. J., Abrahams, N., Mokoena, N. E. and Van Der Spuy, Z. M. (2004). 'You are a man because you have children': experiences, reproductive health knowledge and treatment seeking behaviour among men suffering from couple infertility in South Africa. *Human Reproduction*, **19**(4): 960-967.

Dym, M. (1994). Basement Membrane Regulation of Sertoli Cells. *Endocrine Reviews*, **15**(1): 102115.

Ebadi, M. (2006). *Pharmacodynamic basis of herbal medicine*. CRC press, United State of America. pp 760.

Egbunike, G. N. (1980). Changes in acetylcholinesterase activity of mammalian spermatozoa during maturation. *International Journal of Andrology,* **3**(4): 459-468.

Elftman, H. (1963). Sertoli Cells and Testis Structure. *American Journal of Anatomy,* **113**: 25-33.

Engdal, S., Klepp, O. and Nilsen, O. G. (2009). Identification and exploration of herb-drug combinations used by cancer patients. *Integrative Cancer Therapies,* **8**(1): 29–36.

Ernst, E. (2007). First, do no harm, with complementary and alternative medicine. *Trends Pharmacological Science*, **28**: 48-50.

Ernst, E. and Schmidt, K. (2002). Health risks over the Internet: Advice offered by "medical herbalists" to a pregnant woman. *Wiener Medizinische Wochenschrift*, **152**(7-8): 190–192.

Farnsworth, N. F. (2008). The role of ethnopharrnacology in drug development. *Bioactive Compounds from Plants*, **735**: 2.

Farnsworth, N. R. and Soejarto, D. D. (1991). Global importance of medicinal plants. *The Conservation of Medicinal Plants*, 25-51.

Fawcett, D. W. (1973). Observations on the organization of the interstitial tissue of the testis and on the occluding cell junctions in the seminiferous epithelium. *Advances in Biosciences,* **10**(83): 99-126

Fnimh, A. C. (2001). Encyclopaedia of Medicinal Plants. *London, UK,* 155-156.

Fody, E. and Walker, E. (1985). Effects of drugs on the male and female reproductive systems. *Annals of Clinical Laboratory Science,* **15**(6): 451-458.

Ford, W. C. (2004). Regulation of sperm function by reactive oxygen species. *Human Reproduction Update,* **10**: 387-99.

Fritz, I. B., Louis, B. G., Tung, P. S., Griswold, M., Rommerts, F. G. and Dorrington, J. H. (1975). Biochemical responses of cultured Sertoli cell-enriched preparations to follicle stimulating hormone and dibutyryl cyclic AMP. *Current Topics in Molecular Endocrinology,* **2**: 367-382.

Gao, H. M. (2015). *Zingiber officinale* (Willd.) Rosc. (Jiang, Common Ginger). In *Dietary Chinese Herbs Springer Vienna*, pp. 273-281..

Garner-Wizard, M., Milot, S. H. B., Neustadt, E. J., Oliff, H. S. and Oppel, M. (2006). Ginger, an herbal medicinal product with broad anti-inflammatory actions. *Journal of Medicinal Food*, **8**: 125-132.

Gartner, L. P., Hiatt, J. L. and Strum, J. M. (1998). Cell biology and histology. Wolters Kluwer Health/Lippincott Williams and Wilkins, Belgium. pp 374.

Ghayur, M. N. and Gilani, A. H. (2005). Ginger lowers blood pressure through blockade of voltage-dependent calcium channels. *Journal of Cardiovascular Pharmacology and Therapeutics,* **445**: 74-80.

Giwercman, A., Carlsen, E., Keiding, N. and Skakkebaek, N. E. (1993). Evidence for increasing incidence of abnormalities of the human testis: a review. *Environmental Health Perspectives*, **101**(2): 65.

Goto, C., Kasuya, S., Koga, K., Ohtomo, H. and Kagei, N. (1990). Lethal efficacy of extract from*Zingiber officinale* (traditional Chinese medicine) or [6]-shaogal and [6]-gingerol inAnisakis larvae in vitro. *Parasitology Research*, **76**(8): 653-656.

Govardhan Naik, A., Lakshman, J. and Changamma, C. (2014). A study on the effect of betel leaf stalk extract in male albino rats. *International Journal of Biological and Pharmaceutical Research*, **5**(3): 285-289.

Goyal, R. K. and Kadnur, S. V. (2006). Beneficial effects of *Zingiber officinale* on gold thioglucose induced obesity. *Fitoterapia,* **77**: 160-163.

Grontved, A., Brask, T., Kambskard, J. and Hentzer, E. (1988). Ginger root against seasickness: a controlled trial on the open sea. *Acta Oto-laryngologica,* **105**: 45-49.

Grunwald, J. (1994). The European Phytomedicnes Market Figures, Trends, Analyses. *Herbal Gram,* 6034-6065.

Gurib-Fakim, A. (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow.

Molecular Aspects of Medicine, **27**(1): 1-93.

Hackney, A. C., Moore, A. W., and Brownlee, K. K. (2005). Testosterone and endurance exercise: development of the exercise-hypogonadal male condition. *Acta Physiologica Hungarica*, **92**(2): 121-137.

Hafez, E. S. E. (1980). Embryology and Functional Anatomy of Male Reproductive Organs. In Surgery of the Male Reproductive Tract. Springer Netherland, 5-28.

Hann, M. M., and Keserü, G. M. (2012). Finding the sweet spot: the role of nature and nurture in medicinal chemistry. *Nature Reviews Drug Discovery*, **11**(5): 355-365.

Hess, R. A. (1990). Quantitative and qualitative characteristics of the stages and transitions in the cycle of the rat seminiferous epithelium: light microscopic observations of perfusion-fixed and plastic-embedded testes. *Biology of Reproduction,* **43**(3): 525-542.

Holdcraft, R. W. and Braun, R. E. (2004). Hormonal regulation of spermatogenesis. *International Journal of Andrology,* **27**(6): 335-42.

Hossein, B., Tahereh, E., Farzaneh, D. and Mohammad, R. P. (2013). Stereological study of the effect of ginger's alcoholic extract on the testis in busulfan-induced infertility in rats. *Iran Journal of Reproductive Medicine,* **11**(6):

467-472.

Hughes, I. A. and Acerini, C. L. (2008). Factors controlling testis descent. *European Journal of Endocrinology*, **159**(1): 75-82.

Huhtaniemi, I. and Pelliniemi, L. J. (1992). Fetal Leydig cells: cellular origin, morphology, life span, and special functional features. *Experimental Biology and Medicine*, **201**(2): 125-140.

Hutson, J. M. (2012). Embryology of the human genital tract. In *Disorders of Sex Development*. *Springer Berlin Heidelberg,* 11-21.

Hutson, J. M., Hasthorpe, S. and Heyns, C. F. (1997). Anatomical and Functional Aspects of Testicular Descent and Cryptorchidism 1. *Endocrine Reviews*, **18**(2):

259-280.

Ikechebelu, J. I., Adinma, J. I., Orie, E. F. and Ikegwuonu, S. O. (2003). High prevalence of male infertility in southeastern Nigeria. *Journal of Obstetrics and Gynaecology: The Journal of The Institute of Obstetrics and Gynaecology,* **23**(6):

657-659.

Ilio, K. Y. and Hess, R. A. (1994). "Structure and function of the ductuli efferentes: a review." *Microscopy Research and Technique,* **29**(6): 432-67.

Iqbal, Z., Lateef, M., Akhtar, M. S., Ghayur, M. N. and Gilani, A. H. (2006). In vivo anthelmintic activity of ginger against gastrointestinal nematodes of sheep. *Journal of Ethnopharmacology,* **106**: 285-287.

Ir, N. E. B. (2013). Functional anatomy of the hypothalamic—pituitary—gonadal axis and. *Fertility Preservation in Male Cancer Patients*, **1**.

Isidori, A. M., Pozza, C. Gianfrilli, D. and Isidori, A. (2006). Medical treatment to improve sperm quality. *Journal of Reproductive Biomedicine,* **12**: 704 -714.

Jana, U., Chattopadhyay, R. N. and Shaw, B. P. (1999). Preliminary studies on antiinflammatory activity of *Zingiber officinale Rosc*. Vitex negundo Linn and Tinospora Cordifolia (Willd) miers in albino rats. *Indian Journal of Pharmacology,* **31**: 232-233.

Johnson, Q., Syce, J., Nell, H., Rudeen, K. and Folk, W. R. (2007). A randomized, double-blind, placebo-controlled trial of Lessertia frutescens in healthy adults. *PLoS Clinical Trials,* **2**(4): 16.

Josso, N., Belville, C., Di Clemente, N. and Picard, J. Y. (2005). AMH and AMH receptor defects in persistent Müllerian duct syndrome. *Human Reproduction Update*, **11**(4): 351-356.

Kamtchouing, P., Mbongue Fandio, G. Y., Dimo, T. and Jatsa, H. B. (2002). Evaluation of androgenic activity of *Zingiber officinale* and Pentadiplandra brazzeana in male rats. *Asian Journal of Andrology,* **4**(4): 299-301.

Karangiya, V. K., Savsani, H. H., Patil, S. S., Garg, D. D., Murthy, K. S., Ribadiya, N. K. and Vekariya, S. J. (2016). Effect of dietary supplementation of garlic, ginger and their combination on feed intake, growth performance and economics in commercial broilers. *Veterinary World*, **9**(3): 245.

Kaul, P. N. and Joshi, B. S. (2001). Alternative medicine: Herbal drugs and their critical appraisalpart II. *Progress in Drug Research,* **57**: 1–75.

Kavlock, R. J., Daston, G. P., DeRosa, C., Fenner-Crisp, P., Gray, L. E., Kaattari, S. and Miller, R. (1996). Research needs for the risk assessment of health and environmental effects of endocrine disruptors: a report of the US EPA-sponsored workshop. *Environmental Health Perspectives*, **104**(4): 715.

Kerr, J. B., Loveland, K. L., O'Bryan, M. K. and De Kretser, D. M. (2006). Cytology of the testis and intrinsic control mechanisms. *Knobil and Neill's physiology of reproduction. J. D. Neill. San Diego, Elsevier Academic press,* 827-948.

Khaki, A., Fatemek, F., Mohammad, N., Amir, A. K., Chelar, C. O., Marefat, N. and Mohammad,

H. (2009). The effects of ginger on spermatogenesis and sperm parameters. *Iranian Journal of Reproductive Medicine,* **7**(1): 7-12.

Kidd, S. A., Eskenazi, B. and Wyrobek, A. J. (2001). Effects of male age on semen quality and fertility: a review of the literature. *Fertility and Sterility*, **75**(2): 237-248.

Kirtikar, K. R. and Basu, B. D. (1993). Indian Medicinal Plants. *2nd Edition International Book Distributors, Rajpur Road, Dehradun, India,* **4**: 2435-2438.

Kogan, S. J. and Hafez, E. S. (2012). Pediatric andrology Edition. Springer Science and Business Media. *Clinics in Andrology*, **7**: 232.

Koopman, P., Gubbay, J., Vivian, N., Goodfellow, P. and Lovell-Badge, R. (1991). Male development of chromosomally female mice transgenic for Sry. *Nature*, **351**(6322): 117-121.

Koppen, S. and Berne, L. (2008). Physiology. 6th Edition Copyright by Mosby, an imprint Incorporation, St. Louis. **120**(1): 29-37.

Krinke, G. J. (2000). "History, Strains and Models". The Laboratory Rat (Handbook of Experimental Animals). Gillian R. Bullock (series ed.), Tracie bunton (series edition*). Academic Press,* 3-16.

Leblond, C. P. and Clermont, Y. (1952). Spermiogenesis of rat, mouse, hamster and guinea pig as revealed by the periodic acid-fuchsin sulfurous acid technique. *American Journal of Anatomy,* **90**(2): 167-215.

Lee, P. A., Coughlin, M., T. and Bellinger, M. F. (2001). Inhibin B: Comparison with Indices of

Fertility among Formerly Cryptorchid and control men. *The Journal of Clinical Endocrinology and Metabolism,* **86**(6): 2576- 2584.

Leeson, T. S. and Cookson, F. B. (1974). "The mammalian testicular capsule and its muscle elements." *Journal of Morphology,* **144**(2): 237-254.

Lewington, A. (1993). A Review of the Importation of Medicinal Plants and Plant Extracts into Europe. TRAFFIC International, Cambridge, UK. https://portals.iucn.org/library/node/6651 (Accessed 2015 March 23 at 9:00 GMT).

Lohsiriwat, S., Rukkiat, M., Chaikomin, R. and Leelakusolvong, S. (**2010).** Effect of ginger on lower esophageal sphincter pressure. *Journal of Medical Association of Thailand,* **93**(3): 366-72

Ludwig, M., Schroeder-Printzen, I., Lüdecke, G. and Weidner, W. (2000). Comparison of expressed prostatic secretions with urine after prostatic massage a means to diagnose chronic prostatitis/inflammatory chronic pelvic pain syndrome. *Urology*, **55**(2): 175-177.

Mader, S. S. (2004). Understanding human anatomy and physiology 5th edition. McGraw-Hill Higher Education. pp 1100-1103.

Makker, K., A garwal, A. and Sharma, R. (2009). Oxidative stress and male infertility. *Indian Journal of Medical Research*, **129**(4): 357-367.

Mann, T. (1974). Secretory function of the prostate, seminal vesicle and other male accessory organs of reproduction. *Journal of Reproduction and Fertility*, **37**(1): 179-188.

Mansour, M. S., Smeets, A. and Lejeune, M. P. (2012). Ginger consumption enhances the thermic effect of food and promotes feelings of satiety without affecting metabolic and hormonal parameters in overweight men: A pilot study. *Metabolism,* **61**(10): 1347-1352.

Maregesi, S. M., Ngassapa, O. D., Pieters, L. and Vlietinck, A. J. (2007). Ethnopharmacological survey of the Bunda district, Tanzania: Plants used to treat infectious diseases. *Journal of Ethnopharmacology*, **113**(3): 457-470.

Mathur, P. P. and Chattopadhyay, S. (1982). Involvement of lysosomal enzymes in flutamideinduced stimulation of rat testis. *Andrologia,* **14**(2): 171-176.

McKay, D. L. and Blumberg, J. B. (2007). A review of the bioactivity of South African herbal teas: rooibos (Aspalathus linearis) and honeybush (Cyclopia intermedia). *Phytotherapy Research,* **21**(1): 1-16.

McLachlan, R. I., O'Donnell, L., Meachem, S. J., Stanton, P. G., De Kretser, D. M. and Pratis, K. (2002). Identification of specific sites of hormonal regulation in spermatogenesis in rats, monkeys, and man. *Recent Progress in Hormone Research*, **57**: 149-179.

Meirow, D. and Nugent, D. (2001). The effects of radiotherapy and chemotherapy on female reproduction. *Human Reproduction Update*, **7**(6): 535-543.

Meistrich, M. L., Finch, M., Da Cunha, M. F., Hacker, U. and Au, W. W. (1982). Damaging effects of fourteen chemotherapeutic drugs on mouse testis cells. *Cancer Research*, **42**(1):122131.

Memudu, A. E., Akinrinade, I. D., Ogundele, O. M. and Duru, F. (2012). Effects of long and short term treatment of aqueous extract of ginger (*Zingibier Officinale*) on male reproductive function of adult Sprague dawley rats. *Journal of Medicine and Medical Science*, **3**(11): 692-696.

Merlin, H. E. (1972). Azoospermia caused by colchicine a case report. *Fertility and Sterility,* **23**(3): 180-181.

Metzger, D. A. (1998). A physician's perspective. Infertility and involuntary childlessness: Helping couples to cope. New York, Norton. (Accessed 2015 September 13 at 2:00 GMT).

Mi, T., Abbasi, S., Zhang, H., Uray, K., Chunn, J. L., Xia, L. W. and Xia, Y. (2008). Excess adenosine in murine penile erectile tissues contributes to priapism via A 2B adenosine receptor signaling. *The Journal of Clinical Investigation*, **118**(4): 1491-1501.

Mitchell, J. W. and Myers, G. E. (1968). An analytical model of the counter-current heat exchange phenomena. *Biophysical Journal*, **8**(8): 897.

Mohamed, A. B., Al-Rubaee, M. A. and Jalil, A. Q. (2012). Effect of ginger (*Zingiber officinale*) on performance and blood serum parameters of broiler. *International Journal of Poultry Science*, **11**(2): 143-146.

Momeni, R. H., Mehranjani, M. S., Mohammad, A. H. and Mahmoodi, M. (2009). Effects of vitamin E on sperm parameters and reproductive hormones in developing rats treated with paranonylphenol. *Iranian Journal of Reproductive Medicine*, **7** (3):111-116.

Morakinyo, A., Achema, P. and Adegoke, O. (2010). Effect of *Zingiber Officinale* (Ginger) on Sodium Arsenite-Induced Reproductive Toxicity in Male Rats. *Africa Journal of Biomedical Research,* **13**: 39-45.

Murphy, C. (2014). A functional assessment of the sperm membrane: a multi-species approach. (Accessed 2015 August 23 at 14:00 GMT).

Nashwa, A. A., Kawkab, A. A. and Samar, M. M. (2011). The protective effect of Ginger and N-Acetyl Cysteine on Ciprofloxacin-Induced Reproductive Toxicity in Male Rats. *Journal of American Science,* **7**(7): 741-752.

Nassiri, M., Khaki, A., Ahmadi-Ashtiani, H. R. Rezazadeh, S. H., Rastgar, H. and Gharachurlu, S.

(2009). Effects of ginger on spermatogenesis in streptozotocin-induced diabetic Rat. *Journal of Medicinal Plants,* **8**(31):118-124.

Natarajan, K. S., Narasimhan, M., Shanmugasundaram, K. R. and Shanmugasundaram, E. R. B. (2006). Antioxidant activity of a salt-spice-herbal mixture against free radical induction. *Journal of Ethnopharmacology,* **105**: 76-83.

Nelson, L. (1972). Quantitative evaluation of sperm motility control mechanisms. *Biology of Reproduction,* **6**: 319–324.

Neumann, F., Berswordt-Wallrabe, R. V., Elger, W., Steinbeck, H., Hahn, J. D. and Kramer, M. (1970). Aspects of androgen-dependent events as studied by antiandrogens. *Recent Progress in Hormone Research,* **26**: 337-410.

Nishina, M. S., Sato, D. M., Nishijima, W. T. and Mau, R. F. L. (1992). Ginger root production in Hawaii. Honolulu (HI): University of Hawaii. Commodity Facts Sheet. pp 4.

Nistal, M., Paniagua, R., González-Peramato, P. and Reyes-Múgica, M. (2015). Perspectives in Pediatric Pathology, Chapter 4. Pubertal and Adult Testis. *Pediatric and Developmental Pathology*, **18**(3): 187-202.

Nistal, M., Santamaria, L. and Paniagua, R. (1984). Mast cells in the human testis and epididymis from birth to adulthood. *Acta Anatomica (Basel),* **119**(3): 155-160.

Obeten, K. E.,Victoria, N. I., Udo-Affah, G., Ede, P. O. and Obasee, P. P. (2014). Effect of Ethanolic Extract of Ginger on the Micro Anatomy of the Testis of Adult Wistar Rats. *Journal of Biology, Agriculture and Healthcare,* **4**(15): 2224-3208.

O'Brien, C. P. (1997). A range of research-based pharmacotherapies for addiction. *Science*, **278**(5335): 66-70.

Ochsenkühn, R. and De Kretser, D. M. (2003). The contributions of deficient androgen action in spermatogenic disorders. *International Journal of Andrology*, **26**(4): 195-201.

O'Donnell, L., Robertson, K. M., Jones, M. E. and Simpson, E. R. (2001). Estrogen and spermatogenesis. *Endocrine Review,* **22**(3): 289-318.

Onyeagba, R. A., Ugbogu, O. C., Okeke, C. U. and Iroakasi, O. (2004). Studies on the antimicrobial effects of garlic (Allium sativum Linn), ginger (*Zingiber officinale Roscoe*) and lime (*Citrus aurantifolia Linn*). *African Journal of Biotechnology*, **3**(10): 552-554.

Owiredu, W. K. B. A., Amidu, N., Amissah, F. and Woode, E. (2007). The effects of sphenocentrum jollyanum Pierre root extract on sexual behaviourand hormonal levels in rodents. *Journal of Science and Technology,* **27**(2): 9-27.

Oyewo, O. O., Onyije, F. M., Ashamu, E. A., Akintude, O. W. and Akinola, A. E. (2012). Evaluation of Ethanolic Extract of Ginger On The Histology of The Testes And Sperm of Adult Wistar Rats**.** *International Journal of Scientific and Technology Research,* **1**(5): 2277-8616.

Pandey, G. and Jain, G. C. (2013). A review on toxic effects of aluminium exposure on male reproductive system and probable mechanisms of toxicity. *International Journal of Toxicology and Applied Pharmacology*, **3**(3): 48-57.

Park, W. S., Shin, D. Y., Kim, Do. R., Yang, W. M., Chang, M. S. and Park, S. K. (2007). Korean ginseng induces spermatogenesis in rats through the activation of cAMP-responsive element modulator (CREM). *Fertility and Sterility,* **88**: 1000-1002.

Pryor, J. L., Hughes, C., Foster, W., Hales, B. F. and Robaire, B. (2000). Critical windows of exposure for children's health: the reproductive system in animals and humans. *Environmental Health Perspectives*, **108**(3): 491.

Qian, S. Z. and Wang, Z. G. (1984). Gossypol: a potential antifertility agent for males. *Annual* Review of *Pharmacology and Toxicology,* **24**: 329-360.

Qureshi, S., Shah, A., Tariq, M. and Ageel, A. (1989). Studies on herbal aphrodisiacs used in Arab system of medicine. *American Journal of Chinese Medicine,* **17**: 57–63.

Rahmatullah, M., Hasan, A., Parvin, W., Moniruzzaman, M., Khatun, A., Khatun, Z. and Jahan, R. (2012). Medicinal plants and formulations used by the Soren clan of the Santal tribe in Rajshahi district, Bangladesh for treatment of various ailments. *African Journal of Traditional, Complementary and Alternative Medicines*, **9**(3): 350-359.

Rao, P. K. and Burnett, A. L. (2013). Development of the male reproductive system. *In Clinical Urologic Endocrinology,* Springer london, pp 11-24.

Rates, S. M. K. (2001). Plants as source of drugs. *Toxicon*, **39**(5): 603-613.

Razi, M., Hasanzadeh, S., Najafi, G., Feizi, S., Moshtaghi, M., Janbaz, H. and Amin, M. (2010). Histological and anatomical study of the White Rooster: testis, epididymis and ductus deferens. *Iranian Journal of Veterinary Medicine*, **4**(4).

Rey, R. A. and Grinspon, R. P. (2011). Normal male sexual differentiation and aetiology of disorders of sex development. *Best Practice and Research Clinical Endocrinology and Metabolism*, **25**(2): 221-238.

Rheubert, J. L., Sever, D. M., Geheber, A. D. and Siegel, D. S. (2010). Proximal testicular ducts of the Mediterranean Gecko (Hemidactylus turcicus). *The Anatomical Record*, **293**(12): 21762192.

Rhode, J., Fogoros, S., Zick, S., Wahl, M., Griffith, K. A., Huang, J. and Liu, J. R. (2007). Ginger inhibits cell growth ad modulates angiogenic factors in ovarian cancer cells. *Biomedical Centre Complementary and Alternative Medicine,* **7**(44):

1-9.

Rowland, D. L. and Tai, W. (2003). A review of plant-derived and herbal approaches to the treatment of sexual dysfunctions. *Journal of Sex and Marital Therapy*, **29**(3): 185-205.

Runganga, A., Pitts, M. and McMaster, J. (1992). The use of herbal and other agents to enhance sexual experience. *Social Science and Medicine,* **35**(8): 1037-1042.

Saad, B. (2015). Integrating traditional Greco_Arab and Islamic diet and herbal medicines in research and clinical practice. *Phytotherapies: Efficacy, Safety, and Regulation*, 142.

Sadeghi, G. H., Karimi, A., Padidar Jahromi, S. H., Azizi, T. and Daneshmand, A. (2012). Effects of cinnamon, thyme and turmeric infusions on the performance and immune response in of 1-to 21-day-old male broilers. *Revista Brasileira de Ciência Avícola*, **14**(1): 15-20.

Saeid, J. M., Shanoon, A. K. and Mossa, M. M. (2011). Effects of *Zingiber officinale* aqueous extract on semen characteristic & some blood plasma and semen parameters in Broilers breeder male. *International Journal of Poultry Science,* **10**(8): 629-633.

Sajjad, Y. (2010). Development of the genital ducts and external genitalia in the early human embryo. *Journal of Obstetrics and Gynaecology Research*, **36**(5): 929-937.

Sakr, A. S. and Gamal, M. B. (2011). Effect of ginger (*Zingiber officinale R*.) on metiram-inhibited spermatogenesis and induced apoptosis in albino mice. *Journal of Applied Pharmaceutical Sci*ence, **1**: 131-136.

Sathiyaji, K., Sivaraji, A., Vinothkumar, P., Devi, K. and Senthil, K. B. (2010). Spermicidal Activity of *Azadirachta indica* (Neem) Aqueous Leaf Extract on Male Albino Rats. *International Journal of PharmTech Research,* **2**(1): 588-591.

Schmid, R., Schick, T., Steffen, R., Tschopp, A. and Wiik, T. (1994). Comparison of Seven Commonly Used Agents for Prophylaxis of Seasickness. *Journal of Travel Medicine,* **1**(4): 203-206.

Schrade, S. M. (2003). Man and the workplace: assessing his reproductive health. *Chemical Health Safety,* 11-16.

Sekiwa, Y., Kubota, K. and Kobayashi, A. (2000). Isolation of novel glucosides related to gingerdiol from ginger and their antioxidative activities. *Journal of Agricultural and Food Chemistry,* **48**: 373-377.

Senger, P. (2003). Pathways to Pregnancy and Parturition*, 2nd edition.* Moscow: Current Conceptions, Incorporation. pp 272.

Setchell, B. P. and Breed, W. G. (2006). Anatomy, Vasculature, and Innervation of the Male Reproductive Tract*. Knobil and Neill's Physiology of Reproduction. John Neill. San Diego, Elsevier Academic Press,* 771-826.

Setchell, B. P., Maddocks, S. and Brooks, D. E. (1994). The Reproductive System. The Male: Anatomy, vasculature, innervation and fluids of the male reproductive tract. *In: E. Knobil and J.D. Neill (ed) ^ (eds). The Physiology of Reproduction,* **1**: 1063-1175.

Shamsi, S. and Tajuddin, S. H. (2010). Spice and medicine: *Zingiber officinale*. *International Journal of Applied Biology and Pharmaceutical Technology,* **1**(3): 0976-4550.

Sharma, H., Chandola, H. M., Singh, G. and Basisht, G. (2007). Utilization of Ayurveda in health care: an approach for prevention, health promotion, and treatment of disease. Part 1-Ayurveda, the science of life. *The Journal of Alternative and Complementary Medicine*, **13**(9): 1011-1020.

Sharma, P. K. and Agarwal, A. (1996). A Role of reactive oxygen species in male infertility. *Urology,* **48**: 835-850.

Sharpe, R. M. and Skakkebaek, N. E. (1993). Are Oestrogens involved in falling sperm counts and disorders of the male reproductive tract? *The Lancet*, **341**(8857): 1392-1396.

Shokri, S., Hemadi, M. and Aitken, R. J. (2012). Transmission electron microscopy for the quantitative analysis of testis ultra-structure. INTECH Open Access Publisher. (Accessed 2015 June 20 at 12:00 GMT).

Shukla, Y. and Singh, M. (2007). Cancer preventive properties of ginger. A brief review. *Food and Chemical Toxicology,* **45**: 683-690.

Siiteri, P. K. and Wilson, J. D. (1974). Testosterone formation and metabolism during male sexual differentiation in the human embryo. *The Journal of Clinical Endocrinology and Metabolism*, **38**(1): 113-125.

Sikka, S. C. (2001). Relative impact of oxidative stress on male reproductive function. *Current Medicinal Chemistry*, **8**(7): 851-862.

Srivastava, J., Lambert, J. and Vietmeyer, N. (1996). Medicinal plants: an expanding role in development. *World Bank Technical Paper,* **320**: 328.

Steinberger, A. and Steinberger, E. (1971). Replication pattern of Sertoli cells in maturing rat testis in vivo and in organ culture. *Biology of Reproduction,* **4**(1): 84-87.

Sundby, J. (1997). Infertility in the Gambia: traditional and modern health care. *Patient Education and Counseling,* **31**(1): 29-37.

Surh, Y. J., Park, K. K., Chun, K. S., Lee, L. J., Lee, E. and Lee, S. S. (1999). Anti-tumor-promoting activities of selected pungent phenolic substances present in ginger. *Journal* of *Environmental Pathology*, *Toxicology* and *Oncology,* **18**(2):

131–139.

Surveswaran, S., Cai, Y. Z., Corke, H. and Sun, M. (2007). Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants*. Food Chemistry,* **102**: 938–995.

Sutovsky, P., Moreno, R., Ramahlho-Santos, J., Dominko, T. and Thompson, W. (2001). A putative, ubiquitin-dependent mechanism for the recognition and elimination of defective spermatozoa in the mammalian epididymis. *Journal of Cell Science,* 1665-1675.

Takahashi, O. and Oishi, S. (**2001).** Testicular toxicity of dietary 2.2-bis (4hydroxyphenyl) propane (bisphenol A) in F344 rat. *Archives of Toxicology,* **75**:

42-51.

Tekeli, A., Kutlu, H. R. and Celik, L. (2011). Effects of Z. officinale and propolis extracts on the performance, carcass and some blood parameters of broiler chicks. *Current Research in Poultry Science*, **1**(1): 12-23.

Telisman, S., Cvitkovic, P., Jurasovic, J., Pizent, A., Gavella, M. and Rocic, B. (2000). Semen quality and reproductive endocrine function in relation to biomarkers of lead, cadmium, zinc and copper in men. *Environmental Health Perspective,* **108**: 45-53.

Ternes, T. A. (1998). Occurrence of drugs in German sewage treatment plants and rivers. *Water Research*, **32**(11): 3245-3260.

Tharakan, B. and Manyam, B. V. (2005). Botanical Therapies in Sexual Dysfunction. *Phytotherapy Research,* **19**: 457-463.

Tortora, G. J. and Grabowski, S. R. (1996). Principles of anatomy and physiology. (8th Edition). New York, Harper Collins.

Van der Putte, S. C. J. (2005). Development of the Male Perineum. The Development of the Perineum in the Human*: A Comprehensive Histological Study with a Special Reference to the Role of the Stromal Components*, 81-118.

Vicker, J. (2016). Dietary pattern and metabolic syndrome among urban slum women, Accra Ghana (Doctoral dissertation, University of Health and Allied Sciences),*http://lib.ugent.be/fulltxt/RUG01/002/275/167/RUG01002275167_2016_0001_AC.pdf* (Accessed 2016 June 10 at 12:00 GMT).

Vickers, A. J. (2007). Which botanicals or other unconventional anticancer agents should we take to clinical trial. *Journal of the Society for Integrative Oncology*, **5**(3): 125.

Waleed, A. A. M. and Wisam, S. N. (2012). The effect of Ginger on semen parameters and serum FSH, LH and testosterone of infertile men. *Tikrit Medical Journal,* **18**(2): 322-329.

Wang, F., Zhang, Q., Cao, J., Huang, Q. and Zhu, X. (2008). The microtubule plus end-binding protein EB1 is involved in Sertoli cell plasticity in testicular seminiferous tubules. *Experimental Cell Research*, **314**(1): 213-226.

Wang, L. (1994). Marriage and family therapy with people from China. *Contemporary Family Therapy*, **16**(1): 25-37.

Weibel, R. S. (1979). Stereological Methods, Practical Methods for Biological Morphometry. Volume 1. London Academic Press. pp 1-40.

Wensing, C. J. G. (1988). The embryology of testicular descent. *Hormone Research in Paediatrics*, **30**(4-5): 144-152.

Wilkinson, J. M. (2000). Effect of ginger tea on the fetal development of Sprague-Dawley rats. *Reproductive Toxicology,* **14**(6): 507–512.

William, M. A. (1977). Quantitative methods in biology. In: Practical Methods in Electron Microscopy. Glauert, A. M. (Ed.). Amsterdam, North-Holland.

Wing, T. Y. and Christensen, A. K. (1982). Morphometric studies on rat seminiferous tubules. *American Journal of Anatomy,* **165**(1): 13-25.

Winters, S. J., Banks, J. L and Loriaux, D. L. (1979). Cimetidine is an antiandrogen in the rat. *Gastroenterology,* **76**(3): 504-508.

Wong, A., Chuan, S. S., Patton, C. W., Jacobson, D. J., Corselli, J. and Chan, J. P. (2008). Addition of eosin to the aniline blue assay to enhance detection of immature sperm histones. *Fertility and Sterility*, **90** (5): 1999-2002.

Woode, E., Alhassan, A. and Abaidoo, C. S. (2012). Effect of ethanolic fruit extract of Xylopia aethiopica on reproductive function of male rats. *International Journal of Pharmaceutical and Biomedical Research,* **2**(3): 161-165.

World Health Organization (WHO) (1999). Laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 4th Ed. Cambridge University press. (Accessed 2015 July 17 at 12:00 GMT).

Yamamoto, Y., Isoyama, E., Sofikitis, N. and Miyagawa, I. (1998). Effects of smoking on testicular function and fertilizing potential in rats. *Urological Research,* **26**(1): 45-48.

Yang, H. S., Han, D. K., Kim, J. R. and Sim, J. C. (2006). Effect of alpha- tocopherol on cadmium induced - toxicity in rat testis and carcinogenesis. *Journal of Korean Medical Science,* **21**: 445451.

Zancan, K. C., Marques, M., O., Petenate, A. J. and Meireles, M. A. (2002). Extraction of ginger (*Zingiber officinale Roscoe*) oleoresin with CO_2 and co-solvents: A study of the antioxidant action of the extracts. *Journal Supercritical Fluids*, **24**(1): 57-76.

Zancan, K., Marques, M., Petenate, A. and Meireles, M. (2000). Extraction of ginger *(Zingiber officinale*) oleoresin with CO_2 and co-solvent: a study of the antioxidant action of the extracts. *Journal of Supercritical Fluids,* **24**: 57–76.

Zhang, C., Yeh, S., Chen, Y. T., Wu, C. C., Chuang, K. H., Lin, H. Y. and Lardy, H. (2006). Oligozoospermia with normal fertility in male mice lacking the androgen receptor in testis peritubular myoid cells. *Proceedings of the National Academy of Sciences*, **103**(47): 17718-17723.

Zhou, H. L., Deny, Y. M. and Xie, Q. M. (2006). The modulatory effects of the volatile oil of ginger on the cellular immune response in vitro and in vivo in mice. *Journal of Ethnopharmacology,* **105**: 301-305.

Zini, A., De Lamirande, E. and Gagnon, C. (1993). Reactive oxygen species in semen of infertile patients: levels of superoxide dismutase- and catalase-like activities in seminal plasma and spermatozoa. *International Journal of Andrology,* **16**: 183-188.