UNIVERSITY FOR DEVELOPMENT STUDIES

VARICOCELE AND HYPOGONADISM AMONG ADULTS: A PROSPECTIVE STUDY AMONG INFERTILE MEN IN TAMALE, GHANA

BY

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A THESIS SUBMITTED TO THE DEPARTMENT OF BIOMEDICAL LABORATORY SCIENCE, SCHOOL OF ALLIED HEALTH SCIENCES, UNIVERSITY FOR DEVELOPMENT STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY DEGREE IN CHEMICAL PATHOLOGY

JULY, 2022

DECLARATION

Student

I hereby declare that this thesis is the result of my original work and that no part of it has been presented for another degree in the University or elsewhere:

Candidate

Signature  Date: 10/10/2022

Name: **Adams Yussif**

Supervisors

I hereby declare that the preparation and presentation of the thesis was supervised following the guidelines on supervision of thesis laid down by the University for Development Studies.

Principal Supervisor

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ABSTRACT

Varicocele is a disorder of venous return caused by abnormal enlargement of pampiniform plexus draining the testicles. This condition is common among men seeking medical attention for fertility problems, sexual dysfunction or complains of continuous scrotal discomfort. Varicocele repair has been recommended for varicose patients with such complaints, however, the effect of microsurgical sub-inguinal varicocelectomy on semen parameters, gonadotropins or factors to predict which of the patients are most likely to benefit from the surgery has not been determined in Ghana, hence the aim of this study. This was an interventional study conducted at the Urology Unit of the Tamale Teaching Hospital, Tamale, Ghana between September 2017 to August 2021. Participants included in the study were randomised into surgery group and observed group and were aged between 36 – 69 years old. Duplicate semen samples (mean values adopted) were collected at the onset, after 6-,9- and 12 months, and assessed following the criteria as established by World Health Organization (WHO). Blood samples were collected and serum total testosterone, follicle-stimulating hormones (FSH), luteinizing hormone (LH) were assayed at onset (baseline), 12-,24-, 36-, and 48-months intervals (4 years follow-up). Testicular hemodynamics (peak systolic velocity, end-diastolic velocity and resistive index) were measured using color duplex Doppler ultrasonography (CDUS) at each interval and recorded. Varicocelectomy was performed for the surgery group and no intervention was given to the observed group after baseline measurements. The data was computed using GraphPad Prism (v8.0) at an alpha of 0.05. ***Paper I*** *involved the effect of the varicocelectomy on semen parameters after 180 days. It was found that long-standing varicocele may affect semen parameters and this may be seen by causing a further decrease in semen volume, total sperm count, concentration of spermatozoa, motility, or normal sperm morphology.* ***Paper II*** *involved the*

*effect of varicocelectomy on gonadotropins after 48 months follow-up. The study found that varicocele may cause Leydig cell damage and this may be seen by causing a further decrease in total testosterone and a concomitant rise in follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Again, it was observed that serum total testosterone, FSH, and LH observed spike changes within the first- and second-year in the surgery group but changes were marginal from the third year onwards (**Paper II**). **Paper III** found that the significant predictive factors associated with improved semen characteristics following microsurgical sub-inguinal varicocelectomy in infertile men were; pre-operative low serum FSH, high testosterone, and low left capsular resistive index (L_RIcap). In varicocele patients, blood supply to the testicular tissues is significantly reduced evidenced by the increase in a resistive index (RI) in capsular arteries of the observed group and this appears to improve after surgery as observed by a reduced RI_cap in the surgery group (**Paper IV**). Given the predictive factors associated with improved semen parameters in patients with varicocele, it is worth recognizing that improvement on the semen quality post varicocele repair does not guarantee patients to father children. Further studies on a larger population of varicocele patients with pregnancy rate as the primary outcome will help to conclusively determine the effectiveness of microsurgical sub-inguinal varicocelectomy. Also, varicocelectomy plus supraphysiologic dosages of human chorionic gonadotropin (hCG) therapy have found significant superiority in infertile men with varicocele and could be a topic of further studies.*

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DEDICATION

To my wife

Fouzia

and our children

Hekimat (in loving memory)

Hebatuallah

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LIST OF PAPERS

The thesis was compiled from works that have already been published, under consideration for publication, or yet to be submitted to a journal.

Paper I Effect of varicocelectomy on semen parameters of men seeking infertility treatment in Tamale, Ghana (Open Journal of Urology (2022), DOI: https://doi.org/10.4236/oju.2022.121002)

Paper II Effect of varicocelectomy on gonadal function among patients reporting with sexual dysfunction in Ghana (Open Journal of Urology (2022), DOI: https://doi.org/10.4236/oju.2022.126031)

Paper III Factors associated with improved semen characteristics following microsurgical sub-inguinal varicocelectomy in infertile patients (Asian Journal of Research and Reports in Urology (2022), 5(1), 17-26. https://www.journalajrru.com/index.php/AJRRU/article/view/30158

Paper IV Changes in testicular arterial hemodynamic, gonadotropin levels, and semen parameters among varicocele patients randomized to varicocelectomy or observed in Tamale, Ghana (SAGE Urologia Journal, (2022), 1-9. DOI: https://doi.org/10.1177/03915603221127116

There have been modifications made to the papers that have been published for purposes of the thesis formatting guidelines. However, the changes did not affect the content and the interpretation of the results.

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Chapter 1

INTRODUCTION

1.1 GENERAL INTRODUCTION

This chapter is divided into two sections for easy reading and comprehension. The first section outlines the background to the study while the second section gives overview of the thesis chapters as per the graduate handbook for University for Development Studies.

1.2 BACKGROUND

Varicocele is the enlargement of pampiniform venous plexus draining the testicle, with reflux of venous blood (Clavijo *et al.*, 2017; Bertolotto *et al.*, 2020). It is a common problem in men who seek medical attention for fertility problems, sexual dysfunction, or complain of continuing scrotal discomfort (Paick and Choi, 2019).

Varicocele has been identified in 15% of healthy men (Alsaikhan *et al.*, 2016) but the prevalence ranges from 35% to 45% among men seeking medical attention for primary infertility and 75% to 81% among patients seeking care for secondary infertility (Gorelick and Goldstein, 1993; Jarow *et al.*, 1996; Agarwal *et al.*, 2016; Çayan *et al.*, 2020).

Due to the nature of varicocele, which can affect the semen parameters, it is believed that it can cause infertility (Agarwal *et al.*, 2016). However, the exact cause of this condition is not known. Various factors such as the quality of the sperm and the anatomical anomaly of varicocele can affect the development of spermatogenesis (Xue *et al.*, 2012; Kadioglu *et al.*, 2014), increased scrotal temperature (Shiraishi *et al.*, 2012), adrenal hormone and gonadotoxic metabolite refluxes (Inci and Gunay, 2013), epigenetics changes (Seidel, 2015), and increased production of reactive oxygen species (ROS) in the scrotum which results in sperm DNA damage (Agarwal *et al.*, 2014).

Again, studies involving humans have reported that varicocele causes progressive time-dependent testicular damage (Russell, 1957; Lipshultz and Corriere Jr, 1977; Sakamoto and Ogawa, 2009). The function of the testicular tissue varies depending on the duration of varicocele. During the early stages of development, it is usually normal. However, it drops significantly after the age of 18 (Agarwal *et al.*, 2016). One of the most common theories about the effects of varicocele on the testicular function is the cooling effect of the blood stream through the meshwork of spermatic veins (Lü and Chen, 2008). However, persons confirmed with varicocele lack this mechanism, hence, causing elevated scrotal temperature.

Varicocele is a disorder in which the pampiniform plexus draining the testicle is abnormally dilated, with reflux of venous blood (Clavijo *et al.*, 2017; Bertolotto *et al.*, 2020). The consequence of this venous abnormality is often arrest of ipsilateral testicular growth, thus arterial 'insufficiency' and hypoperfusion of testicular tissues. The demand and supply of blood during spermatogenesis is of significant importance. Blood supply to the testes is derived from testicular arteries which arise from the aorta, although, other sources include; the cremasteric artery which supplies the peri-testicular tissues, and the deferential artery which supplies the epididymis and vas deferens (Al-Naffakh, 2012; Kang *et al.*, 2021). Adequate blood supply to the testes is needed for spermatogenesis and decreased blood supply may cause ischemia.

In most studies, information about blood flow within the testicles are obtained using Doppler indices with a recently introduced ultrasonic parameter, resistive index (RI), which shows testicular parenchymal perfusion and microcirculation function (Zolfaghar-Khani *et al.*, 2020). This index is calculated using the S-D/S formula, where 'S' stands for peak systolic velocity (PSV), and 'D' represents end-diastolic velocity (EDV). In the testicles, high values of RI (> 0.6) imply disruption in microcirculation and thus hypoxia in the testicles (Al-

Naffakh, 2012; Halpern *et al.,* 2016). Afoko *et al.* (2010) reported a significant reduction in arterial perfusion of testicular tissues evidenced by the increase in the resistive index (RI) in an observed group compared with improved testicular perfusion evidenced of decreased RI in the surgery group among adolescents with left-sided varicocele. Increased RI in the testes is associated with disruptions in microcirculation as a result of a significant reduction in testicular blood flow (Al-Naffakh, 2012; Gloria *et al.*, 2018; Zolfaghar-Khani *et al.*, 2020).

Varicocelectomy is widely used for the treatment of patients with male fertility factors reporting varicocele. The ultimate aim of this surgical procedure is to improve couples' chances of achieving a pregnancy and live birth. Several ligation methods are used but the microsurgical repair approach has become popular among other varicocele treatment methods because it is associated with improved Leydig cell function (Çayan *et al.*, 2020; Kang *et al.*, 2021; Adams *et al.*, 2022) and reduced complications and/ or recurrence of varicocele (Cayan *et al.*, 1999). The two approaches are; microsurgical sub-inguinal and inguinal varicocelectomy, but vascular anatomy gets more complicated as we get through the sub-inguinal region to the inguinal region, thus leaving the microsurgical sub-inguinal (lymphatic- and artery sparing) varicocelectomy the most appropriate choice (Tarhan *et al.*, 2011).

There are conflicting reports on the effect of varicocele repair on male fertility. Some studies have attempted to clarify the efficacy of surgical remediations on sperm density, concentration, motility, morphology, and gonadotropins. Zini *et al.* (2005) reported that infertile men presented improved spermiogram six months after microsurgical varicocele repair. Similar findings were observed by Kadioglu *et al.* (2014) who concluded that all seminal parameters significantly improved post-surgery when compared with preoperative values. Counterrally, Krause *et al.* (2002) in a randomized study on varicocele treatment in

infertile men, found no significant increase in pregnancy rate in the treated group compared with controls. Again, Breznik *et al.* (1993) and Rageth *et al.* (1992) showed that varicocelectomy bears no influence on male fertility.

Furthermore, Leydig cells function to produce testosterone but this is controlled by luteinizing hormone. FSH functions to promote the beginning of testosterone production; in the process, LH is maintained. Hence, there is crosstalk with the changes of serum testosterone, FSH, and LH.

1.3 PROBLEM STATEMENT/JUSTIFICATION

In most men presenting with varicocele, varicocelectomy results in improved semen parameters but not all published data agrees with this (Redmon *et al.*, 2002; Baazeem *et al.*, 2011). To determine whether or not infertility-related treatment following varicocele repair is achieved, the endpoints commonly analyzed are semen parameters (that is; semen volume, sperm count, sperm concentration, motility, and/ or morphology), pregnancy rate (PR), and/ or integrity of sperm DNA. But most studies consider semen parameters to be the primary outcome parameter of varicocele therapy (Inci and Gunay, 2013). Based on current evidence, the guidelines and the protocol by the American Society for Reproductive Medicine (ASRM), the American Urological Association (AUA), and the European Association of Urology (EAU) recommend varicocele repair for patients with palpable varicocele with one or more semen parameter abnormalities' whether or not they are attempting to conceive a child (Shridharani *et al.*, 2012; Kang *et al.*, 2021). However, factors to predict which of the patients are likely to benefit from the varicocele repair in terms of improved semen quality and characteristics have proven to be very difficult.

Also, long-standing varicocele might worsen Leydig cell functions; a significant risk factor for hypogonadism. Lotti *et al.* (2009) in a study found that patients with severe varicocele

had increased serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) with lowered testicular volume. Increased serum FSH and LH levels in infertile men diagnosed with varicocele have resulted in the hypothesis that varicocele causes Leydig cell dysfunction (Tian *et al.*, 2018).

Clinical studies suggest that repair of the varicocele may improve gonadal function in men with varicocele (Li *et al.*, 2012; Tiseo *et al.*, 2016; Çayan *et al.*, 2020). Çayan *et al.* (2020) reported that approximately 60%-80% of men with low serum testosterone had normalized testosterone levels after varicocele repair. Li *et al.* (2012) in a meta-analysis found that post varicocelectomy, the mean serum testosterone concentration increased. Nonetheless, there are conflicting reports on whether varicocele and varicocelectomy result in changes in serum FSH and LH levels or not. Some studies reported no significant changes in the levels of serum FSH and LH (Su *et al.*, 1995; Salem and Mostafa, 2009), yet others noted decreased serum FSH and LH levels following varicocelectomy (Sathya Srini and Belur Veerachari, 2011; Tian *et al.*, 2018). Varicocelectomy is commonly done for patients reporting with varicocele however, there is scarcity of data on its impact/effect on semen characteristics, male fertility hormones and hemodynamic parameters in our locality, hence, the need for the study.

1.4 AIMS AND OBJECTIVES

1.4.1 Aim

The study aims to determine the effect of microsurgical sub-inguinal varicocelectomy on fertility, gonadotropins, and hemodynamic parameters among adults with fertility problems, Ghana

1.4.2 Specific objectives

The specific objectives are as follows; to determine

- the effect of varicocelectomy on semen parameters of men seeking infertility treatment in Tamale, Ghana
- the effect of varicocelectomy on gonadal function among patients reporting with sexual dysfunction in Ghana
- factors associated with improved semen characteristics following microsurgical sub-inguinal varicocelectomy in infertile patients
- the changes in testicular arterial haemodynamic, gonadotropin levels and semen parameters among varicocele patients randomized to varicocelectomy or observed in Tamale, Ghana

1.5 GENERAL METHODS

1.5.1 Ethics and human subjects' issues

The Institutional Ethics and Review Board of University for Development Studies (Number: UDS/RB/004/22) and the Ethics and Review Board of the Department of Research and Development of Tamale Teaching Hospital (TTH) (Number: TTH/R&D/SR/119) approved this study. Thus, the study therefore has been performed following the standards laid down protocol in the 1964 Declaration of Helsinki.

1.5.2 Consent form

Informed consent was obtained from all the participants before the study. Participation in this study was voluntary, participants were kept anonymous, and information obtained remained confidential to the researchers only. Only blood samples intended for the study were drawn

and information that was deemed as important to the management of the patient was communicated to the patient.

1.5.3 Study design

This was an intervention study designed to evaluate the effect of a specific therapy in a well-defined population. Intervention studies can be categorized into two; therapeutic studies and preventive studies and in this case, therapeutic study design was adopted. Therapeutic studies test the effect of therapies such as surgery, novel drugs or vaccine, in population of already known disease condition. In this study, all participants had varicocele and were randomized into two groups; the surgery group (n = 127) and the observed group (n = 114). The study was to test the effect of microsurgical sub-inguinal varicocelectomy on fertility, gonadotropins and testicular hemodynamics of patients presenting with varicocele.

1.5.4 Study and participants recruitment

The study was conducted at the Urology Unit of Tamale Teaching Hospital (TTH), Tamale Metropolis, Ghana from September 2017 to August 2021. All consented participants were sexually active men, aged between 30 – 67 years.

1.5.5 Inclusion criteria

Participants eligible for inclusion were offered the option of immediately undergoing microsurgical sub-inguinal varicocelectomy (surgery group) or being observed for one year with a subsequent re-evaluation of the management plan and possibly delayed the operation (observed group). Based on the willingness to equally accept either option, eligible participants were assigned at a balanced one-to-one ratio to either the observed group or the surgery group.

To qualify for inclusion, participants had to have male factor fertility, alterations of spermiogram, and/or had varicocele but had fathered at least one child. Male factor fertility

was defined by a man's inability to make a normal female partner or spouse pregnant after one year of unprotected sexual intercourse (i.e., female with normal ovulation, normal reproductive history, and tubal patency) (Nallella *et al.*, 2006).

Again, participants who had complained of any form of sexual dysfunction including; weak sex drive, impotency, premature ejaculation, infrequency of having sexual intercourse, avoidance of sexual intercourse, non-communication about sex with a partner, and dissatisfaction after sexual intercourse (Rust and Golombok, 1985) were also included.

All consented participants were sexually active men who for at least 2 years, had maintained an unchanging heterosexual relationship were allowed to participate in the study. A stable heterosexual relationship was considered as one in which the man was involved and maintained sexual relations, regardless of marital status.

Selection strategies were adopted depending on the Unit at the recruitment centre of Tamale Teaching Hospital. After a patient's arrival at the Urology Specialist Clinic, pre-assessment checks were made and those who do not qualify for the study were made to continue with their routine medical examinations. If a patient met the eligibility criteria, informed consent was obtained and a questionnaire administered to be completed independently. Where participants were not able to read and write, the queries on the questionnaire were translated verbatim in the common dialect. Participants were made to see specialist for medical examination.

1.5.6 Exclusion criteria

Participants who had no sexual dysfunction complaints, excessive alcohol abuse (chronic alcoholics i.e., men who takes more than 4 a day, or 14 or 15 in a week), cigarette smoking history (as smoking is an independent risk factor for infertility), incomplete/inconclusive questionnaires were excluded. Participants with a history of uncontrolled diabetes (glycated

hemoglobin >7%), uncontrolled hypertension (bp ≥ 140/90 mmHg), undescended testis, mumps orchitis, use of anti-estrogen and/or testosterone replacement therapy, or orchidectomy as well as patients on long term statins were all excluded.

1.5.7 Clinical examination

Each consenting participant was clinically examined by a urologist. Dubin and Amelar (1970) approach was employed to detect, confirm and clinically grade varicocele. Varicocele was categorized into three (3); grade I, II and III. Grade I was confirmed if participant had a dilated spermatic venous plexus obvious by palpation through Valsalva manoeuvre. Abnormal dilation of the spermatic venous plexus evident by palpation at upright position was considered Grade II while enlargement of the venous plexus evident visually was confirmed Grade III. Scrotal ultrasound was used to diagnosed the non-palpable enlargement of the venous plexus of the spermatic tone (Marsman and Schats, 1994).

1.5.8 Scrotal Ultrasound Evaluation

Participants were examined using two phases of scrotal duplex Doppler ultrasound scan; the first phase was with participants in the supine position with penis resting on suprapubic region and the second, in an upright position. A color Doppler ultrasonography (CDUS) of the testes (Samsung Medison Accuvix V20 scan, Samsung Electronics, South Korea) with measurement of PSV (peak systolic velocity), EDV (end-diastolic velocity), and RI (resistive index) for capsular arteries were calculated and recorded for both testes and to evaluate blood reflux along the pampiniform plexus, the extent of any fluid collections or testicular malposition. This CDUS has 83% to 95% sensitivity and 94% specificity for identifying subclinical varicocele compared with the physical examination which has only 70% specificity (Trum *et al.*, 1996; Rehman *et al.*, 2019). Images obtained from the region of

interest were delineated over both the left and right hemiscrota, femoral artery, and femoral muscles.

1.5.9 Data collection

1.5.9.1 Pre-testing questionnaire

A pilot-test of the questionnaires were carried out among 20 apparently healthy adult males working at University for Development Studies for internal consistency and understanding of the questions. Participants were asked to read the questions, respond and explain what they believed the questions were asking. This process was to ensure clarity and eliminate ambiguity in answering questions. Expert review and participants feedback from the pilot-test were utilized to edit the questionnaires accordingly. Pre-testing of questionnaires were done to ensure that, the questionnaires were easy to understand, has face validity and serve its intended purpose.

1.5.9.2 Questionnaire administration

Sociodemographic data, cigarette smoking, and medical history were gathered with a structured pre-tested questionnaire. Questions on sexual response were assessed using the Golombok Rust Inventory of Sexual Satisfaction (GRISS) questionnaire which measures specific sexual behaviours, attitudes, and beliefs (Rust and Golombok, 1985). These standardized questionnaire was chosen because it easy to administer and score, substantially inexpensive, and relatively unobtrusive (Rust and Golombok, 1985).

1.5.9.3 Anthropometric measurement

Anthropometric measurements were done on all study participants. The Seca 213 portable Stadiometer (Seca Corp., Hamburg, Germany) were used to measure the height of the participants to the nearest 0.1 cm. To measure the height, the stadiometer was set up

according to the manufacturer's instructions. Eligible participants were asked take off footwears including socks and measurement was taken.

The measurement of weight, calculation of BMI and the assessment of body fat composition was done using the Omron HBF-516B Body Composition Analyzer and Electronic Scale (Omron Corp., USA). Body fat and muscle mass was calculated as a percentage of the total body weight at intervals of 0.1% (Quaye *et al.*, 2019).

1.5.9.4 Blood pressure measurements

The Omron blood pressure monitor (www.omron.com) was used to measure the blood pressure of the participant. These included; systolic blood pressure (SBP), diastolic blood pressure (DBP) and pulse rate, and categorization of normotension (SBP<140mmHg/DBP<90mmHg) and hypertension (SBP>140mmHg/DBP>90mmHg) was based on WHO cut-offs as cited by Mittal and Singh (2010).

1.5.9.5 Blood samples collection

Venous blood samples (4mls) were collected from each participant within the hours of 8:00 am – 11:00 am after at least 8 - 12 hours of fast by a phlebotomist using standard venepuncture methods. A check-list was given to each consenting participant to tick the number of hours fasted to enable us rescheduled those who could not meet the time.

Venous blood samples collected were dispensed into 5 millilitre vacutainer containing gel separator. Blood samples was centrifuged at 8000rpm for 5minutes to yield serum and cells. The serum was aliquoted and stored at -20^{o}C until assay.

1.5.9.6 Hormonal measurement

Serum FSH (follicle-stimulating hormone) and LH (luteinizing hormone) were measured by electrochemiluminescence with a Hitachi-Roche analyzer (Cobas 6000, Roche Diagnostics, IN, USA). Serum total testosterone was analysed by radioimmunoassay.

1.5.10 Semen analysis

1.5.10.1 Semen sample collection

As part of the protocol for semen analysis as stated by WHO, (2010), a clear written and spoken instruction on semen sample collection was given to each consenting participant. Participants were made to abstain from all forms of sexual intercourse for a minimum of 2 days and a maximum of 5 days.

A clean sterile wide-mouthed plastic container confirmed to be non-toxic for spermatozoa was given to each participant to produced semen samples by masturbation (two semen samples – mean value adopted) after participants made to refrain from sexual activity for 2 to 5 days. In order to minimize temperature fluctuations and control the time between semen sample collection and analysis, semen specimens were collected in a private room near the laboratory. All semen analyses were carried out following the guidelines by WHO, 2010 (Organization, 2010).

1.5.10.2 Macroscopic examination

Specimen containers containing semen samples were inspected for labels, identification numbers, data and time of sample collection, and whether the content of semen sample were in the container or not.

1.5.10.3 Liquefaction

Specimen containers containing semen samples were placed on a two-dimensional shaker and kept in incubator (37 ^{o}C) for 30 minutes for it to liquefy. The time for sample liquefaction were extended to 60 minutes for samples that delayed in liquefaction within the 30 minutes for complete process before semen analysis.

1.5.10.4 Viscosity of semen

Viscosity of semen were assessed by introducing a clean-sterile rod into the sample and observing the length of thread that was formed upon withdrawal of the rod. Abnormal viscosity was reported when thread exceeded 2 cm.

1.5.10.5 Appearance of semen ejaculate

The appearance of semen ejaculate was assessed and recorded. A homogeneous grey-opalescent semen after liquefaction was considered normal and any other sperm colour (eg: haemospermia or yellow sperms) was considered abnormal (WHO, 2010).

1.5.10.6 Semen volume

Each empty specimen container was pre-weighed by Electronic Precision Balance Digital Weighing Scale to nearest 0.01grams (Shanghai Puchun Measure Instrument Co., Ltd. Shanghai, China), and weight labelled by a permanent marker pen on the specimen container before given to each consenting participant. Specimen containers containing semen brought to the laboratory were weighed and the weight of the specimen container subtracted from the initial weight (WHO, 2010).

1.5.10.7 Semen pH

A sterile wide-bore (approximately 1.5mm diameter) disposable plastic pipette was used to aspirate and return samples of approximately 8 to 10 times to mix samples uniformly in the specimen container. This was done slowly and not vigorously to ensure air bubbles were not being created. A drop of semen was evenly spread onto the pH test strip (QwikCheck^{TM} Test Strip) and results reported within 30 to 45 seconds by comparing the colour with the calibration strip. The pH test strip reports a range of 5.0 to 8.5 and the accuracy were checked by running control semen samples from manufacturers (QwikCheck^{TM} Test Strip).

1.5.10.8 Wet preparation

Semen samples were thoroughly mixed before a standard volume of 10uL aliquots onto a clean glass slide and covered with 22 mm x 22 mm coverslip. Thus, 10µL (i.e., volume of 10.0 x 10^3 µmL) of semen delivered onto a glass slide and covered with 22 mm x 22 mm coverslip (i.e., area of 484mm^2) provides a chamber depth of 20.66 µm. According to studies by Lannou *et al.* (1992) a chamber depth of <20µm constrains spermatozoa rotational movement.

1.5.10.9 Microscopic examination of wet preparation

Freshly made wet prepared microscopic slides were examined using Olympus BX43F contrast microscope by two specialized trained microscopists and each reported independently on the same sample. Samples were initially scanned with a total magnification of x100 (i.e., x10 objective lens with x10 ocular lens combination) for mucus strand formation, sperm agglutination or aggregation and the presence of cells other than spermatozoa such as 'round cells', epithelial cells, sperm heads or tail, etc.

Samples were further examined at x200 magnification (i.e., x20 objective lens with x10 ocular lens combination) for sperm mortality and accurate counting of number spermatozoa. Reporting of wet preparation was based on the protocol of semen analysis by WHO, (2010).

1.5.10.10 Counting the number of spermatozoa in semen

A 100µm-deep disposable Neubauer haemocytometer chamber was loaded with a well-mixed liquefied semen sample, covered with a coverslip allowing spermatozoa to settle in the chamber. Samples were examined at x200 magnification (i.e., x20 objective lens with x10 ocular lens combination) and only spermatozoa with head and tail were counted and reported.

1.5.10.11 Assessment of semen morphology

Sperm morphology were determined according to Kruger criteria using *Nigrosin 8%* staining technique (Nigrosin, Water Soluble, Darmstadt, Germany) (Elder and Dale, 2020).

1.5.11 Interventions (Microsurgical sub-inguinal varicocelectomy)

Participants were counselled about their condition, and the exact nature of the problem was explained to them by a urologist. A microsurgical open sub-inguinal varicocelectomy procedure as described by Marmar *et al.* (1985) was performed for the surgery group. Surgery was performed under spinal anaesthesia, using microsurgical instruments and magnification with an operating microscope KARL CAPS SOM 82, Germany. The lymphatic vessels and testicular artery were spared, and both internal and external spermatic veins ligated and divided. The spermatic fasciae were closed using PGA 3/0 running sutures. The wound was closed in layers and a subcuticular skin stitch was applied using 4/0 PGA sutures. Wound dressing was removed after 24 hours. No antibiotics were employed and the pain was managed by using 1-gram of rectal paracetamol during the period of recovery and followed by oral paracetamol 1-gram tid for the next 24 hours.

1.5.12 Follow-up

Follow-ups depended on the objective of each paper. In paper I, both groups were followed for 180 days after the day of surgery (surgery group) or the day of the last baseline semen analysis (observed group). Participants in the observed group were advised not to use any form of contraceptives during sexual intercourse, and to abstain from tobacco/cigarette smoking. Participants in the surgery group were advised to abstain from any form of sexual activity until the surgical wound was properly healed. All participants were reassessed every 90 days to confirm that; the participant was not smoking, and was clinically examined to confirm the absence of genital infection, recurrence of varicocele, formation of hydrocele,

and increased testicular size. Duplicate semen samples were collected (mean values adopted) for repeated analysis at 180 days of follow-up.

In paper II, both groups were followed for 48 months (4 years) after the day of surgery (surgery group) or the day of the last baseline hormone analysis (observed group). Participants in the observed group were advised not to use any form of contraceptives during sexual intercourse, and to abstain from tobacco/cigarette smoking. Participants in the operated group were advised to abstain from any form of sexual activity until the surgical wound was properly healed. All participants were reassessed every 90 days to confirm that; the participant was not smoking, and was clinically examined to confirm the absence of genital infection, formation of hydrocele, recurrence of varicocele, and increased testicular size. Participants were asked to revisit the clinic after 6 months and 12 months. Blood samples were drawn for repeated measurement of serum total testosterone, FSH, and serum LH at follow-up months 12, 24, 36, and 48 respectively.

In paper III, the pre-operative semen specimens were collected at least 2 weeks before the surgery while the post-surgery specimens were collected at 9 months and 12 months intervals respectively.

Lastly, paper IV involved three groups: Surgery group, varicocele patients who had microsurgical sub-inguinal varicocelectomy (n = 127); Observed group, varicocele patients whose spermatic veins were preserved (n = 114); and healthy controls (n = 33). All three (3) groups were followed for 12 months after the day of the last baseline semen analysis. Participants in the surgery group were reassessed every 90 days to confirm the absence of genital infection, formation of hydrocele, recurrence of varicocele, and change in testicular size. Hormonal measurements (FSH, LH, and Total testosterone), seminal fluid analysis, and

testicular hemodynamic indices (PSV, EDV, and RI) were repeated at 12 months of the follow-up.

1.5.13 Data analysis strategies

Data were entered into Microsoft Excel version 10 (www.microsoft.com) and exported to GraphPad Prism version 8.0 (www.graphpad.com) and statistical package for social sciences (SPSS) version 23 (www.ibm.com) for analysis. Categorical data were presented as frequency, percent, and charts, and parametric data presented as mean ± standard deviation (SD) or mean ± standard error of the mean (SEM). Kolmogorov-Smirnov test were performed on parametric data to check whether or not the data were normally distributed. To compare two groups, the Chi-square test was used for categorical variables, and unpaired student t-test was used for parametric data. Variables before and after the operation in each patient was compared using paired *t*-test. Group means were compared using one-way ANOVA followed by Newman-Keul's test as post hoc. A two-tailed p-value less than 0.05 was considered statistically significant.

1.6 OVERVIEW OF THE THESIS CHAPTERS

This PhD thesis by publication is arranged in a comprehensive and logical order to enable easy reading and understanding. The chapters in this thesis are mostly manuscripts that have either been published or are under consideration for publication in reputable journals.

The Chapter 1 in this study is divided into two sections; the background of the study and the overview of the thesis chapters per the graduate handbook by University for Development Studies. The overview of the thesis chapters describes the general format and arrangement of the chapters of the thesis. The background introduces the subject of varicocele, the prevalence worldwide, link between varicocele and infertility, summary of the historical

milestones, hypothesis, surgical repair of varicocele, and the effect of varicocelectomy on gonadal function and spermatogenesis. In the background, arguments in favour and the critiques of the subject matter by various authors have been highlighted including reasons advanced by both sides. Chapter 1 also includes the problem statement, the main aim, the specific objectives of the study, and ends with the summary of the general methods/methodology.

In Chapter 2, an in-depth review of the literature has been presented. The chapter opens with an overview of varicocele, theories and discoveries of varicocele origin, pathophysiology of varicocele, global incidence and prevalence, varicocele and infertility, effects of varicocele on gonadal hormones, and pressure flow hemodynamic of varicocele. Where conflicting or null results have been found, these have been stated with the arguments indicated by the authors. The chapter ends with a review of the effects of varicocelectomy on gonadal function and semen parameters.

Chapter 3 involved hypothesis testing. This chapter sought to investigate the effect of microsurgical sub-inguinal varicocelectomy on semen parameters among men seeking infertility treatment in Tamale, Ghana. In Chapter 4, the study aims to determine the gonadal functions pre-and post-microsurgical sub-inguinal varicocelectomy among aging men reporting sexual dysfunction. This was a time-series analysis where participants were followed for 48 months after the initial (baseline) data collected. Factors associated with improved semen characteristics following microsurgical sub-inguinal varicocelectomy were determined in Chapter 5. In Chapter 6, the study determined the Changes in testicular arterial haemodynamic, gonadotropin levels and semen quality among varicocele patients using color Doppler ultrasonographic indices (CDUS).

The general discussion and summary of the findings of the study including the conclusions were presented in Chapter 7. The limitations of the study and the general recommendations for future studies were also presented in Chapter 8.

1.7 CONCEPTUAL FRAMEWORK

A total of 274 participants were included in the study; consisting of 241 varicocele patients and 33 apparently health controls. The varicocele group were randomised to surgery (n = 127) and observed group (n = 114). Objective I involved 138 varicocele participants reporting male fertility factor and randomised to surgery (n = 75) and observed (n = 63) and were followed for 6 months. Objective II involved 103 varicocele participants who had fathered a child and reported with sexual dysfunction randomised to surgery (n = 52) and observed group (n = 51) and followed for 48 months. Objective III were varicocele participants whom had undergone surgery (n = 127) grouped based on total motile count as responders (n = 69) and non-responders (n = 58). These groups were followed for 12 months. Objective IV included all groups; varicocele patients who had undergone surgery (n = 127), varicocele patients who were observed without going through surgery (n = 114) and apparently healthy controls (n = 33) (Figure 1.1).

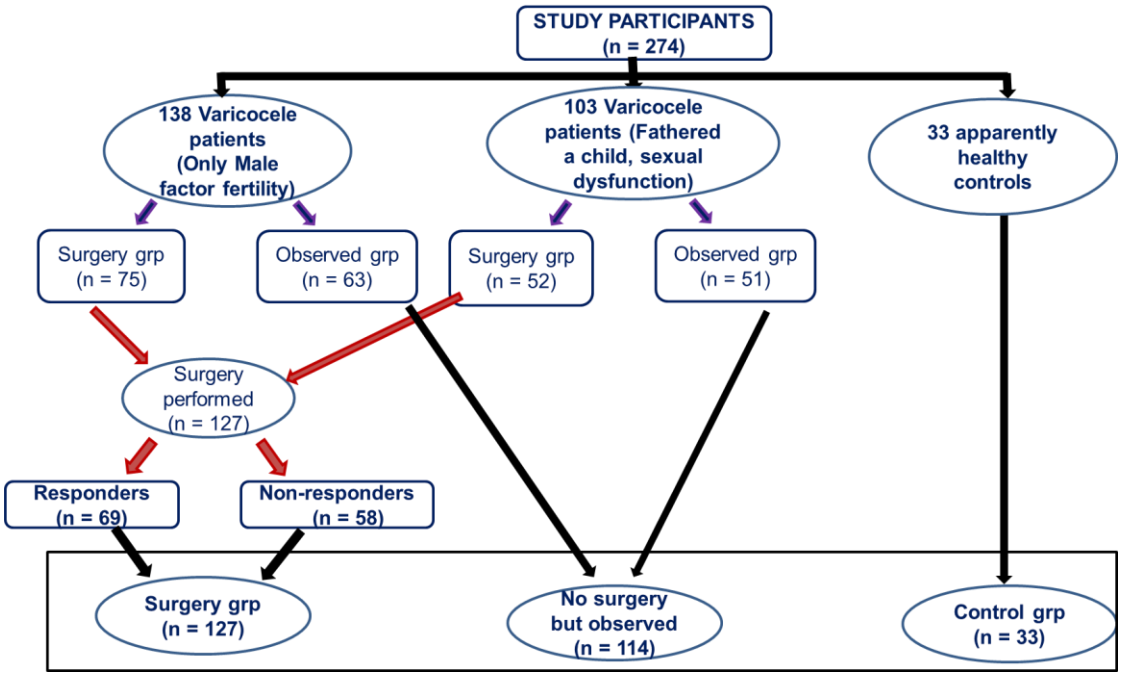

Figure 1.1: Conceptual framework

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Chapter 2

LITERATURE REVIEW

2.1 OVERVIEW OF VARICOCELE

Pampiniform plexus is a web of veins that drains blood from the testicles. Ergün *et al.* (1997) used a light-microscopic examination of cast preparations and a three-dimensional computer-aided reconstruction to show that this network of veins can be separated into two bundles; a group of veins firmly wrapped around the testicular artery and the other in the adjacent fatty tissue (**Figure 2.1**). The two veins merge into the inner spermatic vein at the level of internal inguinal ring.

Abnormal enlargement of the pampiniform plexus draining the testicle is considered to be a varicocele (Clavijo *et al.*, 2017). Varicocele can also be defined as vascular lesion in the spermatic veins that presents with dilation and tortuosity usually found in adolescents and young male adult (Mohammed and Chinegwundoh, 2009).

In 16th century, Ambroïse Paré a French surgeon first defined varicoceles to be as a result of "a compact of vessels filled with melancholic blood" (Steeno, 2013). Later, a link between varicoceles and infertility was proposed by Barfield, a British surgeon (Barfield *et al.*, 1979). Agarwal *et al.* (2012) later defines varicocele as a venous malfunction that results in retrograde of blood into the internal spermatic veins. More recently, varicocele is defined as a pathologic enlargement of the pampiniform plexus or of the cremasteric venous system that causes regressive flow of blood into the venous system when there is an increase in the intra-abdominal pressure (Beretta, 2015).

Varicocele can also be subclinical/nonpalpable. Subclinical varicocele is defined by reversal of flow of venous blood flow with the Valsalva maneuver with a diameter of 3mm or more on color Doppler ultrasonography (Comhaire and Monteyne, 1976; Meacham *et al.*, 1994). This large range could be due to the various ultrasonographic criteria used to describe a

varicocele. While some authors believe that a varicocele should be detected when the vessels are greater than 3mm in diameter, others argue that a 2mm vein diameter cut-off provides a 95% sensitivity in varicocele detection. To add to the complexity, no reference value for the ultrasonographic diagnosis of varicocele has been suggested because backward blood flow can be found in veins less than 2 mm in diameter (Gonda Jr *et al.*, 1987; Atasoy and Fitoz, 2001; Kulis *et al.*, 2012).

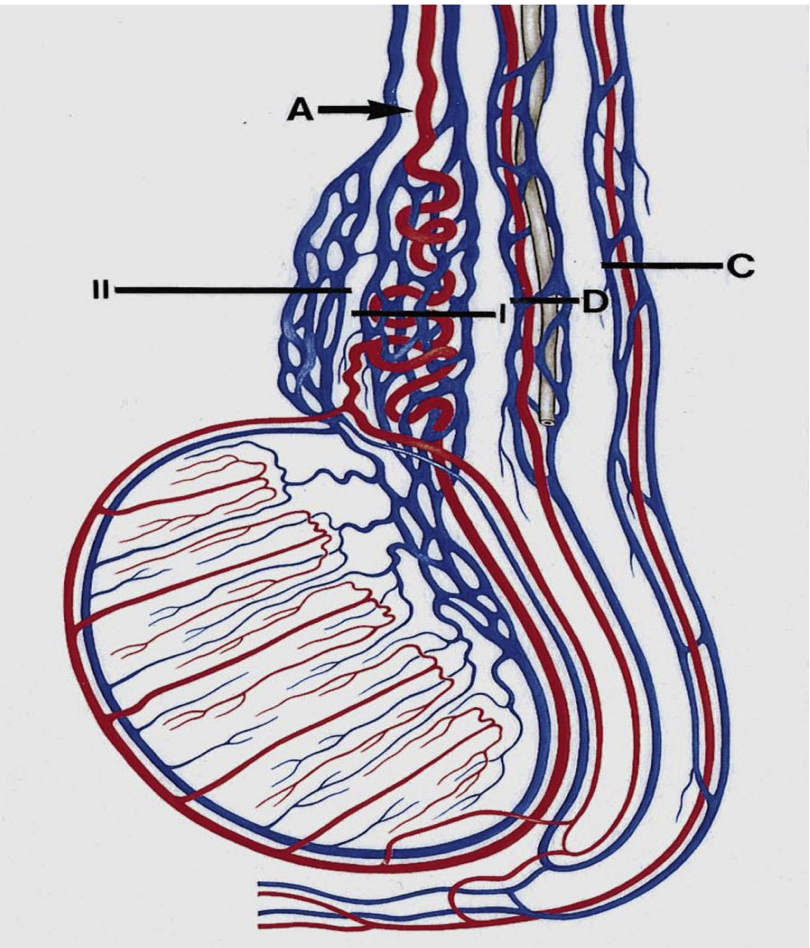

Figure 2.1: Testicular venous drainage system copied from Ergün *et al.* (1997). I - testicular drainage veins strongly wrapped around testicular artery; II: testicular drainage veins dispersed from testicular artery; A: testicular artery; C: cremasteric venous drainage with artery; D: deferential venous drainage with artery

2.2 VARICOCELE ORIGIN

In determining the origin of varicocele, three non-mutually exclusive theories have been proposed: The first is the 90^0 - insertion of the left testicular vein into the left renal vein,

increasing hydrostatic pressure that is eventually conveyed to the pampiniform plexus (Naughton *et al.*, 2001; Miyaoka and Esteves, 2011).

The second theory relies on venous valves that are incompetent (or non-existent) before birth, causing retrograde flow and dilatation (McClure and Hricak, 1986; Miyaoka and Esteves, 2011). Venography and colour Doppler experiments have backed up this idea. Two pathophysiologic subtypes, shunt, and stop, have been defined; a stop-type varicocele is evident when the incompetent valves are solely placed above the level of the connecting veins, which accounts for 14% of all varicocele. And a short reversing flow from the internal spermatic vein characterizes the stop-type varicocele. Because distal valves are present and functionally competent, there is no orthograde venous blood flow and reflux towards the communication veins. By compensating the reflux-producing incompetent valve in contradiction of valves from the residual normal venous drainage system, surgical closure of the stop-type varicocele cures the varicocele (Sigmund *et al.*, 1987). Mohseni *et al*. (2011) found that the shunt-type varicocele was linked with a higher incidence of testicular hypotrophy than the stop-type varicocele. Furthermore, the scientist found that when a shunt-type varicocele was treated by the retroperitoneal method rather than the inguinal technique, the recurrence incidence was greater (Mohseni *et al.*, 2011).

The third theory is the nutcracker effect, in which the left renal vein between the abdominal aorta and the superior mesenteric artery are compressed raising hydrostatic pressure inside the pampiniform plexus due to partial obstruction of the flow of blood through the left testicular vein, (Gat *et al.*, 2010). The nutcracker phenomenon causes a progressively rising renocaval pressure gradient, which then refluxes down the internal spermatic vein, causing collateral venous routes to form (Mali *et al.*, 1986; Carl *et al.*, 1993; Kim *et al.*, 1996). Hemodynamic investigations in adults and children with varicocele offered evidence to support this notion. Mali *et al.* (1986) found a link between renospermatic reflux and the

renocaval pressure gradient in adults, demonstrating that severe compression of left renal vein in the upright position impacts the velocity of backward flow in the left spermatic vein and the extent of the varicocele. In diagnosing nutcracker effect, the gold standard is measuring the selective left renal venography by measuring the pressure gradient between the left renal vein (LRV) and inferior vena cava (IVC). The usual length and diameter of the left renal vein (LRV) are 6–10 cm and 4–5 mm, respectively (Akbay *et al.*, 2000). During Valsalva maneuver, this angle lowers even further, compressing the LRV even more. In patients with suspected nutcracker syndrome, Doppler ultrasonography might be utilized as the first diagnostic test. Left renal vein entrapment syndrome was diagnosed using a combination of B-mode sonographic measurements of the LRV's diameter and Doppler sonographic measurements of the LRV's peak velocity (PV) (Kim *et al.*, 1996; Park *et al.*, 2002).

Finally, a varicocele may be caused by a mixture of all of these mechanisms, which are exacerbated by an upright position. Incompetent venous valves, narrowing of the aortomesenteric angle, and absence of fat support around the left renal vein have been demonstrated to cause varicocele formation in thin, tall athletic people (Rais *et al.*, 2013).

2.3 TESTICULAR VEIN ANATOMY

Testicular veins arise from the testis' mediastinum to form the pampiniform plexus, which is grouped into; anterior, middle, and posterior veins. The posterior group drains into the external pudenda and cremasteric veins and runs behind the spermatic cord. The middle group drains into the internal iliac vein after passing via the vas deferens. While the anterior drains into the inferior epigastric vein. The internal spermatic artery runs through the anterior group. This complex divides into three or four tributaries that enter the pelvis at the superficial inguinal ring. These veins gradually coalesce into two, then into a single internal spermatic vein that runs alongside the testicular artery in front of the ureter. The primary

venous channel usually has medial and lateral branches, with the latter terminating in the renal capsular, retroperitoneal or colonic veins. Just below the right renal vein, the right internal spermatic vein enters the inferior vena cava. Lateral to the spinal column, the left internal spermatic vein connects with the under surface of the left renal vein (Lechter *et al.*, 1991) (**Figure 2.2**).

In around 20% of cases, there is a variation in anatomy. Flow from the right interior spermatic vein into the right renal vein (8–10%) and numerous terminal spermatic veins (15–20%) are notable malformations. Internal spermatic veins have valves in most, but not all (Lechter *et al.*, 1991; Valji, 2011) (**Figure 2.2**).

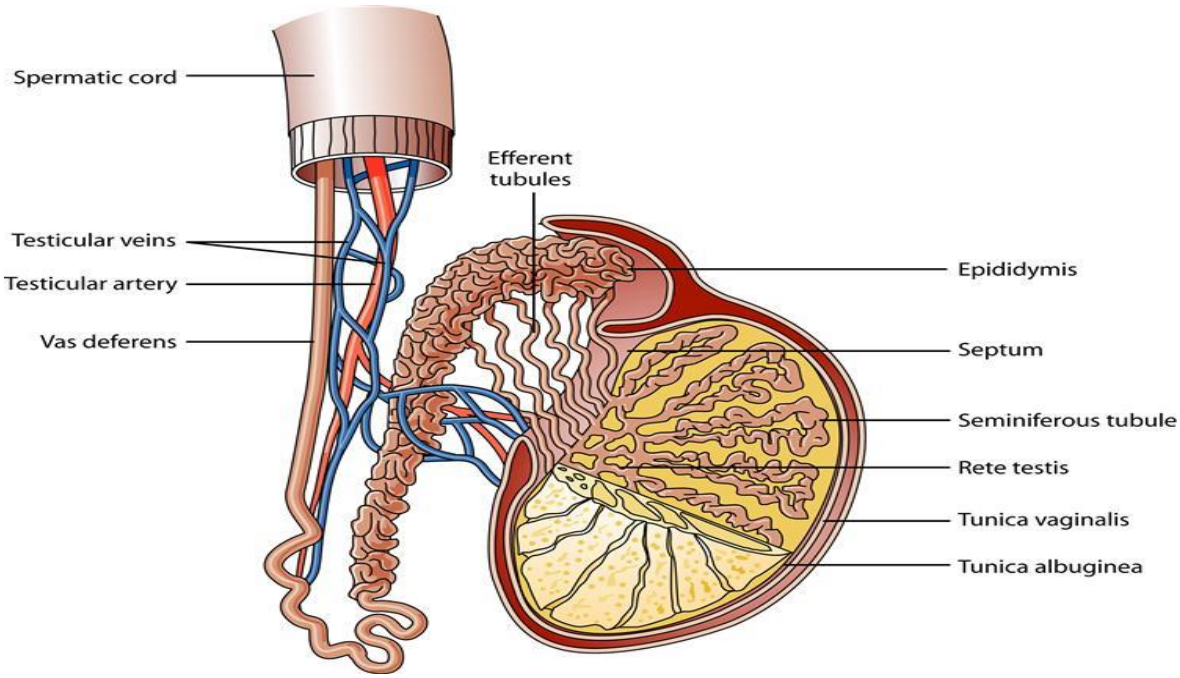

Figure 2.2: Anatomy of testicular vein, artery and vas deferens copied from anatomynotes.com

2.4 PATHOPHYSIOLOGY OF VARICOCELE FORMATION

The pathophysiology of varicocele shows that varicocele is venous inability that allows pathological blood ebb into the testicular vein. The severity of vein structural anomalies varies, but dilatation of the internal spermatic veins to the point of eventual drainage into the

left renal vein or the inferior vena cava is usually the case (Ivanissevich, 1960; Marsman and Schats, 1994).

Vascular dilatation may be attributed to so many reasons; first is the anatomical position of the internal spermatic vein. Several post-mortem examinations and venography studies show that, drainage of the left internal spermatic vein goes directly to renal vein in a perpendicular fashion (Asala *et al.*, 2001). This drainage system together with the observation that, the left spermatic vein has a relatively elongated drainage tract and thus experiences larger venous differences in pressure may sort to explain why left-sided varicoceles are more predominant than right-sided or bilateral varicoceles (Coolsaet, 1980; Shafik and Bedeir, 1980; Gat *et al.*, 2005b; Damsgaard *et al.*, 2016). In addition, Kang *et al.* (2021) postulated that, compression of the inferior vena cava (IVC) or the right gonadal vein may lead to right-sided varicocele while the left-sided varicocele is due to insertion of the left gonadal vein into the left renal vein which is positioned at ninety-degree angle (90^0) (**Figure 2.3**).

Secondly, Lewis *et al.* (2015) reported that varicocele can be cause by left renal vein compression. Although a rare mechanism, compression of the aorta and superior mesenteric artery of internal spermatic vein or left renal vein classically called 'nutcracker syndrome' has been postulated as a possible source of left renal insufficiency and several studies are associating this condition to varicocele formation (Handel *et al.*, 2006; Vanlangenhove *et al.*, 2015).

Thirdly, Ahlberg *et al.* (1965) in the early days studied cadavers and found that there was absence or incompetence of internal spermatic vein valves in half of studied participant which might have predisposed them to varicocele. More recent studies have reported incompetence or complete absence of spermatic vein valves in adolescents (Vanlangenhove *et al.*, 2015) and some adults with varicoceles (Dennison and Tibbs, 1986). In addition to the incompetence of venous valves, the accessory connections between the internal spermatic

vein and systemic venous flow that lack anti-refluxing mechanism may be attributed to the presence of varicoceles (Sze *et al.*, 2008; Vanlangenhove *et al.*, 2015). The anatomical variety in drainage of spermatic vein may be attributed to the significant higher rate of varicocele relapse after surgical procedures such as laparoscopic of selective internal spermatic vein ligation or percutaneous venous embolization internal spermatic vein (Al-Kandari *et al.*, 2007; Halpern *et al.*, 2016).

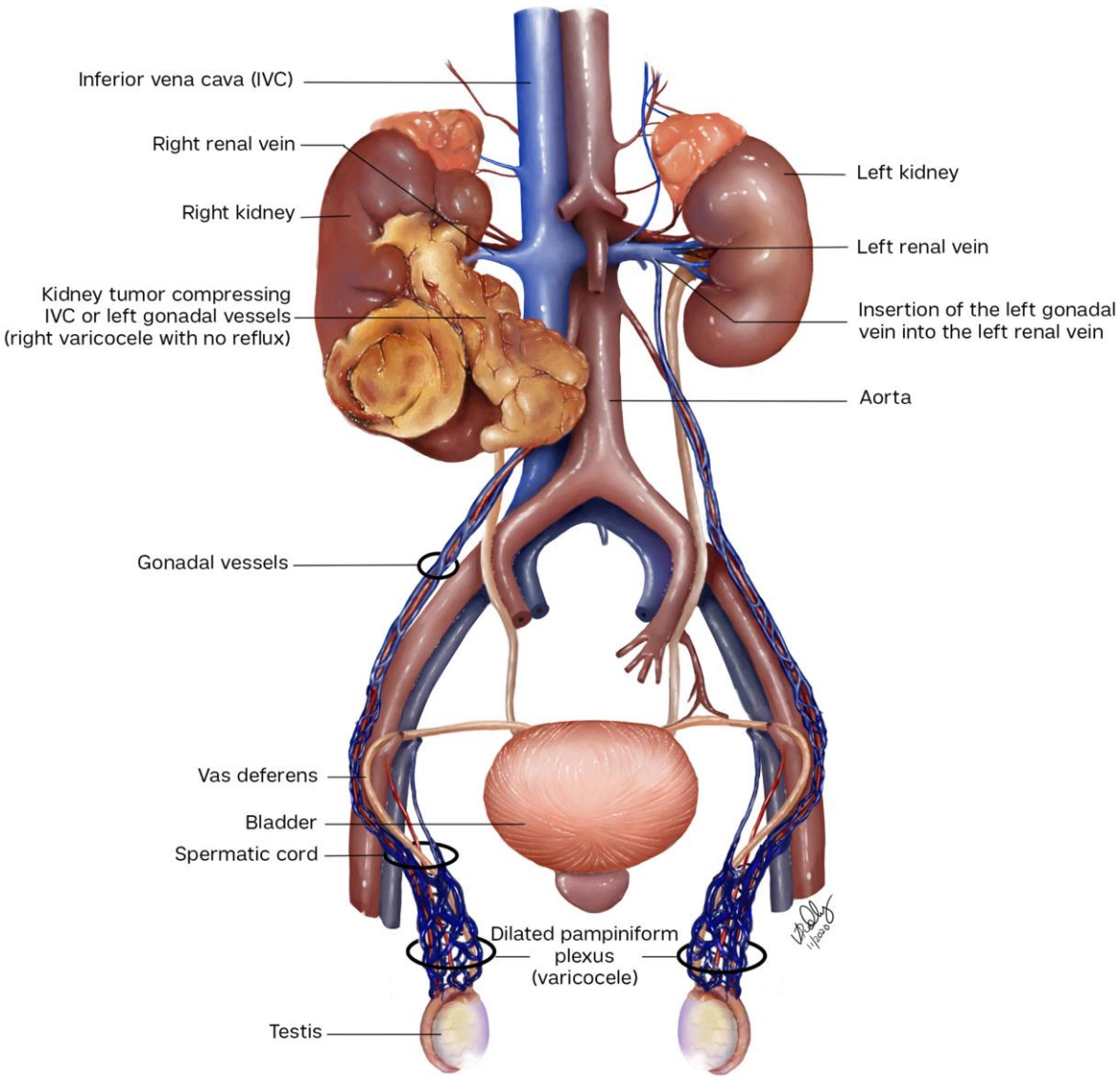

Figure 2.3: Pathophysiology of varicocele (Kang *et al.*, 2021)

2.5 VARICOCELE AS UNILATERAL OR BILATERAL DISEASE

Varicoceles are more commonly found on the left side of the body than on the right, and they are more often unilateral than bilateral. Due to anatomical position, about 90% of varicoceles are unilateral left-sided and this may be due to a hydrostatic column of blood within the left gonadal vein created as a result of the vertical insertion into the left renal vein leading to more turbulent flow and back pressure, which causes abnormal dilation in the left gonadal vein (Damsgaard *et al.*, 2016).

Men may also present with bilateral varicoceles which arise secondary to abnormal anti-reflux valves causing abnormal vein dilation and backflow of venous blood (El-Saeity and Sidhu, 2006; Kantartzi *et al.*, 2007). In the past, 85–90% of all clinical varicoceles were categorized as unilateral left-sided varicoceles. Recent data, however, shows that bilateral palpable varicocele is observed in more of affected men (Cvitanic *et al.*, 1993; Akbay *et al.*, 2000). The prevalence of bilateral varicocele has been reported to be 1.1% with isolated right-sided varicocele ranging from 0.2% to 2% in secondary infertility (Gat *et al.*, 2005b; Damsgaard *et al.*, 2016). Only 2% of patients have a solitary right-sided varicocele, which may be accompanied by the occurrence of an obstructive lesion such as pelvic compressive mass or a retroperitoneal (Gat *et al.*, 2005b).

2.6 DIAGNOSIS OF VARICOCELE

Computer tomography (CT), Ultrasound, Angiography, Magnetic resonance imaging (MRI) are examples of radiological techniques used for diagnosing varicoceles. As stated by Trum *et al.* (1996) physical examination is the first tool used clinically for diagnosing varicocele and this is then later confirmed by color Doppler ultrasonography (CDUS) which is the gold standard diagnostic tool.

Asala *et al.* (2001), reported that varicocele is diagnosed during physical examination and categorized as; grade I, II, and III. Grade I varicocele is confirmed if there is an enlarged

spermatic venous plexus obvious only by palpation during Valsalva manoeuvre. Abnormal dilatation of the spermatic venous plexus evident by palpation at standing/upright posture is considered second grade while dilatation of the spermatic venous plexus evident visually is confirmed Grade III (Dubin and Amelar, 1970). Palpable varicoceles upon touch feel like a "bag of worms" which disappears significantly when the patient is lying down (Medicine, 2008). Most cases of varicoceles are in grade 1 and about 35% is either grade 2 or 3 (Steeno *et al.*, 1976). The World Health Organization later expanded the grading system to include zero (0) or 'subclinical' varicocele, which is absence of varicocele upon physical examination (both at rest and during Valsalva manoeuvre) and can only be detected by color Doppler ultrasonography (CDUS) or thermography (Rowe *et al.*, 2000).

The imaging diagnoses is superior to physical examination because of its ability to assessed the seriousness of the varicoceles by characterizing venous diameter, the presence and absence of blood reflux, and other indices. The sensitivity and specificity of CDUS in identifying subclinical varicocele is 83% and 95% respectively. The assessment mostly include backflow of blood on Valsalva manoeuvre (Dhabuwala *et al.*, 1989). Studies by Taşçi *et al.* (2001) showed that, varicoceles can be diagnosed from the elevation in intra-abdominal pressure due to the reversal in the direction of blood flow in varicocele veins. Different grades have been used to assess the backflow pattern during Valsalva manoeuvre. These grades start from grade I (reflux <2.0s) to grade III (reflux >2.0s) (Ortapamuk *et al.*, 2005). Studies by Kim *et al.* (2012) proposed that the outcome from colour Doppler ultrasonography is superior to the other diagnostic imaging as results of its ability to measure utmost total cross-sectional area and the reflux velocity of the implicated veins of the testes. This function of CDUS can be used to deduce the number of internal spermatic veins that will need to be ligated during microsurgical subinguinal varicocelectomy (Kim *et al.*, 2012).

2.7 PREVALENCE OF VARICOCELE

Globally, varicocele has been reported to range from 4.4% to 22.6% in healthy men with an average of 15% in general population (Saypol, 1981; Alsaikhan *et al.*, 2016). In pre-adolescent ages (2 – 10 years), varicocele is less frequent but the prevalence varies from 6% to 26% in adolescent (øster, 1971; Risser and Lipshultz, 1984; Steeno, 1991; Vasavada *et al.*, 1997). In a population-based studies of 1.3 million Israeli teenage males aged 10 –19 years, the prevalence of 4% to 39% was reported (Kumanov *et al.*, 2008; Rais *et al.*, 2013). Again, Akbay *et al.* (2000) observed that among male children, varicocele occurs in 7.2% of the 4,052 boys studied. In his study unilateral varicocele was seen in 89.7% of the children with varicocele; all left-sided with only 1 person (0.38%) have right-sided varicocele, while 10.8% bilateral varicocele was reported (Akbay *et al.*, 2000).

Among men seeking medical attention for primary infertility, the prevalence of varicocele ranges from 35% to 45% (Gorelick and Goldstein, 1993; Jarow *et al.*, 1996), and in secondary infertility, the value ranges from 78% to 81% (Alsaikhan *et al.*, 2016). Also, Miyaoka and Esteves (2011) and Agarwal *et al.* (2012) also found that varicoceles are present in 15% to 20% among normal adults male and about 35% among men with primary infertility. About 90% of varicocele affect the left side in most patients with 10% presenting with bilateral varicocele (Gat *et al.*, 2004). Canales *et al.* (2005) reported varicocele in 42.9% in a study population; with bilateral in 19.8%, left sided only in 22.0%, and right sided only in 1.1%.

In Africa and the sub-region, Seraphin *et al.* (2019) found the prevalence of varicocele in Africa to be 5.47%. A total of 2,724 teenagers from two university hospitals with incidence of unilateral and bilateral varicoceles being 79.2% and 20.8% respectively by Fiogbe *et al.* (2013). Varicoceles were classified by Seraphin and colleagues into three grades: grade I, II, and III with incidence rates of 38.9%, 44.3%, and 16.8% respectively. Another study was

conducted in West Africa to assessed abnormal scrotal findings of men by Tijani *et al.* (2014), who found that varicoceles was the commonest cause of abnormal scrotal findings with incidence rate of 12% in the fertile and 55% in sub-fertile men. In this study, a population size of 249 was used (Tijani *et al.*, 2014). In Ghana, a prospective study involving 110 infertile men visiting urology outpatient department (OPD) at the tertiary hospital reported a prevalence of varicoceles as 22.6% (Gyasi-Sarpong *et al.*, 2017). However, a recent meta-analysis done by Abebe *et al.* (2020) estimated the incidence of 19.2% among infertile men in Africa.

2.8 EFFECT OF VARICOCELE ON FERTILITY

Patients with varicocele must have varying levels of innate vulnerability, resulting in the different impacts of varicocele on male fertility (Miyaoka and Esteves, 2011). Male infertility can be explained as a combination of male and female factors with a fully functional female reproductive system compensating for male factor deficits resulting in unsuccessful conception. In another context, male factor fertility was defined man inability to make a normal female partner or spouse pregnant after one year of unprotected sexual intercourse (i.e., female with normal ovulation, normal reproductive history, and tubal patency) (Nallella *et al.*, 2006).

Infertility has been associated with larger varicocele which are very easy to palpate (Medicine, 2008). It is usually present in individuals with scrotal discomfort and infertility (Paick and Choi, 2019). Though most men suffering from varicocele in the general population are fathers even though there is substantial evidence regarding the negative impact varicocele have on male fertility (Beretta, 2015). In a study, Evers and Collins (2004) concluded that there is clinical evidence to support the association between male infertility and varicocele, but the evidence suggest that getting the right treatment can improve the individual's chance of pregnancy. It is also reported that varicocele is correctable and when

left untreated in infertile men which may lead to decline of spermatogenesis, testicular volume, and Leydig cell function which may all result in abnormal semen parameters (Dubin and Amelar, 1977; Chehval and Purcell, 1992). Several data from experimental studies in clinical and animal models points to varicocele having an adverse effect on spermatogenesis, with venous reflux and testicular hyperthermia playing vital roles in testicular dysfunction caused by varicocele (Medicine, 2008).

Varicocele affects spermatogenesis and studies conducted earlier suggested three (3) mechanisms; i) slow circulation in varicose veins of the leg, and as the leg varicosities can lead to local skin destruction, so, varicoceles destroy the germinal epithelium less dramatically. ii) Also, the large volume of slowly circulating blood may act as a radiator surrounding the testicles, thus reproducing the experimental scrotal insulation. iii) Lastly, the sheer bulk of the varicocele holds the testicle in one position, thereby preventing the normal physiological cooling mechanism from working efficiently (Glover, 1955; Scott and Young, 1962). In recent times, however, there is limited information on the mechanism to conclusively explain the infertility in men with varicocele with several possible intermediaries being; anatomical anomaly of varicocele (Xue *et al.*, 2012; Kadioglu *et al.*, 2014), increased scrotal temperature (Shiraishi *et al.*, 2012), an adrenal hormone, backflow of toxic metabolites which may lead to failure of spermatogenesis (Inci and Gunay, 2013), epigenetic changes (Seidel, 2015), and increased production of reactive oxygen species (ROS) in the scrotum which results in sperm DNA damage (Agarwal *et al.*, 2014). These related factors maybe act individually or synergistically affecting spermatogenesis in varicocele patients (**Figure 2.4**).

Infertile men (21–41%) have a higher prevalence of palpable varicocele than the overall male population (4.4–22.6%) (Trum *et al.*, 1996). Among the infertile men with aberrant semen parameters, this figure has grown to 25.4% (World Health Organization, 1992).

Furthermore, males with varicocele have observed a decline in sperm concentration and motility over time. Varicocele is substantially more common in men with secondary infertility (81%) than in men with original infertility (35%) (Chehval and Purcell, 1992; Gorelick and Goldstein, 1993; Raman *et al.*, 2005; Canales *et al.*, 2006). As a result of these findings, it appears that varicocele has a gradual influence on spermatogenesis, with testicular damage as a final result.

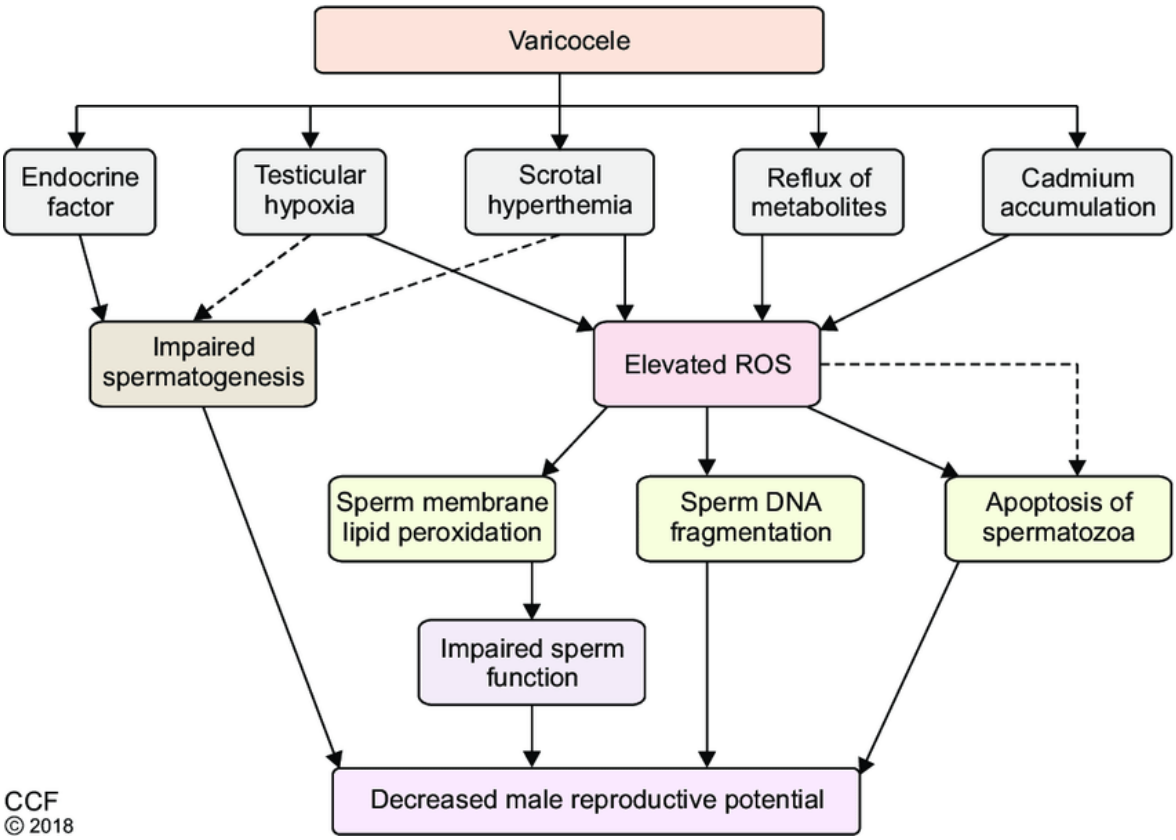

Figure 2.4: Effects of varicocele on spermatogenesis copied from Selvam and Agarwal (2020)

2.9 VARICOCELE AND AZOOSPERMIA

Azoospermia is the entire lack of sperm from the ejaculate without assuming a cause. This disorder affects about 1% of all men and up to 15% of infertile men (Jarow *et al.*, 1989; Maduro *et al.*, 2003). Azoospermia can be caused by a mechanical obstruction along the reproductive tract, including the vas deferens, ejaculatory duct, and epididymis, but it's more

commonly linked to a range of severe and fatal conditions that cause intrinsic testicular impairment known as non-obstructive azoospermia (NOA) (Esteves *et al.*, 2011). Varicocele is seen in 5% of males with non-obstructive azoospermia, but the extent to which it affects azoospermia is still unknown (Esteves and Glina, 2005). The introduction of assisted reproductive technologies, particularly intracytoplasmic sperm injection (ICSI), has revived awareness of varicocele with NOA, as improved testicular function may be crucial for infertile men with NOA to get viable sperm.

A meta-analysis done to determine the impact of surgical correction in 233 men with palpable varicocele and non-obstructive azoospermia shows that motile spermatozoa were discovered in 39 % of individuals after an average follow-up of 13 months, and pregnancy was achieved in around 26 % of males with sperm in the ejaculate, 60 % unassisted (14 cases), and 40 % using *in vitro* fertilization procedures (Weedin *et al.*, 2010).

Another meta-analysis that included five studies and 90 individuals with varicocele and non-obstructive azoospermia backed the previous findings. Hypospermatogenesis was seen in 30 of 90 men, Sertoli-cell-only in 34 of 90 men, and maturation arrest in 26 of 90 men (Elzanaty, 2014). Men with hypospermatogenesis or maturation arrest had a significantly greater rate of effective spermatozoa appearance in the ejaculate after varicocele repair than those with Sertoli-cell-only (18 out of 30 vs one out of 34, $p = 0.001$; 12 out of 26 vs one out of 34, $p = 0.002$), respectively. When hypospermatogenesis was compared with maturation arrest, the success rate of varicocele repair did not change (18 out of 30 vs 12 out of 26, $p = 0.65$) (Elzanaty, 2014).

Comparative research involving varicocele-treated and untreated people is rare when it comes to sperm retrieval. Sperm retrieval rates in azoospermic men who underwent varicocele repair were similar (60%) vs those who did not, according to one study (Schlegel and Kaufmann, 2004). Others, though, suggested that involvement could be beneficial. In a

study of 96 males, Inci *et al.* (2009) discovered that sperm retrieval success was 2.6 times higher in treated men than in untreated men (OR: 2.63, 95% CI: 1.05–6.60). Haydardedeoglu *et al.* (2010) observed better SR success in males who received varicocele surgery before sperm retrieval (61 %) compared to untreated men (38 %; p 0.01) in another trial comprising 66 men. Although it is possible to argue that a control group would stay azoospermic, it is not uncommon to see NOA males ejaculate tiny amounts of motile sperm even when there is no intervention.

2.10 EFFECTS OF VARICOCELES ON GONADOTROPINS

The hypothalamic-pituitary-testicular (HPT) axis through neuroendocrine activity controls spermatogenesis. In human males, HPT axis control of spermatogenesis occurs in three phases; neonatal-infantile, juvenile/childhood and the pubertal initiation phases (Ramaswamy and Weinbauer, 2014). The neonatal-infantile phase is characterized with hypothalamic-pituitary-testicular axis activity but spermatogenic activity is conspicuously absent. This is followed by a long period of dormancy in the childhood phase before spermatogenesis is initiated at the pubertal phase resulting in normal adult testicular functions (Plant *et al.*, 2005; Witchel and Topaloglu, 2019). Gonadotropin releasing hormone (GnRH); a ten-peptide hormone secreted pulsatile to travel the short distance of the hypopheaseal system to the pituitary is the driving signal of spermatogenesis from the hypothalamus. In response, the gonadotropes secrete two glycoprotein hormones or gonadotropins i.e. the follicle stimulating hormone (FSH) and the luteinizing hormone (LH) (Ramaswamy and Weinbauer, 2014). These hormones directly or indirectly exert their actions on spermatogenesis.

In the testes, FSH and LH respectively bind to receptors predominantly expressed in the Sertoli cells and interstitial Leydig cells. Leydig cells in response to LH produces testosterone, a steroid hormone secreted in a pulsatile manner while the Sertoli cells in

responding to FSH stimulation secretes inhibin B in a non-pulsatile fashion. These series of events in the Leydig, Sertoli, and peritubular cells of the seminiferous tubules stimulates spermatogenesis (O'Donnell *et al.*, 1994). Testosterone is suggested to have an inhibitory effect on inhibin B secretion and together, they serve as feedback signals in order to maintain normal physiologic functions of the hypothalamic-pituitary-testis axis (Babu *et al.*, 2004). Also, FSH plays a major role in DNA synthesis in spermatogonia (Rodriguez Peña *et al.*, 2009; Shabana *et al.*, 2015) with FSH, LH and testosterone being essential in the quantitative production of spermatozoa (Shabana *et al.*, 2015). And the evaluation of the serum levels of these spermatogenesis-related reproductive hormones is useful in detecting and managing male infertility (Glassberg *et al.*, 2008). There is wide variation in testosterone concentration in peripheral circulation and intratesticular fluid with intratesticular testosterone being several folds higher (Sharpe, 1994; Jarow and Zirkin, 2005; Page, 2011). Some have reported intratesticular testosterone to be about 100-fold higher than serum levels and this may be as result of local production of testosterone by the Leydig cells (Swerdloff and Walsh, 1975; Jarow *et al.*, 2001; Jarow *et al.*, 2005). Also, significant variations in testicular testosterone concentrations within a given species and studies has been reported (Sharpe, 1994; Polepalle *et al.*, 2003; Jarow and Zirkin, 2005). These high levels are required to support normal spermatogenesis (Coviello *et al.*, 2004**).** However, the amount of testicular testosterone needed to initiate, sustain or reinitiate normal spermatogenesis is still not clearly defined (Sharpe, 1994).

During puberty, gonadotropins function mainly by recruiting units of Leydig, Sertoli and stem germ cells and priming them for normal spermatogenesis during adulthood. Consequently, hormonal abnormality or deprivation during puberty affects the development of the adult testis while in adults, the effects are predominantly on the germ cells (Ramaswamy and Weinbauer, 2014). Few studies have reported on the role of LH, FSH and

testosterone in male infertility, however not much has been done with regards to the subgroups of male infertility including varicoceles (Shabana *et al.*, 2015; Neto *et al.*, 2016). Further, inconclusive results have been reported in studies that seek to compare the reproductive hormones in infertile men with varicoceles with infertile men without varicocele. Presently, data available on the impact of varicoceles on reproductive hormones and semen quality is mainly from studies among sub-fertile and infertile men and men referred to fertility clinics (Gorelick and Goldstein, 1993). A study by Shabana *et al.* (2015) reported a significantly increased serum FSH, LH mean values in male with varicocele but not testosterone levels when compared with controls. Also, (Gorelick and Goldstein, 1993) and (Ishikawa and Fujisawa, 2005) respectively recorded elevated FSH and LH levels in infertile men with varicocele. In a study among 7035 young healthy men, varicocele even in its mildest form (grade 1) was associated with poor semen quality. LH levels were higher in men with varicocele and this indicates a Leydig cell dysfunction usually associated with varicocele. A 28% increase in serum FSH was found in men with varicocele grade 3 compared with men without varicocele.

Testosterone levels were not significantly different from the reference group. Also, lower levels of serum inhibin B have been reported in men with varicocele compared to controls (Kumanov *et al.*, 2006). A steady decreased in Inhibin B was observed with increasing grade of varicocele by Hormonal changes in varicocele article with men with grade 3 varicocele presenting with a 15% decreased in serum Inhibin B levels compared with normal controls. In men with varicocele, Weiss and colleagues, reported evidence of decreased testicular testosterone production as testicular tissue from some of these men were less efficient compared to normal men in converting 3H-pregnenolone to 3H-testosterone (Al Bakri *et al.*, 2012). This support the hypothesis that there is primary testicular dysfunction in

spermatogenesis and testosterone synthesis in men with varicoceles. In primary testicular failure, there is a high or elevated FSH and LH level (Shabana *et al.*, 2015).

2.11 EFFECTS OF VARICOCELE ON HEMODYNAMIC

2.11.1 Pressure Flow Dynamics of Varicocele

In testicular veins of healthy people, low-pressure dynamics can be seen. Retrograde blood flow does not occur in these people. When the venous valves are lacking or ineffective, significant intravenous pressure can be transmitted from the internal spermatic veins to the testes and this has been attributed to high intra-abdominal pressure (Gat *et al.*, 2006). The backflow of blood in the internal spermatic veins is identical to the backflow of blood in other veins, regardless of their branching arrangement. However, investigations by Kim *et al.* (2012) found that when blood velocity approaches the testes, blood velocity decreases in these branches of veins.

Ischemia is observed in men with varicocele, this occurs as a results of high hydrostatic pressure seen in veins of varicoceles which is greater than the pressure in the microcirculation of the testicular arteries (Gat *et al.*, 2005b). However, definitive results have not been seen from animal studies. Some researchers report elevation or decreased in blood flow to the testes after induced varicocele (Naughton *et al.*, 2001). This could be as a result of the differences in pathophysiology phenomenon of human and animal diseases, hence distinction in the presentation of same disease in humans and animals.

In men with varicocele, ischemia develops as a result of high hydrostatic pressure in varicocele veins that is greater than the pressure in the testicular arteries' microcirculation (Gat *et al.*, 2005b).

Clinically, varicoceles are commonly seen in patients with upper motor neuron lesion and spastic paralysis of the abdominal muscles whiles it is less common in patients with lower

motor neuron lesion with flaccid paralysis (Ünsal *et al.*, 2006). This may be attribute to the high pressure in the abdomen produced by spastic paralysis whiles flaccid paralysis does not. Research by Gat *et al.* (2005b) proves that hydrostatic pressure in veins of varicocele men rely on the height of the column of blood rather than the venous diameter.

The high incidence and severity of left-sided varicocele in tall people could be as a results of elevated hydrostatic pressure in their veins due to the length of their internal spermatic veins (Hassanzadeh *et al.*, 2011). Most of the patients diagnosed with varicocele had absent of internal spermatic valves during sclerotherapy (Braedel *et al.*, 1992). The transmission of the pathological hydrostatic pressure to the testicular venous circulation occurs as a result of the destruction of the valves. There is greater hydrostatic pressure in varicoceles veins than the arterior pressure. This pressure is at times 5times higher, which often leads to hypoxia.

Studies by Gall *et al.* (1986) proposed that varicocele could be a shunt type or pressure type. The shunt type occurs as a result of the incompetency of deferential vein or and cremasteric vein whiles pressure-type occurs as a result of incompetency of valves in the internal spermatic vein. The researchers also found out that, induced reflux was associated with the pressure type. The pressures were commonly observed in subclinical varicoceles (Gall, 1983; Gall, 1987).

The "Nutcracker syndrome" is another suggested phenomenon used to explain the occurrence of varicoceles. The compression of the left renal vein situated between the superior mesenteric artery and the abdominal is the cause of varicocele as explained by this phenomenon. This phenomenon is mostly observed in children (Barnes *et al.*, 1988; Kim *et al.*, 2006; Liu *et al.*, 2012). Studies by Dong *et al.* (2014) found out that after performing shunt anastomosis of the inferior epigastric vein and proximal aspect of spermatic vein in 12 adults with varicoceles, symptoms disappeared. These patients were treated by joining of the left spermatic vein. From the same study, it was observed that after the varicocelectomy, the

peak velocities and diameters of the renal left vein were reduced whiles there was a rise in the testicular left volume.

Epididymis and testicular venous outflow are usually distorting in varicoceles this leads to compensatory hydrodynamic changes as observed in experimental anatomic studies. Extravasation and testicular venous infarction occur as a result of impairment of intra-organ integrity due to the elevation in intra-testicular pressure. Localized inter-arterial shunting and arterial hypertension then occurs. This leads to secondary ischemia of the arteries in the testis and epididymis. This disorder is mostly seen in circumstances where there is blockade in testicular venous blood flow. This blockade needs to be severe in addition to cause blockage of testicular cremasteric veins, which results in the collapse of hydrodynamic veins (Artyukhin, 2007). Two studies have observed that there is distortion in blood flow to the testis in varicoceles due to higher intravascular pressure in varicocele vein than the other veins (Carl *et al.*, 1993; Tarhan *et al.*, 2003). Research by Shafik and Bedeir (1980) observed that varicose men with venous pressure in the left spermatic vein are higher by 22mmHg and 19.7mmHg during Valsalva's maneuver and rest respectively, as compare to normal population.

Baranov *et al.* (2004) reported an increase of 61.1% ± 10.0% in femoral vein caliber during Valsalva test in varicocele patients where as in normal adult it is elevated to 22.6% ± 5.7%. These researchers suggested that the ileo-spermatic reflux in varicoceles may be due to the formation of tension chamber in the femoral vein. Several studies have reported that varicoceles cause severe damage to the testis. This damage can be improved through microsurgical varicocelectomy (Tarhan *et al.*, 2011). Sperms have been shown to occur in about 34.6% of varicocele patients with non-obstructed azoospermia during ejaculation after microsurgical varicocelectomy (Lee *et al.*, 2007).

2.11.2 Hemodynamic Parameters

Several studies have shown that infertility is one of the commonest disorders which affects averagely 15% of couples in their reproductive ages (Makar and Toth, 2002; Polis *et al.*, 2017; Tabatabaeichehr *et al.*, 2018; Sun *et al.*, 2019). Further studies, have proven that males contribute to about 50% of this infertility (Frey, 2010; Choy and Eisenberg, 2018; Öztekin *et al.*, 2019). Primary or secondary varicocele are reported to be the main common cause of infertility in men. The primary cause of varicocele has been suggested to be the non-functioning valves in the internal spermatic vein. This results in retrograde blood flow to internal spermatic vein from the left renal vein. This observation is called the nutcracker phenomenon (Kim *et al.*, 1992; Carl *et al.*, 1993; Braedel *et al.*, 1994). Several studies have reported the development of collateral venous pathway by constant rise in renocaval pressure gradient and ebb down the internal spermatic vein is associated with the nutcracker phenomenon (Nishimura *et al.*, 1986; Th *et al.*, 1986; Hohenfellner *et al.*, 1991; Kim *et al.*, 2006). Good correlation between renospermatic reflux and renocaval pressure gradient was observed by Th *et al.* (1986). These researchers also showed that the velocity of backward flow of blood in the left spermatic vein and varicocele size depends on the severity of the left renal vein compression in the upright position.

The assessment of hemodynamic in varicoceles patients may be done using testicular venography, Color Doppler ultrasonography (CDUS) or scrotal scintigraphy. The use of venography is an invasive procedure and many at times patients are uncomfortable undergoing such treatment. Venography treatment are most at times requested with embolization procedure.

The advent of modern technology in ultrasonography in assessing male infertility is increasing. Ultrasonography can be used to assess echogenicity, testicular volume, macro

vascular and microvascular blood flow and others (Schurich *et al.*, 2009; Tijani *et al.*, 2014; Lotti and Maggi, 2015; Sihag *et al.*, 2018).

In routine studies the use of CDUS is regarded as the most reliable and quick method of measuring blood flow, adding anatomical and velocity data. Clinical information on vascular impedance and blood flow are obtain from Doppler parameters. Doppler indices rest on Peak Systolic volume (PSV), End Diastolic Volume (EDV) and mean velocity measurement. The most commonly assessed parameters are the pulsatility index (PI) and resistive index (RI). The RI has been used in both human and animal studies to assess intra-testicular blood flow (Biagiotti *et al.*, 2002). Resistive Index (RI) and Peak systolic volume are some ultrasonic indices that measures testicular parenchymal perfusion and microcirculation function. Resistive index is calculated from Peak Systolic Volume (PSV) and end diastolic volume (EDV), by using the formula, PSV-EDV/PSV. The rise in resistive index in the testicles is associate with an obstruction in microcirculation and hence a decrease in flow of blood to the testis (Halpern *et al.*, 1998; Tanriverdi *et al.*, 2006; Al-Naffakh, 2012b; Gloria *et al.*, 2018). Research by Bude and Rubin (1999) found out that RI is affected by vascular compliance. Vascular compliance refers to the change in vessel volume with pressure change (Bude and Rubin, 1999).

Studies by Schurich *et al.* (2009) demonstrated that in varicocele there is rise in RI and PI of the capsular branches of testicular arteries on unenhanced CDUS. This may be as a result of reduction in testicular arterial flow of blood in varicocele patients, this may then decrease spermatogenesis. Research has shown that spermatogenesis depends on constant and precise flow of blood, when there is a distortion in this blood flow, the production of sperm will be significantly affected (Holstein *et al.*, 2003; Cheng and Mruk, 2010; Neto *et al.*, 2016). Several studies have proven a correlation between RI of testicular function and intratesticular arteries by using colour Doppler ultrasonography (Unlu *et al.*, 2007; Dudea *et al.*, 2010;

Hillelsohn *et al.*, 2013; Rebik *et al.*, 2019). Resistivity index have been used as a biomarker to determine male infertility because a lot of pitfalls has been associated with microscopic semen analysis. Additionally, masturbation as a way of producing semen sample for sperm analysis by males has been questioned and others do not find it suitable when is being done at the clinical laboratory. It is very imperative to develop more user friendly and accurate diagnostic test such as resistive index (Elzanaty and Malm, 2008; Vasan, 2011; Wang and Swerdloff, 2014; Pottinger *et al.*, 2016).

Several studies have concluded that RI is a predictive parameter for spermatogenesis (Pinggera *et al.*, 2008; Al-Naffakh, 2012b). Past studies by Al-Naffakh (2012b) showed that there are differences in RI by men with normal sperm count from those with abnormal sperm count. A total of 42 normal dogs on clinical examination were studied by Saypol *et al.* (1981) using CDUS. These researchers established the normative value of blood flowing to the testes using the RI. Also, experimental studies on 14 dogs done to assess the testicular blood flow of contra-lateral testes, observed that the reduction in contra-lateral blood flow to the testes is not caused by unilateral testicular torsion (de Souza *et al.*, 2015). Furthermore, ischemia of the testes have been associated with an increased RI (Tarhan *et al.*, 2000). Studies by Zolfaghar-Khani *et al.* (2020) found that infertile men had an increased RI of intratesticular and capsular arteries than healthy men. Tarhan *et al.*, (2000) also found out that RI of the capsular artery is of lower positive predictive value (PPV) and specificity as compared to intratesticular artery.

On the other hand, RI of capsular artery had higher sensitivity and negative predictive value (NPV) than intratesticular artery. Previous study by Biagiotti *et al.*, (2002) found out that higher values RI are commonly seen in men with varicocele than fertile men whilst within the different grades of varicoceles men no significant RI values were indicated. Studies by Biagiotti *et al.* (2002) also reported that men with oligoasthenispermia had lower RI as

compared to men with normal sperm analysis. These researchers were of the view that PSV and RI are reliable indicator for clinical diagnoses of men with infertility (Biagiotti *et al.*, 2002). A study by Pinggera *et al.* (2008) showed RI with values >0.6 may indicate pathological sperm count.

In diagnosing men with sub-fertility with spermatogenesis disorders, a diagnostic value of RI >0.06 were predicted (Hillelsohn *et al.*, 2013). Hille and coleagues divided their study participants into those with RI ≤ 0.6 and those with RI > 0.6. They found out that the sensitivity and likelihood ratio of RI above 0.6 to determine sperm motility in spermatogenesis were 63.27% and 1.56 respectively. These researchers were of the view that impaired spermatogenesis was associated with intratesticular artery. They further concluded that intratesticular RI > 0.6 was linked with impaired spermatogenesis. The testicular RI in men with spinal cord injury was assessed by Krebs *et al.* (2015). These researchers found that testicular RI and sperm concentration was positively correlated ($p < 0.001$).

Studies by Rehman *et al.* (2019) reported negative correlation between sperm progressive motility and RI of intraparenchymal ($r = -0.28$; $p = 0.02$) and subscapular arteries ($r = -0.236$; $p = 0.07$). Significant relationship between PSV in testicular arteries and semen count in men with varicoceles has been reported by (Semiz *et al.*, 2014). These researchers were not able to establish any relationship between hydrodynamic pattern of blood flow and semen analysis indices. Current studies have confirmed an inverse relationship between intravascular pressure and testicular blood flow, sperm morphology and motility (Ur Rehman *et al.*, 2018). Correlation between resistive index of testicular blood flow and progressive motility of sperm has been reported by Rehman *et al.* (2019). Zhang and colleagues studied the effects of the different types of varicocelectomy on arterial blood flow. They observed that sperm parameters and testicular blood flow were improved after the procedure. These researchers further reported that the mean RI, PI and PSV were reduced

significantly after the procedure but no changes occured in the EDV. They concluded that after varicocelectomy, RI and PI of the intratesticular arterial and capsular artery are vital indices for assessing the prognosis of varicocele (Zhang *et al.*, 2014)**.**

2.11.3 Hemodynamic Grading System

Ultrasonography use in assessment of varicocele is widely accepted in clinical urology practice, and the European Association of Urology's (EAU) 2011 guidelines included recommendations for using color Doppler flow mapping to diagnose venous reflux and subclinical varicocele, as well as assessing testicular size to detect hypoplasia (Stein *et al.*, 2015). However, the use of hemodynamic indicators has yet to be supported by prospective randomized trials or incorporated into paediatric and adolescent patient guidelines. In clinical practice, nevertheless, hemodynamic characterization can aid in the better description of varicocele and the selection of surgical candidates.

The lengthier the time of reflux and the seriousness of the varicocele has been used to establish hemodynamic grading system. The Hirsh *et al.* (1980) categorisation can be used to group the reflux types. This classification clasify reflux into inducible and spontaneous. Using the duration, features, location and inducibility of the reflux, varicoceles can be put into five grades. This type of classification was first suggested by Liguori *et al.* (2004) later confirmed it. Studies by Hussein (2006) observed a negative correlation between the chances of improvement in semen indices after surgery and reflux grade of hydrodynamic. In varicoceles men with grade 3-5 reflux have shown poor improvement as compared to those with grade 1 or 2 (Sarteschi, 1993). Past studies by Schiff *et al.* (2006) observed that an inducible retrograde venous flow was correlated strongly with improvements in semen analysis indices after surgery. This involves about 200% rise in sperm count and a 55% elevation in sperm motility.

2.12 ARE ALL VARICOCELE PATIENTS INFERTILE?

Though oxidative stress indicators in the seminal fluid are increased in varicocele patient with infertile, this does not always mean that fertility is compromised (Hendin *et al.*, 1999; Pasqualotto *et al.*, 2008; Mostafa *et al.*, 2009). As previously stated, 15% of men with varicocele are fertile (Alsaikhan *et al.*, 2016). As a result, it is plausible to assume that some defensive mechanisms are engaged to protect sperm from oxidative stress. Most varicocele patients are fertile which could be due to differences in genetic transcriptional responses to oxidative stress. The genetic response to oxidative stress differs between cell lines and in response to different ROS subtypes and concentrations, according to studies utilizing eukaryotic cells (Dawes, 2006).

Unfortunately, human research into the genomic and proteomic responses of germ cells to oxidative stress is sparse. However, cellular stresses linked to varicocele (such as heat and hypoxia) may elicit identical and divergent genetic responses in germ, epididymal primary, endothelial, Leydig, and Sertoli cells. Increased scrotal temperature and lack of oxygen supply to the testicles cause sperm genetic material and incidence of the disintegrating plasma membrane, damaged or non-existent acrosome, aberrant nuclear morphologies with disordered chromatin, periaxonemal cytoskeletal structures, and deranged axonemal in spermatozoa from infertile males with varicocele. Infertile men with varicocele had a greater rate of aneuploidy due to meiotic segregation mistakes, resulting in more disomies and diploidies in their spermatozoa than fertile controls, according to fluorescent in situ hybridization (Baccetti *et al.*, 2006).

However, there are some conflicting studies which observed that oxidative stress caused by heat and hypoxia might produce unique cellular genetic responses evidenced by increases in mRNA that counter the deleterious effects of ROS, thereby conferring cellular adaptability to the stressors (Allen and Tresini, 2000).

There is still limited information on the mechanisms by which nuclear or mitochondrial genes activated or repressed in response to varicocele-related cellular stresses. Few studies believe that in addition to constitutively produced cellular antioxidants, the functional genetic response to oxidative stress is an important factor in cell survival (Agarwal *et al.*, 2012). Germ cells can compensate for the higher levels of oxidative stress indicators found in fertile males with varicocele, shielding sperm from harm. These adaptive genetic responses may be overcome in infertile men with varicocele, resulting in sperm malfunction and cell death (Agarwal *et al.*, 2012).

2.13 TREATMENT /MANAGEMENT NODALITY

Depending on the type and grade of varicocele, an appropriate method of correction can be chosen. Surgery and percutaneous embolization are the two approaches to varicocele repair, each with merits and demerits. Generally, varicocelectomy (surgery) is indicated in clinically significant varicoceles if there is infertility with impaired semen parameters or sperm quality, hypogonadism and scrotal pain and testicular hypotrophy (Chan, 2011).

2.13.1 Percutaneous Embolization

First described over 30 years ago, percutaneous embolization involves blocking the refluxing spermatic or gonadal vein(s) with an embolizing material and can be performed using two approaches; the retrograde and anterograde techniques (Chan, 2011). A major advantage of percutaneous embolization is a short time for return to normal activities compared to other treatment methods and it is also ideal for treating persistent varicoceles after surgical repair (Sze *et al.*, 2008; Chan, 2011). However, arterial puncture, hydroceles, infection, dislodging of embolizing material, and side pains at higher rate (up to 30%) are some of the disadvantages of percutaneous embolization (Chan and Goldstein, 2002; Bittles and Hoffer, 2008).

2.13.2 Antioxidants and Anti-Inflammatory Agents

Oral antioxidants as a therapeutic alternative or an adjuvant treatment to varicocele repair in varicocele-related infertility have lately been investigated. The use of a NOS inhibitor (aminoguanidine) in a rat model of varicocele resulted in improved semen characteristics and decrease in sperm DNA fragmentations (Abbasi *et al.*, 2011). Vitamin E has also been proven in an experimental rat varicocele model to drastically lower seminal ROS levels (Cam *et al.*, 2004).

In humans, daily oral folic acid, zinc, and pentoxifylline supplementation for three months increased sperm morphology for at least four weeks after therapy ended (Oliva *et al.*, 2009). Cavallini *et al.* (2003) investigated the effects of a 6-month course of the antioxidants L-carnitine (1 g/day) and Acetyl-L-carnitine (2 g/day) combined with the anti-inflammatory cinnoxicam (30 mg suppository administered every 4 days) in oligozoospermic infertile males with or without varicocele. Males with low-grade varicocele and/or idiopathic oligoasthenoteratozoospermia responded better to the combination than to placebo or simply the antioxidants, according to the study. A 1-year course of cinnoxicam proved to boost the semen quality of patients with low-grade varicocele in the second research by the same group (Cavallini *et al.*, 2003). The findings confirm the theory that higher oxidative stress causes decreased fertility in males with varicocele, as well as the favorable effect of exogenous antioxidant treatment in boosting the antioxidant defense system.

Similarly, after surgical varicocele correction another study found increases in both seminal parameters, including oxidative stress reduction, and conception rates in 30 infertile men who received the Chinese remedy Jingling (herbal-derived material with antioxidant benefits) (Yan *et al.*, 2004). Despite the possible benefits of oral antioxidant medications for varicocele patients, there is no definitive conclusion about their use at this time. The majority of published articles have poor design and lack adequate controls, necessitating the urgent

need for well-designed trials. Despite the lack of clear data supporting its routine prescription, it is reasonable to expect that treating patients with infertility with or without varicocele will continue to offer alternative therapy (Esteves and Agarwal, 2011).

2.13.3 Surgical repair (Varicocelectomy)

The current guidelines and the protocol by the American Urological Association (AUA), the European Association of Urology (EAU), and the American Society for Reproductive Medicine (ASRM) recommend varicocele repair for patients with palpable varicocele with one or more semen parameter abnormalities' whether or not they are attempting to conceive a child (Shridharani *et al.*, 2012; Kang *et al.*, 2021). The most common method of varicocele repair remains surgical which is achieved either by the orthodox open varicocelectomy, laparoscopy or microsurgical varicocelectomy (Diamond, 2007; Chan, 2011). The conventional/ orthodox open varicocelectomy includes the retroperitoneal high ligation, inguinal and sub-inguinal ligation (Chan, 2011). However, conventional open varicocelectomy presents with a 5-30% risk of complications such as testicular atrophy, hydroceles and injuries to the vas deferens and epididymitis and infection (Chan, 2011). The risk of persistence or recurrence is also higher with this method compare to other treatment options and due to the risk of intraperitoneal complications such as injuries to the bowel and bladder, laparoscopy is least favoured for varicocele repair (Mishra, 2008).

Microsurgical varicocelectomy is currently the best method for varicocele repair and has been adapted by many male infertility specialists as the routine standard treatment due to its favourable outcomes (Chan, 2011). This repair approach has become popular among other varicocele treatment methods because it is associated with improved Leydig cell function (Çayan *et al.*, 2020; Kang *et al.*, 2021) and reduced complications and/or recurrence of varicocele (Cayan *et al.*, 1999). The two surgical approaches are microsurgical sub-inguinal and inguinal varicocelectomy (Diegidio *et al.*, 2011) but vascular anatomy gets more

complicated as we get through the sub-inguinal region to the inguinal region, thus leaving the microsurgical sub-inguinal (lymphatic- and artery sparing) varicocelectomy the most appropriate choice (Tarhan *et al.*, 2011). Also, this method is associated with higher improvement in sperm count and sperm motility as with many surgical procedures, the experience and skills of the surgeon plays an important role in the outcome of the process (Chan, 2011). In a study, Hsiao and colleagues found significant changes in sperm count and concentration in study participants following microsurgical varicocelectomy (Hsiao *et al.*, 2011). These changes were especially significant in men between the ages of 40 and 60 years. Choi *et al.* (2009) also found improved sperm motility to be correlated with lower age at the time a patient underwent varicocelectomy. However, some studies have reported that age at varicocelectomy has no impact on the improvement of semen parameters (Ishikawa and Fujisawa, 2005; Shabana *et al.*, 2015)**.**

For couples with male associated infertility due to varicocele, the Practice Committee of the American Society for Reproductive Medicine (PCASFM) recommends; varicocelectomy, *in vitro* fertilization, and IUI are the management options (Meng *et al.*, 2005). However, varicocelectomy is the widely used procedure in treating male infertility in patients with clinical varicocele. This surgical method helps reduce intratesticular temperature to 'within normal range', hence improves spermatogenesis (Esteves, 2015). As opposed to IUI and IVF, varicocelectomy has the ability to reverse abnormal semen parameters and provide a permanent solution for infertility and it is also highly cost effective (Shabana *et al.*, 2015). Furthermore, the complications associated with varicocele repair are rare and are usually mild. Scrotal numbness, hydrocele, wound infection, rarely testicular atrophy and persistence of varicocele are some of the possible complications of varicocele repair (Cayan *et al.*, 1999).

For couples attempting to get pregnant, treatment of varicocele is indicated after the following conditions are met; 1) presence of a palpable varicocele on physical examination; 2) female partner is having normal fertility; 3) a known infertility with the couple, and 4) the male partner should be presenting with abnormal semen parameter after at least two semen analyses (Shridharani *et al.*, 2012; Kang *et al.*, 2021). However, for persons presenting subclinical varicocele with normal semen parameters, treatment is not indicated. Also, only large varicoceles, which are usually palpable, have been shown to be associated with male infertility (Medicine, 2008).

For a patient presenting palpable varicocele, atypical semen parameters, and a wish for future procreation, varicocele repair is recommended. And for young adults with normal semen in the presence of varicocele, monitoring is done by performing semen analyses yearly as there is a risk of progressive testicular dysfunction. This allows for early detection of reduced spermatogenesis. Male adolescents with unilateral or bilateral varicoceles and reduced testicular size are also viable candidates for varicocele repair (Yamamoto *et al.*, 1995; Cocuzza *et al.*, 2008). In the absence of reduced testicular size, annual follow-up with measurements of testis size and/or semen analyses should be done to detect early signs of varicocele-related testicular injury and varicocele repair performed upon detection of semen abnormality (Medicine, 2008).

2.13.3.1 Effect of varicocelectomy on semen quality, gonadotropins, and testicular hemodynamics

The reasons why the reproductive potential of patients who have undergone varicocele repair do not always improve are unknown, and there is a lack of solid evidence to obtain prognostic markers that could help identify the best candidates for therapy. According to the scant data available, infertile men who have grade I or II varicoceles are more likely to benefit from varicocele repair (Schlesinger *et al.*, 1994; Matkov *et al.*, 2001; Colpi *et al.*,

2006). In terms of age, an intriguing study found that advanced paternal age has no bearing on the fertility results of males with varicocele-related infertility. However, it is important to note that these findings may be biased due to the fact that older men (over 40 years) may have considerably larger number of participants presenting secondary infertility compared with younger men (Zini *et al.*, 2008b).

2.13.3.2 Effect of varicocelectomy on semen parameters

The relationship between impaired spermatogenesis and varicocele has been well studied (Dubin and Hotchkiss, 1969; Terquem and Dadoune, 1981). Sertoli cell changes, premature sloughing of germ cells into the seminiferous tubules and different degrees of hypo-spermatogenesis had been the report of most authors. Tulloch (1952) was the first author to publish the benefits of varicocele repair to male fertility. The study observed spontaneous conception after varicocelectomy in men with azoospermia. After those years, varicocelectomy has been the most recommended surgery for male fertility factor. Several studies in the past decade have reported the importance of varicocelectomy in non-obstructive azoospermic men located at physical assessment (Mehan, 1976; Kadioğlu *et al.*, 2001; Pasqualotto *et al.*, 2003; Gat *et al.*, 2005a). This surgery is the choice made by men with poorer baseline features (Zini *et al.*, 2008a). Moreover, couples who do not prefer varicocelectomy may go in for ICSI to increase their probability of given birth (Pasqualotto *et al.*, 2012).

Studies by Zini *et al.* (2005) observed that varicocelectomy improves spermatogenesis and sperm function. It has been widely noted that there is recovery of sperm production after varicocelectomy. This recovery is said to be suboptimal. A study involving 95 men with a unilateral/bilateral varicocele and non-obstructive azoospermia observed a significant rise in sperm retrieval rate after varicocelectomy (Inci *et al.*, 2009). However, these researchers came into conclusion that there is not enough quality sperm to obtain conception with ICSI,

intercourse or intrauterine insemination without the use of testicular sperm extraction after varicocelectomy in azoospermic and varicoceles patients. Additionally, elevated sperm retrieval rate had been reported in subjects who underwent varicocelectomy than those who do not partake in varicocelectomy (Haydardedeoglu *et al.*, 2010). These authors further observed that the rate of live births and pregnancy were higher in the varicocelectomy group. They further concluded that non-obstructive azoospermic patients undergoing an ICSI cycle, it is advisable to use varicocele repair.

According to Stechel and colleagues, after varicocelectomy of men with larger varicoceles there is superior improvements in sperm motility, concentration and fertility index (Steckel *et al.*, 1993; Su *et al.*, 1995). In the same vein, studies by Steckel *et al.* (1993) have shown that greater improvement in sperm count after varicocelectomy were associated with larger varicoceles. Several research has found no correlation between clinical grade of varicoceles and elevation in serum testosterone after varicocele repair (Su *et al.*, 1995; Hsiao *et al.*, 2013; Abdel-Meguid *et al.*, 2014). Another study reported about 82% improvement in sperm concentration, morphology and motility in azoospermic and oligoteratoastenospermic patients after embolization of sinternal vein (Gat *et al.*, 2005a) . These authors were of the view that treatment of varicoceles may improve spermatogenesis in azoospermic patients.

In roughly 65 % of the treated males, varicocelectomy studies show a significant improvement in one or more semen parameters (Schlesinger *et al.*, 1994). In a meta-analysis, Agarwal *et al.* (2007) used sophisticated approaches to minimize selection bias in 17 observational studies and RCTs, as described by the Potsdam Consultation (Cook *et al.*, 1995). After varicocelectomy, sperm motility, concentration, and morphology all rose by 10%, 9.7 million/mL, and 3%, respectively. After surgery, the average period for improvement in semen parameters and acheiveing natural pregnancy was about 5- and 7 months respectively (Colpi *et al.*, 2006; Agarwal *et al.*, 2007).

Additionally, Abdel-Meguid *et al.* (2014) observed that sperm concentration in pre and post-varicocelectomy were positively correlated with testosterone changes in men who underwent varicocelectomy. They further conclude that improvement of sperm concentration occurs after varicocelectomy. Retrospective studies by Cayan *et al.* (1999) found out that subjects with low sperm counts after varicocelectomy had no improvement in their testosterone and vice versa.

Experimental studies using animal models by Sofikitis *et al.* (1992) found out that varicocelectomy of secondary right varicocele caused an improvement in semen indices. This finding shows the harmful outcome of primary varicocele on the right testis. Using Tygerberg strict classification, sperm morphology and quality shows variation from 2% to 6%, this proves that the functionality of sperm is improved after varicocelectomy (Pasqualotto *et al.*, 2006). On the contrary, despite the improvement of semen indices after varicocelectomy, ART is needed for most couples to initiate conception (Aboulghar *et al.*, 1997). One importance of varicocelectomy is that it allows the provision of sperm by ejaculation in azoospermic men who are about to undergo invasive testicular sperm retrieval (Turek *et al.*, 1997; Silber, 2000).

In aged couples, it is of utmost importance in knowing the timing of improvements in semen parameters after varicocelectomy. Studies have shown that the whole process of spermatogenesis takes about 64 days in humans (Schlegel and Katzovitz, 2020). Two studies that assessed the duration between varicocele surgery and improvements in semen indices unveiled that, this improvement occurs within the first three months after the surgery but no changes occurred after the third month (Al Bakri *et al.*, 2012; Fukuda *et al.*, 2015). Interestingly, improvements occurring within 3-6 months had been suggested by ASRM practice committee (Control and Prevention, 2014). These outcomes help in therapeutic

designing for varicocele candidates and not underscoring other approaches for fertility management.

The improvement in spermatogenesis in azoospermic men undergoing varicocelectomy is attainable, if there is no observation of genetic abnormality (Cayan *et al.*, 2001). Two studies have shown that the area of deletion of Y chromosome is essential predictive indicator for sperm retrieval as compare to a coincident varicocele (Cayan *et al.*, 2001; Foresta *et al.*, 2001). Studies by Schlegel and Kaufmann (2004) involving 21 patients, out of which 14 had Klinefelter syndrome and the rest had partly deletions of Y chromosome reported history of varicocelectomy do not affect the results of TESE in Klinefelter's men. The impact of varicocelectomy on spermatogenesis could be as a results of venous pressure, intratesticular interstitial fluid volume and temperature changes but not changes in only hormones (Su *et al.*, 1995).

2.13.3.3 Effect of varicocelectomy on gonadotropins

Varicocele repair and other forms of varicocele treatment are generally geared towards the prevention of backflow of venous blood via the internal spermatic veins. The maintenance of peripheral autonomic and sensory nerve structure is one of the utmost functions of testosterone. The serum testosterone also plays a role in erectile function by regulating the trabecular smooth muscle (Traish *et al.*, 1999). The partly alteration in function of the hypothalamus-pituitary gonadal axis in patients with varicoceles has been proven by several studies within the past few years. Research by Weiss *et al.* (1978) observed a reduction in *in vitro* production of testosterone in serious oligospermia and varicocele men in relation to normal population. This outcome from their study was later resonanced by Ando *et al.* (1983) who found an elevation in 17-hydroxyprogesterone-to-testosterone ratio.

There have been conflicting studies which shows that serum testosterone did not vary significantly between men with varicoceles and those without varicocele (Swerdloff and

Walsh, 1975; Steckel *et al.*, 1993). Leydig cell hyperplasia have been observed in some studies and this may account for no significant difference in testosterone biosynthesis in varicocele and healthy men (Sirvent *et al.*, 1990). There is controversy as to whether the use of varicocele repair as treatment for varicocele can reverse the hormonal dysfunction. A trend towards rise of serum testosterone was observed in 14 men with varicocele after retroperitoneal varicocelectomy which was not appreciably distinct from preoperative quantities (Hudson *et al.*, 1985). Moreover, not appreciably elevation in blood testosterone were seen in 24 men with varicocele who were treated with ligation of the internal spermatic vein as a treatment procedure (Segenreich *et al.*, 1986). Conversely, 10 sexual inadequate and varicocele patients with an average testosterone levels of 346.2 ng/dl preoperatively were study by Comhaire and Vermeulen (1975). These authors observed that the serum testosterone rise to normal levels after varicocelectomy. Serum testosterone is said to be highly variable in different individuals.

Furthermore, studies by Su *et al.* (1995) observed an elevation in serum testosterone after performing microsurgical inguinal varicocelectomy on 53 patients with preoperative testosterone levels ranging from 122-585 ng/dl. Preoperative and postoperative serum testosterones in 325 patients with visible varicoceles and in 510 men with vasectomy relapses without varicoceles were studied by Tanrikut *et al.* (2011). These authors reported that subjects with varicoceles had reduced testosterone than those without varicoceles. After varicoceles repair, serum testosterone rose to about 2/3 of these patients. The observation of improvement in serum testosterone in infertile men after varicocelectomy had been noticed in other studies (Sathya Srini and Belur Veerachari, 2011b; Zohdy *et al.*, 2011). Moreover, a retrospective study by Hsiao *et al.* (2011) reported increase in blood testosterone levels in men who were infertile after varicocele repair. Additionally, a meta-analysis by Li *et al.* (2012) observed a significant difference in average serum testosterone after varicocelectomy

in infertile men. The authors reported an elevation in serum testosterone by 97.48 ng/Dl (95% CI, 43.73-151.22) after varicocelectomy. According to Su *et al.* (1995) varicocelectomy do not cause elevation of serum testosterone in all varicocele men except those with very low testosterone preoperatively. These authors also observed that men with firm testis have higher hormonal response than those with bilateral soft testis after varicocelectomy. Studies have shown that after varicocelectomy, rise in serum testosterone are often seen in those with smaller varicoceles than those with bigger varicocele preoperatively (Su *et al.*, 1995).

Varicocelectomy has been recommended as the best option to treat and prevent low blood testosterone levels in varicoceles patients even those without outrageous sperm quality (Mehta and Goldstein, 2013). Longitudinal studies by Abdel-Meguid *et al.* (2014) reported that varicocelectomy infertile men resulted in an improvement in total testosterone, with 12.9 percent change from the mean baseline. The authors further explained that more mean change was seen in men with baseline biochemical hypogonadism than those with eugonadism. However, these authors did not observe any significant difference serum testosterone in fertile and infertile varicocele control group during follow ups. This finding is contradictory to the regression toward the mean phenomenon as a possible explanation for the improvement of serum testosterone after varicocelectomy in men with baseline low gonadotropins.

Varicocelectomy may improve gonadal function in men with varicocele (Li *et al.*, 2012; Tiseo *et al.*, 2016; Çayan *et al.*, 2020). Çayan *et al.* (2020) reported that approximately 60 - 80% of men with low serum testosterone had normalized testosterone levels after varicocele repair. Li *et al.* (2012) in a review and meta-analysis reported that the mean serum testosterone concentrations increased after varicocelectomy. Nonetheless, there are conflicting reports on whether varicocelectomy result in changes in serum FSH and LH

levels or not. Some studies reported no significant changes in the levels of serum FSH and LH (Su *et al.*, 1995; Salem and Mostafa, 2009), yet others noted decreased serum FSH and LH levels following varicocelectomy (Sathya Srini and Belur Veerachari, 2011a; Tian *et al.*, 2018). A meta-analysis by Tian *et al.* (2018) showed that serum FSH level (95% CI: 0.19-0.77; p=0.001) and serum LH level (95% CI: 0.25-0.91; p=0.0005) were higher before operation than after varicocelectomy. The Leydig cells function to produce testosterone but this is controlled by luteinizing hormone. Follicle-stimulating hormone functions to promote the beginning of testosterone production; in the process, LH is maintained. Hence, there is crosstalk with the changes of serum testosterone, FSH, and LH.

Again, the use of gonadotropin-releasing hormone as a screening tool to determine whether varicocele patients may or may not undergo varicocelectomy had been suggested by several studies (Hudson and Mckay, 1980; Hudson, 1996). These studies were of the view that the rise in FSH and LH after giving synthetic GnRH to stimulate the hypothalamus-pituitary gonadal axis may be used as a preliminary indictor for varicocelectomy treatment (Hudson *et al.*, 1985; Fisch *et al.*, 1989; Fisch *et al.*, 1990). Most of the studies have reported about 3-5 times elevation in LH levels in severe oligozoospermia and varicocele men whiles 2-3 times rise in FSH in such patients after the injection of synthetic GnRH (Hudson, 1988; Bickel and Dickstein, 1989; Castro-Magana *et al.*, 1990; Fujisawa *et al.*, 1994). Fujisawa *et al.* (1994) further observed that GnRH testing is a good predictor for varicocelectomy treatment in varicocele men. These investigators study involved 121 varicocele infertile men. They conducted a study of GnRH in these patients out of which 73.5% were positive for GnRH. They later performed varicocele repair, in the subjects that tested for GnRH, where 74.1% became GnRH negative. These researchers also observed improvement in semen indices and pregnancies in the wives of those that tested positive for GnRH before varicocelectomy

(Fujisawa *et al.*, 1994). Also, report from Su *et al.* (1995) observed that FSH and LH do not elevate after microsurgical varicocelectomy.

2.13.3.4 Effect of Varicocelectomy on Inhibin

Inhibin is a hormone secreted by the Sertoli cells that negatively regulate FSH production. Inhibin, GnRH, gonadal sex steroids, follistatin, pituitary activins are factors that affects FSH production. Inhibin is said to be a direct predictor of functionality of Sertoli cells whereas FSH is an indirect indicator (Andersson *et al.*, 1998). As stated by Illingworth *et al.* (1996), inhibin is a member of TGF-β, it's a heterodimeric glycoprotein in structure. Its first isolation occurred in 1985 from porcine follicular fluid (Robertson *et al.*, 1985). It is made up of α and β subunit of 53KDa and 43KDa respectively. Inhibin is said to exist in two different forms thus Inhibin A and B. They are different from each other due to the variation in the amino acid chains at NH2-terminal (Ling *et al.*, 1985). Studies by Groome (1991) demonstrated that inhibin B is the most physiological active form of the hormone. In men with abnormal spermatogenesis, negative correlation is said to exist between FSH and Inhibin (Illingworth *et al.*, 1996).

Also, direct correlation between blood inhibin B quantities and testicular volume have been reported by Klingmüller and Haidl (1997). This assessed the amount of inhibin-secreting Sertoli cells. The authors also observed that when the inhibin levels go below 112 pg/ml, there is reduction in sperm concentration. Studies by Fujisawa *et al.* (2001) reported a correlation between testicular volume, sperm concentration and inhibin B levels in infertile men with varicocele. These researchers also found out after varicocele repair there was no difference in inhibin quantities between subjects with elevated sperm concentration and those without elevated sperm concentration. Significant rise in inhibin levels in varicocele patients after varicocele repair had been reported by Pierik *et al.* (2001). Similarly, after varicocelectomy, increase in sperm motility, concentration and inhibin B levels has also been

observed by Ozden *et al.* (2008). Andersson *et al.* (1998) demonstrate a relationship between post pubertal period spermatid germ cell and serum inhibin B.

Additionally, Von Eckardstein *et al.* (1999) demonstrated that serum inhibin quantity is connected to the percentage of Sertoli-cell-only tubules with spermatids. It may be deduced from these studies that there is correlation between inhibin production and spermatids. As stated by Fretz and Sandlow (2002), varicocele impacts negatively on function of the testis by hampering scrotal regulation of heat. Two studies have shown that spermatids are mostly affected by germ type to hyperthermia, after varicocele repair scrotal temperature is said to decrease to the normal range (Jegou *et al.*, 1984; Wright *et al.*, 1997). The improvement in the late phase of spermatogenesis could be explained as a results of the rise in blood inhibin B after varicocelectomy (Ozden *et al.*, 2008).

2.13.3.5 Effect of varicocelectomy on testicular hemodynamics

The objective for the treatment of infertile patients with varicocele repair is to eradicate backward venous flow to the pampiniform plexus from the internal spermatic veins. Studies have showed that about 60-70% men with varicoceles show improvement in seminograms after undergoing retroperitoneal/microsurgical subinguinal ligation (Pryor and Howards, 1987; Madgar *et al.*, 1995). The demand and supply of blood during spermatogenesis is a sensitive and precise process. Adequate blood supply to the testes is needed for spermatogenesis and decreased blood supply may cause ischemia (Al-Naffakh, 2012a; Kang *et al.*, 2021).

In most studies, Doppler indices are used to obtain information about blood flow within the testicles with a recently introduced ultrasonic parameters, resistive index (RI) and pulsatility index (PI), which shows testicular parenchymal perfusion and microcirculation function. Earlier studies reported no significant difference between the mean Doppler indices before and after surgical ligation of the spermatic veins (Študent *et al.*, 1998; Cocuzza *et al.*, 2010).

In recent times, however, Tarhan *et al.* (2011) found a significant improvement in supply of blood to the testis and sperm characteristics after microsurgical sub-inguinal varicocelectomy. In this study, though there was no significant difference in the Doppler indices on the right artery, the mean values of blood flow velocities (peak systolic and end-diastolic) on the left artery increased while the resistive and pulsatility indices decreased significantly after surgery (Tarhan *et al.*, 2011). In another study, Rehman *et al.* (2019) reported a significant negative correlation between progressive motility of spermatozoa and RI of the capsular arteries in sixty prospective patients undergoing microsurgical varicocelectomy.

2.13.3.6 Effect of varicocelectomy on oxidative stress

The key pathogenic mechanism underlying testicular injury in men with varicocele-related infertility is thought to be oxidative stress (OS). Several investigations have looked into the resolution of oxidative stress markers in these patients. Varicocelectomy has been shown to reduce or normalize the common 4977-bp mitochondrial DNA deletion (Bozkurt *et al.*, 2012), 8-OHdG, TBARS, and nitrate plus nitrite content in spermatozoa of infertile men with varicocele (Sakamoto *et al.*, 2008), all of which are elevated in infertile men with varicocele. Varicocelectomy has also been demonstrated to improve seminal and peripheral blood plasma total antioxidant capacity (TAC) as well as seminal antioxidants like alpha-tocopherol, retinol, zinc, ascorbate, and selenium (Marra *et al.*, 1998; Mostafa *et al.*, 2001; Cervellione *et al.*, 2006). Varicocele repair has also been shown to lower total and specific markers of seminal reactive oxygen species (ROS) increase (malondialdehyde, H_20_2, nitric oxide, 8-OHdG, and hexanoyl-lysine) in several trials (Mostafa *et al.*, 2001; Dada *et al.*, 2010). However, one controlled study (Yeşilli *et al.*, 2005) and two uncontrolled trials (Rodriguez Peña *et al.*, 2009; Söylemez *et al.*, 2012) found no evidence of varicocele repair reducing oxidative stress.

In a similar vein, Rodriguez Pea et al. (2009) found no changes in NO levels following varicocele repair. However, their study included varicocele individuals without a history of infertility, which could have skewed their findings. Despite a good effect on sperm DNA integrity, Lacerda et al. (2011) found that varicocele repair was not related to a detectable beneficial benefit of varicocelectomy in lowering seminal levels of malondialdehyde. Because the levels of seminal plasma oxidative stress were not enhanced pre-operatively in their group of adolescents, the authors speculated that varicocele repair was unable to affect them, and that varicocele itself does not affect seminal plasma lipid peroxidation in this subset of patients. However, it is yet uncertain if varicocele has a time-dependent influence on OS markers in adolescents at a later age.

Notably, the amount of time it takes to see any improvement in oxidative stress markers following varicocele surgery varies. Dada *et al.* (2010) found that the decrease in ROS levels following varicocelectomy was proportionate to the length of the postoperative period in one study. Damage to sperm DNA improved only after 6 months in their study, even though it generally takes a long time for it to return to normal. Mostafa *et al.* (2001) found that NO, H2O2, and malondialdehyde were significantly lower 3- and 6-months following varicocele repair, whereas superoxide dismutase, catalase, vitamin C, and glutathione peroxidase were significantly higher. Chen *et al*. (2008) also found that 6 months following varicocele repair, 8-OHdG levels, and sperm mitochondrial DNA deletions were lower, but seminal plasma protein thiols and ascorbic acid levels were higher than preoperative values. Finally, Sakamoto *et al.* (2008) discovered that after varicocele repair, a 6-month time lag is required to produce a significant improvement in seminal ROS indicators such as NO, 8-OHdG, and hexanoyl-lysine.

Two noteworthy findings highlight the necessity of assessing oxidative stress in varicocele and observing oxidative stress relief following varicocele repair. First, these markers could

be used to predict how infertile men will respond to varicocele correction. Shiraishi and Naito (2006) found that men who responded to varicocelectomy had higher basal levels of preoperative testicular modified proteins than non-responders ($p = 0.014$) when analyzing levels of testicular 4-hydroxynonenal-modified proteins before varicocelectomy. More crucially, the researchers discovered that men lacking oxidative stress did not respond as well to varicocelectomy, bolstering the argument that individuals with high levels of oxidative stress should be considered excellent candidates for varicocelectomy (Shiraishi and Naito, 2006). Secondly, several studies have found a link between the resolution of oxidative stress markers and postoperative improvements in semen parameters and sperm quality (Marra *et al.*, 1998; Mostafa *et al.*, 2001). Hurtado de Catalfo *et al.*, (2007), for example, observed increases in both semen parameters (sperm concentration and shape) and blood testosterone levels three months following surgery, which were accompanied by improvements in seminal protein carbonyl, zinc, selenium, and DNA fragmentation. Mostafa *et al.* showed significant improvements in sperm count, motility, and morphology 3 and 6 months after varicocele repair, in agreement with improvements in seminal levels of malondialdehyde, H_2O_2, NO, catalase, vitamin C, superoxide dismutase, and glutathione peroxidase (Mostafa *et al.*, 2001).

In conclusion, current research suggests that varicocele repair can help restore or improve fertility by reducing oxidative stress and normalizing antioxidant defence mechanisms. Furthermore, data suggests that this positive effect is time-dependent, with better results occurring 6 months following surgery. The use of oxidative stress indicators to track postoperative outcomes after varicocele repair could be beneficial. Subjects with palpable varicocele and aberrant semen parameters or sperm function tests, including oxidative stress, appear to be ideal candidates for varicocele correction.

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Chapter 3

PAPER I

3.1 EFFECT OF VARICOCELECTOMY ON SEMEN PARAMETERS OF MEN SEEKING INFERTILITY TREATMENT IN TAMALE, GHANA

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Author Statement

Yussif Adams (Candidate)
Conceived and designed the research, did field sampling and part of the laboratory analysis, analysed and validated the results, drafted and reviewed the manuscript.

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Performed the surgery, supervised the work, provided resource, validated the results, and reviewed the manuscript.

Nafiu Amidu
Supervised the work, validated the results, and reviewed the manuscript

3.2 ABSTRACT

Background*: Spontaneous pregnancy maybe impossible due to infertility and currently in medical practice, impairment of semen parameters suggests that a varicocele might be present. The study aimed to determine the effect of microsurgical sub-inguinal*

varicocelectomy on semen parameters among men seeking infertility treatment in Ghana. ***Methods****: This was an intervention study conducted in Tamale Teaching Hospital in the Tamale Metropolis from September 2017 to August 2021. The study involved two groups; the surgery group (n = 75) and the observed group (n = 63). Duplicate semen samples (mean values adopted) were collected at the onset and assessed according to the criteria established by World Health Organization (WHO), 2010. Varicocelectomy was performed for the surgery group and no intervention was given to the observed group. The two groups were followed for 180 days and repeated semen samples were collected and analyzed. The data was computed using GraphPad Prism (v8.0) at an alpha of 0.05.* ***Results****: All the men had varicocele and were aged between 46.0 and 67.0 years old. There was no difference between semen parameters among the two groups before the surgery. However, after 180 days of follow-up, all of the semen parameters significantly improved in the surgery group (p<0.0001), while sperm concentration (p = 0.0068), progressive motility (p = 0.0281), and normal sperm morphology (p = 0.0015) decreased in the observed group. The surgery group had an overall percent increase in total sperm count (840.7%; p=0.0197), sperm concentration (582.1%; p=0.0125), total viable sperms (155.2%; p<0.0001), and normal sperm morphology (110.9%; p<0.0001) while immotile sperms (-51.71%; p<0.0001) reduced. A pregnancy rate of 25.3% (19/75) was reported among the surgery group but none was reported among the observed group after 180 days.* ***Conclusion****: Microsurgical sub-inguinal varicocelectomy improves semen parameters and hence effective treatment of infertile men with a clinically palpable varicocele. It is recommended to use this choice for similar patients, however, further studies with a larger sample size are needed to provide more evidence to recommend this therapy.*

Keywords: varicocele, sub-inguinal varicocelectomy, male infertility, semen parameters, Ghana

3.3 ABBREVIATIONS AND ACRONYMS

AFLP active forward linear progression

BMI body mass index

DBP diastolic blood pressure

RCT randomized controlled trial

ROS reactive oxygen specie

SBP systolic blood pressure

SD standard deviation

SEM standard error of the mean

TTH Tamale Teaching Hospital

WHO World Health Organization

3.4 INTRODUCTION

Infertility renders spontaneous pregnancy nearly impossible and currently in medical practice, impairment of semen parameters suggests that a varicocele might be present (Agarwal *et al.,* 2016). Varicocele is an abnormal dilatation of the pampiniform plexus draining the testicles with reflux of venous blood (Clavijo *et al.,* 2017; Bertolotto *et al.,* 2020). This medical condition is associated with male infertility as studies have found that approximately 30 to 50% of men with primary infertility (Nashan *et al.,* 1990; Jarow *et al.,* 1996) and 60 to 81% of patients with secondary infertility are reported to have varicocele (Choi and Kim, 2013).

The main cause of infertility in varicocele patients is unknown. Several studies have linked the low or poor quality of sperm production to; anatomical anomaly of varicocele (Xue *et al.*, 2012; Kadioglu *et al.*, 2014), increased scrotal temperature (Shiraishi *et al.*, 2012), an adrenal hormone, and gonadotoxic metabolite refluxes (Inci and Gunay, 2013), epigenetics changes (Seidel, 2015), and increased production of reactive oxygen species (ROS) in the scrotum which results in sperm DNA damage (Agarwal *et al.*, 2014). These related factors may act individually or synergistically affecting spermatogenesis in varicocele patients.

In the treatment of infertility in varicocele patients, varicocele repair is widely used. However, there are conflicting reports on the effect of varicocelectomy on male fertility. Some studies have attempted to clarify the efficacy of surgical remediations on sperm density, concentration, motility, and morphology. Zini *et al.* (2005) reported that infertile men showed improved spermiogram six months after microsurgical varicocele repair. Similar findings were observed by Kadioglu *et al.* (2014) who concluded that all seminal parameters significantly improved post-surgery when compared with preoperative values. Counterrally, Krause *et al.* (2002) in a multicentre, prospective randomized study on varicocele treatment in infertile men found no significant increase in pregnancy rate in the treated group compared with controls. Breznik *et al.* (1993) and Rageth *et al.* (1992) also reported that varicocelectomy bears no influence on male fertility.

To determine whether or not infertility-related treatment following varicocele repair is successful, the endpoints commonly analyzed are semen parameters (that is; semen volume, sperm count, sperm concentration, motility, and/ or morphology), pregnancy rate (PR), and/ or integrity of sperm DNA. But most studies consider semen parameters to be the primary outcome parameter of varicocele therapy (Inci and Gunay, 2013). Therefore, this study aims

to determine the effect of microsurgical sub-inguinal varicocelectomy on semen parameters of men seeking infertility treatment in Tamale, Ghana.

3.5 MATERIALS AND METHODS

3.5.1 Ethical consideration

The Ethics and Review Board of the Department of Research and Development, Tamale Teaching Hospital approved this study (Number: TTH/R&D/SR/119) and has therefore been performed following the standards laid down protocol in the 1964 Declaration of Helsinki. Informed consent was obtained from all the participants before the study. Participants were kept anonymous; participation was voluntary and information obtained remained confidential to the researchers only.

3.5.2 Study design

This was an intervention study design involving two groups; the surgery group (n = 75) and the observed group (n = 63) (**Figure 3.1**). This study was conducted at Tamale Teaching Hospital in the Tamale Metropolis from September 2017 to August 2021.

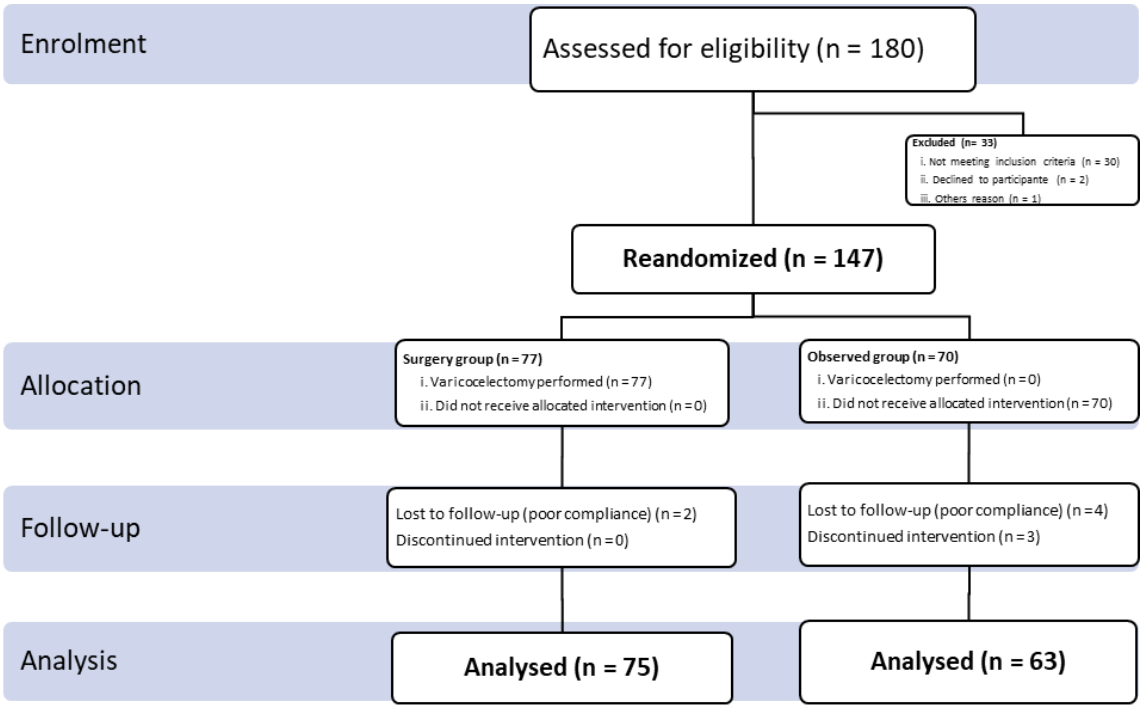

Figure 3.1: Flowchart diagram

A total of 180 participants were initially recruited for the study of which 33 did not meet the eligibility criteria. This reduced the number of participants to 147 who were randomized in to surgery group (n = 77) and the observed group (n = 70). Two (2) from the surgery group and four (4) from the observed group drop-out due to poor compliance and three (3) from the observed group discontinue on personal reasons only known to them. Participants eligible for inclusion were 75 in the surgery group and 63 in the observed group as shown in **Figure 3.1**.

3.5.3 Participant's recruitment

Participants eligible for inclusion were offered the option of immediately undergoing microsurgical sub-inguinal varicocelectomy (surgery group) or being observed for one year with a subsequent re-evaluation of the management plan and possibly delayed the operation (observed group). Based on the willingness to equally accept either option, eligible participants were allocated at a balanced one-to-one ratio to either the observed group or the

surgery group. All consented participants were sexually active men who had maintained a stable heterosexual relationship for at least 2 years. A stable heterosexual relationship was considered as one in which the man was involved and maintained sexual relations, regardless of marital status.

3.5.3.1 Inclusion criteria

Participants with varicocele, male factor fertility, and spermiogram alterations were included in the study. Male factor fertility was defined as the inability of a couple to conceive a child after one year of unprotected sexual intercourse with a normal female partner or spouse (i.e., normal reproductive history, normal ovulation, and tubal patency) (Nallella *et al.*, 2006).

3.5.3.2 Exclusion criteria

Participants with a history of smoking (as smoking is an independent risk factor for infertility), excessive alcohol use (chronic alcoholics i.e., men who takes more than 4 a day, or 14 or 15 in a week), drug consumption, or incomplete/inconclusive questionnaires were excluded. Patients who had a history of mumps orchitis, uncontrolled hypertension (blood pressure ≥ 140/90 mmHg, uncontrolled diabetes (glycated hemoglobin>7%), use of anti-estrogen and/or testosterone replacement therapy, undescended testis, or orchidectomy as well as patients on long term statins were all also excluded.

3.5.4 Clinical evaluation

Diagnosis of varicocele was based on physical examination and was confirmed by ultrasound scan examination. Dubin and Amelar (1970) approach was employed to detect, confirm and clinically grade varicocele. Clinical Grade 1 – varicocele detected by palpation only upon Valsalva maneuver; Clinical Grade II – varicocele detected only upon Valsalva maneuver; and Clinical Grade III – varicocele detected without Valsalva maneuver. Clinical

examination was carried out by one and the same consultant urologist who also performed the surgeries.

Scrotal ultrasound was used to diagnose the non-palpable enlargement of the venous plexus of the spermatic tone (Marsman and Schats, 1994). Two phases of scrotal ultrasound scans were carried out on each participant; the first phase was with participants in the supine position (with penis resting on suprapubic region) and the second in an upright position. The examination was conducted with a Samsung Medison Accuvix V20 scan (Samsung Electronics, South Korea) equipped with linear, high-resolution, and high-frequency (7.5 – 14 MHz) probe keen to the study of soft body parts and with color Doppler for detecting slow flows and scanning surface of at least 5 cm (Hussein, 2006). An ultrasound scan was done to evaluate testicular malposition, blood reflux along the pampiniform plexus, or the extent of any fluid collections.

3.5.5 Data collection

Sociodemographic data, cigarette smoking, and medical history were gathered with a structured pre-tested questionnaire. The Omron blood pressure monitor was used to measure the blood pressure of the participant. These included; systolic blood pressure (SBP), diastolic blood pressure (DBP) and pulse rate, and categorization of normotension (SBP<140mmHg/DBP<90mmHg) and hypertension (SBP>140mmHg/DBP>90mmHg) was based on WHO cut-offs as cited by Mittal and Singh (2010).

Anthropometric measurements were done on all study participants. The Seca 213 portable Stadiometer (Seca Corp., Hamburg, Germany) was used to measure the height of the participants to the nearest 0.1 cm. The Omron HBF-516B Body Composition Analyzer and Electronic Scale (Omron Corp., USA) was used to measure the weight and body mass index

(BMI) calculated. Body fat and muscle mass were recorded as percentages of the total body weight at intervals of 0.1%.

3.5.6 Semen sample collection and analysis

A clean sterile wide-mouthed plastic container confirmed to be non-toxic for spermatozoa was given to each participant to produce semen samples by masturbation (two semen samples – mean value adopted) after 2 to 5 days of sexual abstinence. To minimize temperature fluctuations and control the time between semen sample collection and analysis, samples were collected in a private room near the laboratory.

Macroscopic analysis of the sperm was performed with the observation of liquefaction time, viscosity, semen volume, color, and pH. For microscopy analysis, a 100 µm-deep disposable Neubauer hemocytometer chamber was loaded with a well-mixed liquefied semen sample, covered with a coverslip allowing spermatozoa to settle in the chamber. Sperm concentration count and sperm motility were determined using x200 magnification (i.e., x20 objective lens with x10 ocular lens combination). Only spermatozoa with head and tail were counted and reported. The semen was analyzed according to WHO criteria (WHO, 2010). Vitality was measured using *Eosin Y 0.5% dye* (Eosin Gelblich, Darmstadt, Germany). Sperm morphology was determined according to Kruger criteria using *Nigrosin 8%* staining technique (Nigrosin, Water Soluble, Darmstadt, Germany) (Elder and Dale, 2020).

3.5.7 Interventions (Sub-inguinal microscopy varicocelectomy)

Participants were counselled about their condition, and the exact nature of the problem was explained to them by a urologist. A microsurgical open sub-inguinal varicocelectomy procedure as described by Marmar *et al.* (1985) was performed for the surgery group. Surgery was performed under spinal anesthesia, using microsurgical instruments and

magnification with an operating microscope KARL CAPS SOM 82, Germany. The lymphatic vessels and testicular artery were spared, and both internal and external spermatic veins ligated and divided. The spermatic fasciae were closed using PGA 3/0 running sutures. The wound was closed in layers and a subcuticular skin stitch was applied using 4/0 PGA sutures. Wound dressing was removed after 24 hours. No antibiotics were employed and the pain was managed by using 1-gram of rectal paracetamol during the period of recovery and followed by oral paracetamol 1-gram TID for the next 24 hours.

3.5.8 Follow-up

Both groups were followed for 180 days after the day of surgery (surgery group) or the day of the last baseline semen analysis (observed group). Participants in the observed group were advised not to use any form of contraceptives during sexual intercourse, and to abstain from tobacco/cigarette smoking. Participants in the surgery group were advised to abstain from any form of sexual activity until the surgical wound was properly healed. All participants were reassessed every 90 days to confirm that; the participant was not smoking, and was clinically examined to confirm the absence of genital infection, recurrence of varicocele, formation of hydrocele, and increased testicular size. Duplicate semen samples were collected (mean values adopted) for repeated analysis at 180 days of follow-up.

3.5.9 Statistical Analysis

All statistical analysis was performed using GraphPad Prism version 8.0 (https://www.graphpad.com/) for analysis. Categorical data were presented as frequency, percent, and charts, and quantitative data presented as mean ± standard deviation (s.d) or mean ± standard error of the mean (SEM). Kolmogorov-Smirnov test was performed on quantitative data to check whether or not the data was normally distributed. To compare the

two groups, an unpaired student t-test was used. Values before and after in each group were compared using paired *t*-test. A two-tailed p-value less than 0.05 was considered statistically significant.

3.6 RESULTS

3.6.1 General characteristics of study participants

The general characteristics of the study population are summarized in **Table 3.1-3**. The men were aged between 46.0 and 67.0 years old. The mean ± standard deviation (SD) BMI, body fat muscle mass, and visceral fat were 24.05 ± 2.948 kg/m^2, 18.63 ± 8.037%, 35.52 ± 4.50%, and 7.804 ± 3.513% respectively. The systolic blood pressure (SBP) was between 71.00 and 138.00 mmHg while the diastolic blood pressure (DBP) was between 64.00 and 87.00 mmHg. The baseline total semen parameters (pH, semen volume, total sperm count, sperm concentration, motility, viability, and Kruger) are summarized in **Table 3.1**. There was no significant difference between the age (p = 0.3384), BMI (p = 0.2474) visceral fat (p = 0.2621), SBP (p = 0.5448), and DBP (p = 0.3575) of the surgery group compared with the observed group (**Table 3.2**).

From **Table 3.3**, all the men were married (100.0%), most had formal education (58.7%), none (0.0%) smoked cigarettes, and 21.7% consumed alcoholic beverages. The majority were confirmed with varicocele grade II (76.1%) with left-sided being the predominant type (93.5%). The average total sperm count (baseline) showed that the majority recorded oligozoospermia (93.5%).

Table 3.1: General (continuous variables), anthropometric characteristics, and baseline total semen parameters of study participants

Variable	Minimum	Mean	Std. deviation	Maximum
Age (years)	46.0	55.83	2.567	67.0
Anthropometry				
Weight (kg)	54.0	70.90	15.98	158.0
Height (cm)	82.3	169.3	14.25	183.0
BMI (kg/m^2)	17.9	24.05	2.948	33.0
Body fat (%)	5.9	18.63	8.037	46.9
Muscle mass (%)	23.6	35.52	4.500	43.3
Visceral fat	3.0	7.804	3.513	17.0
Blood Pressures				
SBP (mmHg)	71.0	129.6	8.597	138.0
DBP (mmHg)	64.0	72.76	7.499	87.0
Baseline (Onset) semen parameters (total)				
pH	7.4	7.837	0.226	8.5
Volume/mL	1.5	3.326	0.883	4.3
Sperm Total Count ($x10^6$/ejaculate)	0.0	11.20	3.930	14.7
Sperm Concentration (Million/mL)	0.0	6.457	4.559	20.0
Motility (AFLP) (%)	0.0	13.59	8.671	35.0
Motility (Sluggish) (%)	0.0	10.00	6.236	25.0
Motility (Immotile sperm) (%)	0.0	72.07	18.81	100.0
Viability (% of total)	0.0	23.59	11.77	50.0
Kruger (Normal morphology) (% of total)	0.0	29.13	14.43	65.0
Pus cells/HPF	2.0	7.457	3.710	18.0

Note: Data presented as mean ± standard deviation (SD); Abbreviation: BMI, body mass index

Table 3.2: General (categorical variables) characteristics of study participants

Variable	Frequency	Percent (%)
Married	138	100
Formal education	81	58.7
Consumed alcoholic beverages	30	21.7
Varicocele grade		
II	105	76.1
III	33	23.9
Varicocele type		
Left-sided	129	93.5
Bilateral	9	6.5
Total sperm count (x10^6/ejaculate)		
Normozoospermia	0	0.0
Oligozoospermia	129	93.5
Azoospermia	9	6.5

Note: Data presented as frequency and percent

Table 3.3: Comparison between general (continuous variables) and anthropometric characteristics of study participants

Variable	Surgery group (mean ± sd)	Observed group (mean ± sd)	p-value
Age (years)	50.32 ± 2.456	51.26 ± 2.423	0.3384
Anthropometric measurements			
Weight (kg)	69.93 ± 10.48	72.28 ± 10.82	0.6286
Height (cm)	171.0 ± 5.831	166.8 ± 8.168	0.3299
BMI (kg/m2)	24.48 ± 2.880	23.45 ± 3.015	0.2474
Body fat (%)	18.38 ± 5.070	18.97 ± 6.437	0.8088
Muscle mass (%)	35.44 ± 4.534	35.62 ± 4.572	0.9005
Visceral fat	8.296 ± 2.103	7.105 ± 2.378	0.2621
Blood pressure			
SBP (mmHg)	121.0 ± 11.23	127.6 ± 10.97	0.5448
DBP (mmHg)	71.67 ± 8.444	74.32 ± 10.87	0.3575

Note: Data presented as frequency and percent

3.6.2 Distribution of seminal parameters over 180 days of follow-up

The pre-and post-operative spermiogram parameters over the 180 days follow-up are shown in **Table 3.4**. According to the unpaired t-test statistics, before the operation; sperm with active forward linear progressive (AFLP) motility ($p=0.0433$) and viable sperms (viability as a percent of total) ($p<0.0455$) values were lower in the surgery group compared with the observed group (Table 3). After 180 days follow-up; semen volume ($p<0.0001$), total sperm count ($p<0.0001$), sperm concentration ($p<0.0001$), active forward linear progressive (AFLP) motility ($p<0.0001$), sluggish sperm motility ($p<0.0001$), viable sperms ($p<0.0001$), and morphological normal forms ($p<0.0001$) values increased in patients who had undergone varicocelectomy (surgery group) compared with the observed group whilst the levels of immotile sperms ($p<0.0001$) and pus cells ($p<0.0001$) decreased respectively.

According to the paired t-test analysis showing whether the difference between semen parameters of varicocele patients was significant; semen volume ($p=0.0008$), total sperm count ($p<0.0001$), sperm concentration ($p<0.0001$), active forward linear progressive (AFLP) motility ($p<0.0001$), immotile sperms ($p<0.0001$), sluggish sperm motility ($p<0.0001$), viable sperms ($p<0.0001$), morphological normal forms ($p<0.0001$), pus cells ($p<0.0001$) values differed before and after surgery. However, there was a significant reduction in sperm concentration ($p=0.0068$), active forward linear progressive (AFLP) motility ($p=0.0281$), and morphological normal forms ($p=0.0015$) in patients who were being observed over the 180 days of follow-up (**Table** 3.**4**).

Table 3.4: Pre- and post-operative seminal parameters over 180 days of follow-up

	Semen analysis		
Variable	**Baseline (Pre-operation)**	**180 days of follow-up**	**p-value**
Complete Liquefaction			
Observed group (Control)	46/63 (73.0%)	40/63 (63.5%)	NS
Surgery group	48/75 (64.0%%)	61/75 (81.3%)	NS
p-value	0.7694	0.0.8326	
pH			
Observed group	7.868 ± 0.2540	7.874 ± 0.2469	0.8808
Surgery group	7.815 ± 0.2070	7.793 ± 0.2286	0.7183
p-value	0.4353	0.8737	
Volume/mL			
Observed group	3.079 ± 0.8377	3.211 ± 0.7133	0.4808
Surgery group	3.500 ± 0.8880	4.296 ± 0.6830	0.0008
p-value	0.1123	< 0.0001	
Total sperm count (x10^6/ejaculate)			
Observed group	11.58 ± 2.406	10.11 ± 3.198	0.1298
Surgery group	11.15 ± 1.330	145.2 ± 20.09	< 0.0001
p-value	0.6020	< 0.0001	
Sperm concentration (Million/mL)			
Observed group	5.011 ± 3.407	4.167 ± 2.682	0.0068
Surgery group	5.474 ± 3.034	34.50 ± 15.39	< 0.0001
p-value	0.7607	< 0.0001	
Motility (AFLP) (%)			
Observed group	10.53 ± 7.975	8.421 ± 6.021	0.0281
Surgery group	9.741 ± 6.627	45.00 ± 6.934	< 0.0001
p-value	0.0433	< 0.0001	
Motility (Sluggish) (%)			
Observed group	8.947 ± 4.882	8.684 ± 5.735	0.8041
Surgery group	10.74 ± 7.031	19.81 ± 7.000	< 0.0001
p-value	0.3425	< 0.0001	
Motility (Immotile sperm) (%)			
Observed group	70.00 ± 25.87	72.37 ± 26.74	0.0952
Surgery group	73.52 ± 11.99	35.19 ± 8.490	< 0.0001
p-value	0.5383	< 0.0001	
Viability (% of total)			
Observed group	19.47 ± 10.39	17.11 ± 10.04	0.0952

Surgery group	16.48 ± 11.99	64.81 ± 8.490	< 0.0001
p-value	0.0455	< 0.0001	
Kruger (Normal morphology) (% of total)			
Observed group	31.05 ± 14.20	27.11 ± 11.82	0.0015
Surgery group	27.78 ± 14.70	59.81 ± 8.143	< 0.0001
p-value	0.4546	< 0.0001	
Pus cells/HPF			
Observed group	7.211 ± 4.674	8.053 ± 3.597	0.373
Surgery group	7.63 ± 2.937	2.296 ± 1.409	< 0.0001
p-value	0.7105	< 0.0001	

Note Row comparison done by paired t-test and column comparison done by unpaired t-test. Categorical variables compared with using Chi-square test statistics. P-value <0.05 considered statistically significant. NS = not significant, AFLP = active forward linear progression; HPF = high power field.

3.6.3 Comparison of percentage change in semen analysis between the observed group and operated group over 180 days of follow-up

The total percentage change in semen analysis over 180 days of follow-up between the two groups is shown in **Figure 3.2**. A significant percentage increase in; total sperm count (840.7%; p=0.0197), sperm concentration (582.1%; p=0.0125), active forward linear progressive (AFLP) motility (219.7%; p<0.0001), sluggish sperm motility (95.7%; p=0.0008), viability as a percent of the total (155.2%; p<0.0001), and morphological normal forms (110.9%; p<0.0001) was observed in patients who had undergone the surgery compared with those who were being observed. However, immotile sperms (-51.71%; p<0.0001) were significantly reduced in the surgery group.

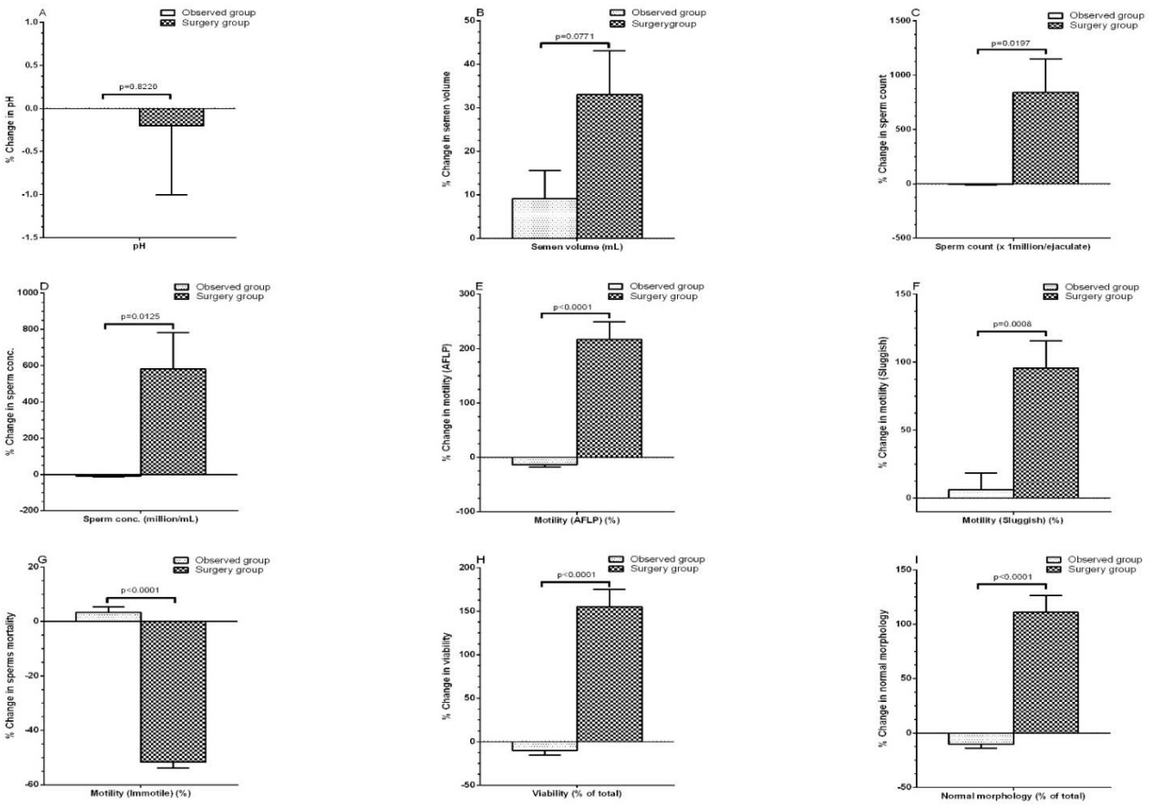

Figure 3.2: Comparison of percentage change in semen analysis between the two groups over 180 days of follow-up (A = % change in pH; B = % change in semen volume; C = % change in sperm count; D = % change in concentration (conc.); E = % change in motility (AFLP); F = % change in motility (sluggish); G = % change in sperms mortality; H = % change in viability; I = % change in morphology)

3.6.4 Post-surgery complications and pregnancy rate

In the surgery group, 4 patients recorded postoperative pain requiring strong opioids, 1 person had post-operative erythema on day 4 of operation, and 1 patient had skin allergy to chlorhexidine cleaning solution 3 days after the surgery (**Table 3.5**).

From **Table 3.5**, all consented participants had male factor fertility at the onset of the study. However, a 25.3% (19/75) pregnancy rate was recorded in the surgery group after 180 days of follow-up but no pregnancy rate was reported in the observed group.

Table 3.5: Post-surgery complications and pregnancy rate

Variable	Frequency	Percent (%)
Post-surgery complications		
Peri-incisional erythema	1	1.3
Immediate postoperative pain requiring opioids	4	5.3
Skin allergy to a chlorhexidine cleaning solution	1	1.3
Pregnancy rate after 180 days of follow-up		
Surgery group	19	25.3
Observed group	0	0.0

Note: Data presented as frequency and percent

3.7 DISCUSSION

To the best of our knowledge, this is the first prospective follow-up study on the effect of varicocelectomy on semen parameters among patients seeking infertility treatment in Tamale, Ghana. Varicocele is found to be responsible for 45-80% of male infertility (Nagler *et al.*, 1993; Witt and Lipshultz, 1993; Agarwal *et al.*, 2007). Choi and Kim (2013) reported 30 to 35% primary infertility and 69 to 81% secondary infertility among patients with varicocele.

In this study, all participants at baseline were clinically confirmed (Hussein, 2006) and diagnosed with varicocele (Dubin and Amelar, 1970). In addition, participants presented with alterations in semen volume, total sperm count, the concentration of spermatozoa, motility, or morphology according to WHO parameters (Cooper *et al.*, 2010). Presently in

medical practice, impairment of semen parameters, most especially sperm concentration, sperm count, morphology, and motility suggests that a varicocele might be present (Agarwal *et al.*, 2016) and this should prompt physical examination and ultrasound studies of the scrotum (Dubin and Amelar, 1970).

Varicocele affects spermatogenesis and studies conducted earlier suggested three (3) mechanisms; 1) slow circulation in varicose veins of the leg, and as the leg varicosities can lead to local skin destruction, so, varicoceles destroy the germinal epithelium less dramatically. 2) Also, the large volume of slowly circulating blood may act as a radiator surrounding the testicles, thus reproducing the experimental scrotal insulation. 3) Lastly, the sheer bulk of the varicocele holds the testicle in one position, thereby preventing the normal physiological cooling mechanism from working efficiently (Glover, 1955; Scott and Young, 1962). In recent times, however, no mechanism has conclusively explained infertility in men with varicocele, with several possible intermediaries being; oxidative stress, scrotal hyperthermia, testicular hypoperfusion, testicular hypoxia, and backflow of toxic metabolites which may lead to failure of spermatogenesis and damage to sperm DNA (Shiraishi *et al.*, 2012; Xue *et al.*, 2012; Kadioglu *et al.*, 2014; Agarwal *et al.*, 2016).

In the treatment of male infertility with varicocele, varicocele repair is widely used. However, the effectiveness of microsurgical ligation of the internal spermatic vein concerning fertility remains to be clarified (Williams *et al.*, 2006). In this study, microsurgical sub-inguinal varicocelectomy was used to correct the varicocele in the surgery group. After 180 days of follow-up; semen volume, total sperm count, sperm concentration, active forward linear progressive (AFLP), sluggish sperm motility, viable sperms, and morphological normal forms values increased significantly. This is in line with several other studies (Barbalias *et al.*, 1998; Khan *et al.*, 2003; Grober *et al.*, 2004; Zini *et al.*, 2005)

including a meta-analysis by Agarwal *et al.* (2016) who found that surgical varicocelectomy is an effective treatment for improving the semen parameters of infertile men with a clinically palpable varicocele. The possible explanation may be that microsurgical sub-inguinal varicocelectomy confers corrections by preventing a retrograde blood flow (Masson and Brannigan, 2014), improves venous circulation at the scrotum by normalizing the counter current heat exchange that involves the pampiniform plexus, and reducing failure of spermatogenesis since no such observation was found in the semen parameters of the observed group.

Despite pregnancy outcomes being central to evaluating fertility status among couples, sperm density may be an important measurement in men especially after several studies linking sperm density to fertility status. A prospective study by Guzick *et al.* (2001) found that semen parameters such as normal sperm morphology less than 9%, motility of less than 32%, and sperm concentration of less than 13.5 x 10^6/mL suggested subfertility with the percentage of normal sperm morphology being the prevailing discriminator between fertile and infertile men. In contrast to this, Nallella *et al.* (2006) reported sperm concentration and motility as superior indicators to the percentage of normal morphology between fertility and infertile population. In this study, not only the sperm density, but a significant increase in the overall percentage change in; total sperm count, sperm concentration, motility (AFLP), and the normal sperms morphology was observed in the surgery group. Aside the different sperm characteristics to distinguish between fertility and infertility, a common observation is that better pregnancy outcomes are associated with better semen parameters.

Clinical varicocele has been studied extensively, however; inconsistent findings have been reported. A 36 to 74 months randomized controlled trial (RCT) of 96 infertile men with left-sided varicoceles (56 had surgery and 46 had no surgery) conducted by Nilsson *et al.* (1979)

found that varicocele repair was not effective since the semen analysis findings and reported pregnancy rates did not vary significantly among study groups. Breznik *et al.* (1993) also reported a higher pregnancy rate in the untreated group (53.7% or 22/41) compared with the surgery group (34.2% or 13/38) in a four-year prospective RCT and concluded that varicocele repairs did not positively affect the semen parameters and pregnancy rate. In this study, however, the surgery group recorded a 25.3% (19/75) pregnancy rate after 180 days of follow-up but none from the observed group. This finding is in line with other studies (Madgar *et al.*, 1995; Krause *et al.*, 2002; Evers and Collins, 2004) who reported a significant improvement in the concentration and motility of the sperm and higher pregnancy rate in the surgery group.

Czaplicki *et al.* (1979) and Witt and Lipshultz (1993) reported a rate of 4.3 to 13.3% azoospermia in varicocele patients. In this study, the semen parameters did not improve in 6.5% of patients with azoospermia. This may imply that spermatogenesis had failed and the surgery was not able to reverse azoospermia. Although the mechanisms leading to failure of spermatogenesis in patients with varicocele are not fully elucidated, some studies have linked it to sperm DNA damage associated with increased scrotal temperature (Agarwal *et al.*, 2014), epigenetics alterations (Seidel, 2015), and increased productions of reactive oxygen species (ROS) and apoptosis (Shiraishi *et al.*, 2012). Not all patients with varicocele will improve following surgery (Lenzi *et al.*, 1998).

In the surgery group, the following postoperative complications were observed: 1) Postoperative erythema 1(1.3%); a mild form of surgical site infection which was noticed on a postoperative day 4. Wound swab for culture and sensitivity yielded negative culture. The wound healed spontaneously without the need for antibiotics. 2) Post-operative pain requiring strong opioids 4 (5.3%); the majority of patients did not experience pain

postoperatively. All patients received paracetamol 1000 mg TID for 24 hours. Four patients, however, experienced severe postoperative pain that was not relieved by paracetamol. They, therefore, were given IM Pethidine 50mg TID to control their pain. 3) Skin allergy to Chlorhexidine 1 (1.3%); one patient had excoriation of the scrotal skin 3 days after the surgery. This was attributed to the use of a Chlorhexidine cleaning solution to prep the skin before surgery. This was treated with skin moisturizing shea ointment and healed spontaneously by postoperative day 7 (Maiwald *et al.*, 2014).

It is recommended to use microsurgical sub-inguinal varicocelectomy for similar patients, however, further studies with a larger sample size are needed to provide more evidence to recommend this therapy.

3.8 CONCLUSION

Long-standing varicocele may affect semen parameters and this may be seen by causing a further decrease in semen volume, total sperm count, concentration of spermatozoa, motility, or normal sperm morphology. This study found that microsurgical sub-inguinal varicocelectomy improves semen parameters and pregnancy rate, hence, effective treatment of infertile men with a clinically palpable varicocele.

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Chapter 4

PAPER II

4.1 EFFECT OF VARICOCELECTOMY ON GONADAL FUNCTION AMONG PATIENTS REPORTING WITH SEXUAL DYSFUNCTION IN GHANA

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Author Statement

Authors **Yussif Adams (Candidate)**, **Akisibadek Alekz Afoko (Supervisor)** and **Nafiu Amidu (Supervisor)** designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author **Akisibadek Alekz Afoko** performed the surgery. Authors **Yussif Adams**, **Lawrence Quaye**, **Simon Bannison Bani, and Peter Paul M. Dapare** managed the analysis of the study, software and did the validation of the results. Authors **Yussif Adams**, **Akisibadek Alekz Afoko**, and **Vivian Afoko** did field sampling and part of the laboratory analysis, analysed the results, and manged the literature searches.

4.2 ABSTRACT

Background: *Long-standing varicocele might worsen Leydig cell functions; a significant risk factor for hypogonadism. The study aims to determine the effect of microsurgical sub-inguinal varicocelectomy on gonadal function among men reporting sexual dysfunction in*

Ghana. ***Methods****: This was an intervention study conducted at the Tamale Teaching Hospital from September 2017 to August 2021. A total of 103 participants were randomized into two groups; the surgery group (n = 52) and the observed group (n = 51). Venous blood samples were collected at baseline, varicocelectomy was performed for the surgery group, and no intervention was given to the other. Blood samples were subsequently collected at 12-, 24-, 36-, and 48-months intervals for assay of serum total testosterone, follicle stimulating hormone (FSH), and luteinizing hormone (LH). The data were analyzed in GraphPad Prism (v8.0) at an alpha value of 0.05.* ***Results****: All the participants had varicocele and were aged between 55.0 to 69.0 years old. At the baseline of the study, all participants presented with sexual dysfunction but a significant improvement ($p < 0.000$) in the Golombok Rust Inventory of Sexual Satisfaction (GRISS) score, and the subscale was observed 12 months after the surgery. The mean ± SD serum total testosterone ($p = 0.6078$), FSH ($p = 0.6522$) and LH ($p = 0.2281$) between the groups at baseline did not vary but those in surgery group had improved values at 12-, 24-, 36- and 48-months post-surgery (p-trend <0.0001). The surgery group had an overall percent increase in serum total testosterone (76.3%, 194.0%, 221.0%, and 231.9%) over 12-, 24-, 36- and 48-months and significant percent reduction in both FSH (-14.7%, -29.9%, -33.8% and -40.8%) and LH (-21.8%, -31.0%, -32.4%, and -36.4%) respectively. These gonadotropins observed annual percentages spike within the first- and second-year but changes were marginal from the third year onwards in the surgery group.* ***Conclusion****: Microsurgical sub-inguinal varicocelectomy improved gonadal function among varicocele patients reporting sexual dysfunction. It is recommended to use this choice for similar patients; however, these findings should be verified by the multi-institutional study to provide more evidence to this choice.*

Keywords: varicocele, sub-inguinal varicocelectomy, gonadal function, sexual dysfunction

4.3 ABBREVIATIONS AND ACRONYMS

ANOVA analysis of variance

BMI body mass index

DBP diastolic blood pressure

FSH Follicle-stimulating hormone

GRISS Golombok Rust Inventory of Sexual Satisfaction

LH Luteinizing hormone

RCT randomize control trials

SBP systolic blood pressure

SUE Scrotal ultrasound evaluation

TTH Tamale Teaching Hospital

WHO World Health Organization

4.4 INTRODUCTION

Varicocele is the enlargement of pampiniform venous plexus draining the testicle, with reflux of venous blood (Clavijo *et al.*, 2017; Bertolotto *et al.*, 2020). It is a common problem in men who seek medical attention for fertility problems, sexual dysfunction or complain of continuing scrotal discomfort (Paick and Choi, 2019).

Varicocele has been identified in 15% of healthy men (Alsaikhan *et al.*, 2016) but the prevalence ranges from 35% to 45% among men seeking medical attention for primary infertility and 80% among patients seeking care for secondary infertility (Gorelick and Goldstein, 1993; Jarow *et al.*, 1996).

Studies involving humans have reported that varicocele causes progressive time-dependent testicular damage (Russell, 1957; Lipshultz and Corriere Jr, 1977; Sakamoto and Ogawa, 2009). Between the ages of 18-20 years, the testicular function is usually normal but declines progressively depending on the duration of the varicocele (Agarwal *et al.*, 2016). Some propositions have sort to explain the lethal effects of varicocele on testicular function with the most accepted postulate related to alterations in the thermal environment of the testicles. The formation of a communicating meshwork of spermatic veins leaving the testicles produces a counter-current heat-exchange mechanism to cool arterial blood (Lü and Chen, 2008). However, persons confirmed with varicocele lack this mechanism, hence, causing elevated scrotal temperature.

Long-standing varicocele might worsen Leydig cell functions and is a significant risk factor for hypogonadism. Lotti *et al.* (2009) in a study found that patients with severe varicocele had increased serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) with lowered testicular volume. Increased serum FSH and LH levels in infertile men diagnosed with varicocele have resulted in the hypothesis that varicocele causes Leydig cell dysfunction (Tian *et al.*, 2018).

Clinical studies suggest that repair of the varicocelectomy may improve gonadal function in men with varicocele (Li *et al.*, 2012; Tiseo *et al.*, 2016; Çayan *et al.*, 2020). Çayan *et al.* (2020) reported that approximately 60%-80% of men with low serum testosterone had normalized testosterone levels after varicocele repair. Li *et al.* (2012) in a meta-analysis found that the mean serum testosterone level increased after varicocelectomy. Nonetheless, there are conflicting reports on whether varicocele and varicocelectomy result in changes in serum FSH and LH levels or not. Some studies reported no significant changes in the levels

of serum FSH and LH (Su *et al.*, 1995; Salem and Mostafa, 2009), yet others noted decreased serum FSH and LH levels following varicocelectomy (Sathya Srini and Belur Veerachari, 2011; Tian *et al.*, 2018).

As already known, Leydig cells function to produce testosterone but this is controlled by luteinizing hormone. FSH functions to promote the beginning of testosterone production; in the process, LH is maintained. Hence, there is crosstalk with the changes of serum testosterone, FSH, and LH. This study, therefore, aims to determine the effect of microsurgical sub-inguinal varicocelectomy on serum total testosterone, FSH, and LH levels among patients reporting sexual dysfunction in Ghana.

4.5 MATERIALS AND METHODS

4.5.1 Ethical consideration

This study was approved by the Ethics and Review Board of the Department of Research and Development, Tamale Teaching Hospital (No: TTH/R&D/SR/119), and has therefore been performed following the standards laid down in the 1964 Declaration of Helsinki. Informed consent was obtained from all the participants before the study. Participation in this study was voluntary, participants were kept anonymous, and information obtained remained confidential to the researchers only. Only blood samples intended for the study were drawn and information that was deemed as important to the management of the patient was communicated to the patient.

4.5.2 Study design

This was an intervention study design in which participants were randomized into two groups; the surgery group (n = 52) and the observed group (n = 51) (**Figure 4.1**). The study

was conducted at Tamale Teaching Hospital in the Tamale Metropolis from September 2017 to August 2021.

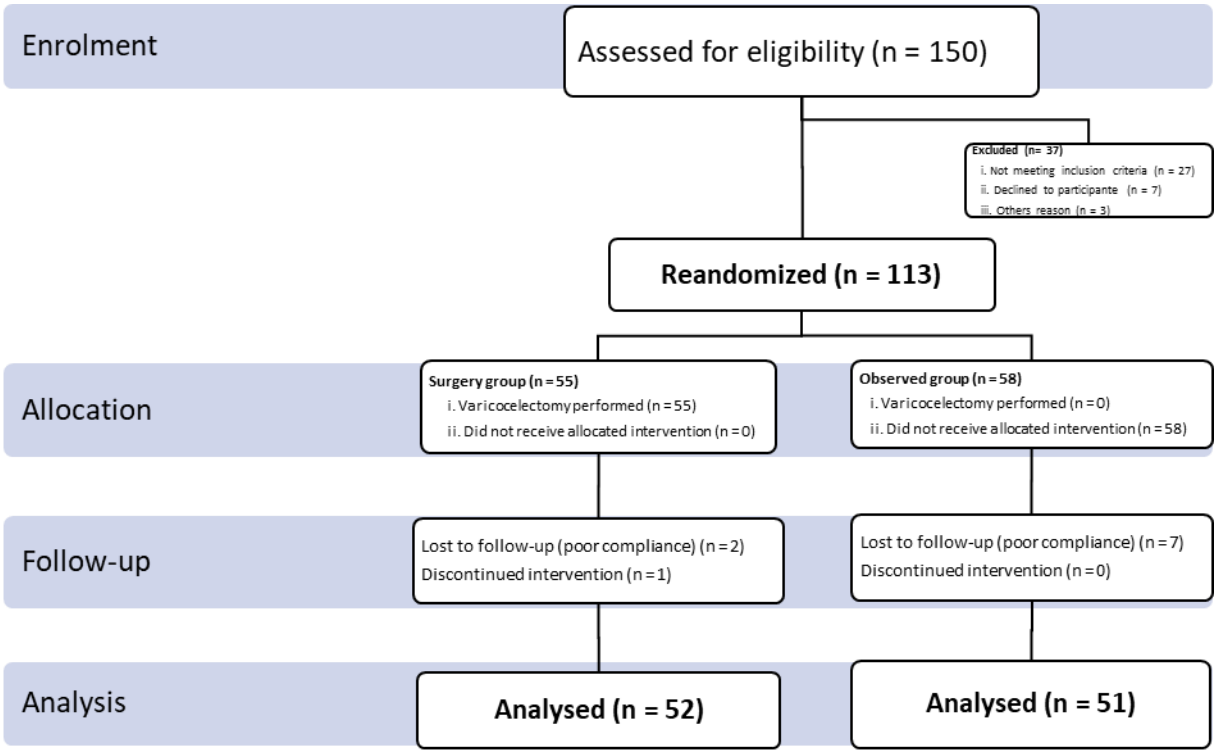

Figure 4.1: Flowchart diagram

4.5.3 Study population

Participants who were eligible for inclusion in this study were given the option of immediately undergoing microsurgical sub-inguinal varicocelectomy or being observed for 12 months with a subsequent reassessment of the management plan and possibly delayed the operation. Based on the willingness to equally accept either option, eligible participants were randomized to the surgery group and observed group. However, neither the investigator nor participants were blinded to the intervention after allocation (Abdel-Meguid *et al.*, 2011).

4.5.3.1 Inclusion criteria

Participants were eligible for inclusion in the study if they had fathered at least one child, and had complained of any form of sexual dysfunction including; weak sex drive, impotency, premature ejaculation, infrequency of having sexual intercourse, avoidance of sexual intercourse, non-communication about sex with a partner, and dissatisfaction after sexual intercourse activity (Rust and Golombok, 1985).

All consented participants were sexually active men who had maintained a stable heterosexual relationship for at least 2 years before participating in the study. A stable heterosexual relationship was considered as one in which the man was involved and maintained sexual relations, regardless of marital status.

Selection strategies were adopted depending on the unit at the recruitment center of the Tamale Teaching Hospital. After a patient arrived at the Urology Specialist Clinic, pre-assessment checks were made according to the criteria stated above and those who did not qualify for the study were made to continue with their routine medical examinations. If a patient met the eligibility criteria, informed consent was obtained and a questionnaire was administered to be completed independently. Where participants were not able to read and write, the queries on the questionnaire were translated verbatim in the common dialect. Participants were made to see the urologist for medical examination.

4.5.3.2 Exclusion criteria

Participants with sexual dysfunction who had not fathered a child (or children), had no sexual dysfunction complaints, excessive alcohol use (chronic alcoholics), cigarette smoking history (as smoking is an independent risk factor for infertility), incomplete/inconclusive questionnaires were excluded. Participants with a history of uncontrolled diabetes (glycated

hemoglobin >7%), uncontrolled hypertension (blood pressure ≥ 140/90 mmHg, undescended testis, mumps, orchitis, use of anti-estrogen and/or testosterone replacement therapy, or orchidectomy as well as patients on long term statins were all also excluded.

4.5.4 Clinical evaluation

Each consenting participant was clinically examined by a urologist. The diagnosis was based on physical examination and was confirmed by ultrasound scan examination. All participants had a varicocele. Dubin and Amelar (1970) approach was employed to detect, confirm and clinically grade varicocele. Varicocele was categorized into three (3) grades; grade I (first grade), II (second grade), and III (third grade). Grade I was confirmed if the participant had an enlarged venous plexus of spermatic tone evident only by palpation during the Valsalva maneuver. Enlargement of the venous plexus of spermatic tone evident only by palpation at the upright position was considered Grade II while enlargement of the venous plexus of spermatic tone evident visually was confirmed Grade III (WHO, 1992). Scrotal ultrasound was used to diagnose the non-palpable enlargement of the venous plexus of the spermatic tone (Marsman and Schats, 1994).

4.5.5 Scrotal Ultrasound Evaluation (SUE)

Two phases of scrotal ultrasound scans were carried out on participants who qualified for the study; the first phase was with participants in the supine position (with penis resting on suprapubic region) and the second in an upright position. The examination was conducted with a Samsung Medison Accuvix V20 scan (Samsung Electronics, South Korea) equipped with linear, high-resolution, and high-frequency (7.5 – 14 MHz) probe keen to the study of soft body parts and with color Doppler for detecting slow flows and scanning surface of at least 5 cm (Hussein, 2006). To evaluate testicular malposition, blood reflux along the

pampiniform plexus, or the extent of any fluid collections an ultrasound scan was done (Adams *et al.*, 2022).

4.5.6 Data collection

4.5.6.1 Questionnaire administration

Sociodemographic data, cigarette smoking, and medical history were gathered with a structured pre-tested questionnaire. Questions on sexual response were assessed using the Golombok Rust Inventory of Sexual Satisfaction (GRISS) questionnaire which measures specific sexual behaviors, attitudes, and beliefs (Rust and Golombok, 1985). The GRISS questionnaire has 28 items on a single sheet and it is used for assessing the existence and severity of sexual problems in heterosexual couples or individuals who have a current heterosexual relationship. All the 28 questions were answered on a five (5) point scale from "never" through "hardly ever", "occasionally" and "usually" to "always". This provided overall scores for the quality of sexual functioning within a relationship. In addition, the subscale scores for infrequency, non-sensuality, dissatisfaction, non-communication, and avoidance were obtained and presented as a profile. The total score and subscale scores were transformed using a standard nine-point scale ranging between 1 and 9, with high scores indicating greater problems. Scores of 5 or more were considered to indicate sexual dysfunction (SD). The GRISS was chosen because it is standardized, easy to administer and score, relatively unobtrusive, and substantially inexpensive (Rust and Golombok, 1985).

4.5.6.2 Blood pressure measurement

The Omron blood pressure monitor was used to measure the blood pressure of the participant. These included; systolic blood pressure (SBP), diastolic blood pressure (DBP) and pulse rate, and categorization of normotension (SBP<140mmHg/DBP<90mmHg) and

hypertension (SBP>140mmHg/DBP>90mmHg) was based on WHO cut-offs as cited by Mittal and Singh (2010). The Omron blood pressure monitor uses the oscillometric method of blood pressure measurement. This means the monitor detects the blood's movement through the brachial artery and converts the movements into a digital reading (www.omron.com).

4.5.6.3 Anthropometric measurement

Anthropometric measurements were done on all study participants. The Seca 213 portable Stadiometer (Seca Corp., Hamburg, Germany) was used to measure the height of the participants to the nearest 0.1 cm. To measure the height, the stadiometer was set up according to the manufacturer's instructions. Participants were asked to take off footwear including socks and measurements taken in the upright position.

The measurement of weight, calculation of BMI, and the assessment of body fat composition were done using the Omron HBF-516B Body Composition Analyzer and Electronic Scale (Omron Corp., USA). The Omron HBF-516B Body Composition Analyzer and Electronic Scale is a tetra-polar bioelectrical impedance analyzer that measures weight to the nearest 0.01kg. It has electrodes on the surface of the scale and on a hand-held device that is attached to the scale by a retractable cord. It works by passing a painless, imperceptible electrical current (500μA) at a fixed frequency of 50 kHz through the body while determining resistance and reactance. Body fat and muscle mass were recorded as percentages of the total body weight at intervals of 0.1% (Adams *et al.*, 2022).

4.5.6.4 Blood samples collection

Venous blood samples (4mls) were collected from each participant within the hours of 8:00 – 11:00 GMT after at least 8 hours of fast by a phlebotomist using standard venipuncture

methods. A check-list was given to each consenting participant to tick the number of hours fasted to enable rescheduling those who could not meet the time.

Venous blood samples collected were dispensed into a 5ml vacutainer containing a gel separator. Blood samples were centrifuged at 8000 rpm for 5minutes to yield serum and cells. The serum was aliquoted and stored at -20^{o}C until assay.

4.5.6.5 Hormonal measurement

Baseline male fertility hormones (total testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH)) were measured by electrochemiluminescence with Hitachi-Roche analyzer (Cobas 6000, Roche Diagnostics, IN, USA).

4.5.7 Interventions (Sub-inguinal microscopy varicocelectomy)

Participants were counseled about their condition, and the exact nature of the problem was explained to them by a urologist. A microsurgical open sub-inguinal varicocelectomy procedure as described by Marmar *et al.* (1985) was performed for the surgery group. Surgery was performed under spinal anesthesia, using microsurgical instruments and magnification with an operating microscope KARL CAPS SOM 82, Germany. The lymphatic vessels and testicular artery were spared, and both internal and external spermatic veins ligated and divided. The spermatic fasciae were closed using PGA 3/0 running sutures. The wound was closed in layers and a subcuticular skin stitch was applied using 4/0 PGA sutures. Wound dressing was removed after 24 hours. No antibiotics were employed and the pain was managed by using 1-gram of rectal paracetamol during the period of recovery and followed by oral paracetamol 1-gram tid for the next 24 hours (Adams *et al.*, 2022).

4.5.8 Follow-up

Both groups were followed for 48 months (4 years) after the day of surgery (surgery group) or the day of the last baseline hormone analysis (observed group). Participants in the observed group were advised not to use any form of contraceptives during sexual intercourse, and to abstain from tobacco/cigarette smoking. Participants in the operated group were advised to abstain from any form of sexual activity until the surgical wound was properly healed. All participants were reassessed every 90 days to confirm that; the participant was not smoking, and was clinically examined to confirm the absence of genital infection, formation of hydrocele, recurrence of varicocele, and increased testicular size. Participants were asked to revisit the clinic after 6 months and 12 months. Blood samples were drawn for repeated measurement of serum total testosterone, FSH, and serum LH at follow-up months 12, 24, 36, and 48 respectively.

4.5.9 Statistical Analysis

Data were entered into Microsoft Excel version 10 (www.ibm.com) and exported to GraphPad Prism version 8.0 (www.graphpad.com) for analysis. Categorical data were presented as frequency, percent, and charts, and parametric data presented as mean ± standard deviation (s.d) or mean ± standard error of the mean (SEM). Kolmogorov-Smirnov test was performed on parametric data to check whether or not the data was normally distributed. To compare two groups, the Chi-square test was used for categorical variables, and the unpaired student t-test was used for parametric data. Variables before and after the operation in each patient were compared using paired *t*-test. Group means were compared using one-way ANOVA followed by Newman-Keul's test as post hoc. A two-tailed p-value less than 0.05 was considered statistically significant.

4.6 RESULTS

4.6.1 Baseline general characteristics of study participants

The general characteristics of the study population are summarized in **Table 4.1-3**. From **Table 4.1**, the majority of the participants were married (83.5%), self-employed (60.2%), and were from the Mole-Dagomba tribe (62.1%). About 46.6% of the participants attained formal education and 19.4% were gainfully employed. The majority were confirmed with varicocele grade II (50.5%) with left-sided being the predominant type (94.2%) (**Table 4.1**).

As shown in **Table 4.2**, participants were aged between 55.0 and 69.0 years old. The mean ± standard deviation (SD) BMI, body fat, muscle mass, and visceral fat were 23.63 ± 2.971 kg/m^2, 17.92 ± 7.814%, 35.82 ± 4.322%, and 7.434 ± 3.467% respectively. The systolic blood pressure (SBP) was between 96.0 and 136.0 mmHg while the diastolic blood pressure (DBP) was between 66.0 and 88.0 mmHg. Before the randomization, the mean ± standard deviations (SD) for the total FSH, LH, and Testosterone were 23.8 ± 7.788 IU/L, 11.85 ± 3.751 IU/L, and 2.128 ± 0.811 nmol/L respectively.

The eligible participants were randomized into; the observed group and the surgery group (those who had undergone varicocelectomy). At baseline, participants in the surgery group recorded significantly higher BMI ($p = 0.0337$) compared with their counterparts. However, there was no significant difference between the age ($p = 0.2294$), body fat ($p = 0.6648$), muscle mass ($p = 0.5235$) and visceral fat ($p = 0.0644$) (**Table 4.3**).

Table 4.1: General (categorical variables) characteristics of study participants

Variable	Frequency (n = 103)	Person (%)
Married	86	83.5
Formal education	48	46.6
Consumption of alcoholic beverage	21	20.4
Ethnicity		
Mole-Dagomba	64	62.1
Other tribes	39	37.9
Occupational status		
Gainful employed	20	19.4
Self-employed	62	60.2
Unemployed	21	20.4
Varicocele grade		
I	16	15.6
II	52	50.5
III	35	33.9
Varicocele type		
Left-sided	97	94.2
Bilateral	6	5.8

Data presented as frequency and percent. Other tribes in Ethnicity included; Dagaati, Frafra, Gonja, Ashanti, Ewe, Ga, Kassena

Table 4.2: General (continuous variables), anthropometric characteristics, and baseline hormonal parameters of study participants

Variable	Minimum	Mean	Std. deviation	Maximum
Age (years)	55.0	60.92	2.487	69.0
Anthropometric measurements				
Weight (kg)	60.1	69.2	15.62	158.0
Height (cm)	82.3	168.9	13.47	183.0
BMI (kg/m2)	17.9	23.63	2.971	33.0
Body fat (%)	6.9	17.92	7.814	45.8
Muscle mass (%)	22.6	35.82	4.322	44.7
Visceral fat	2.0	7.434	3.467	17.0
Blood pressure				
SBP (mmHg)	96.0	121.1	7.190	136.0
DBP (mmHg)	66.0	73.7	5.787	88.0
Pulse (beat/mins)	58.0	66.77	5.250	97.0
Pre-operative hormones				
S-Follitropin (FSH) (IU/L)	4.8	23.8	7.788	38.1
S-Lutroppin (LH) (IU/L)	2.0	11.85	3.751	25.0
Total Testosterone (nmol/L)	0.4	2.128	0.811	4.1

Data presented as mean and standard deviation (SD); Abbreviation: BMI - body mass index; SBP – Systolic blood pressure; DBP – Diastolic blood pressure, FSH – Follicle Stimulating Hormone; LH - Luteinizing Hormones

Table 4.3: General (continuous variables), age, anthropometric characteristics, and blood pressure at baseline of study participants

Variable	Observed Group (n=51)	Surgery Group (n=52)	p-value
Age (years)	61.35 ± 2.262	60.52 ± 2.666	0.2294
Anthropometry			
Weight (kg)	68.43 ± 19.81	69.93 ± 10.48	0.7300
Height (cm)	166.7 ± 18.23	171.0 ± 5.831	0.2509
BMI (kg/m^2)	22.76 ± 2.857	24.48 ± 2.880	0.0337
Body fat (%)	17.44 ± 8.634	18.38 ± 7.070	0.6648
Muscle mass (%)	36.21 ± 4.142	35.44 ± 4.534	0.5235
Visceral fat	6.538 ± 2.420	8.296 ± 4.103	0.0644
Blood Pressures			
SBP (mmHg)	121.2 ± 13.59	121.0 ± 11.23	0.9697
DBP (mmHg)	72.81 ± 4.77	71.67 ± 4.444	0.1247
Pulse (beat/min)	66.15 ± 5.12	68.22 ± 6.11	0.2986

Data presented as mean ± standard deviation (SD); quantitative variables compared using Unpaired t-test statistics and a two-tailed p-value less than 0.05 considered statistically significant. Abbreviation: BMI, body mass index; SBP – Systolic blood pressure; DBP – Diastolic blood pressure

4.6.2 Baseline score of sexual dysfunctions among participants according to Golombok Rust Inventory of Sexual Satisfaction (GRISS)

The sexual function scores of the participants for each GRISS scale are shown in **Figure 2**. The Stanine scale depicting sexual dysfunction for the overall male score ranges from 6 – 9, impotence (5 – 9), premature ejaculation (4 – 9), non-sensuality (5 – 9), avoidance (4 – 9), dissatisfaction (4 – 9), in frequency (5 – 7), and non-communication (6 – 9) with the prevailing value for each of these scales being a score of 5 or above.

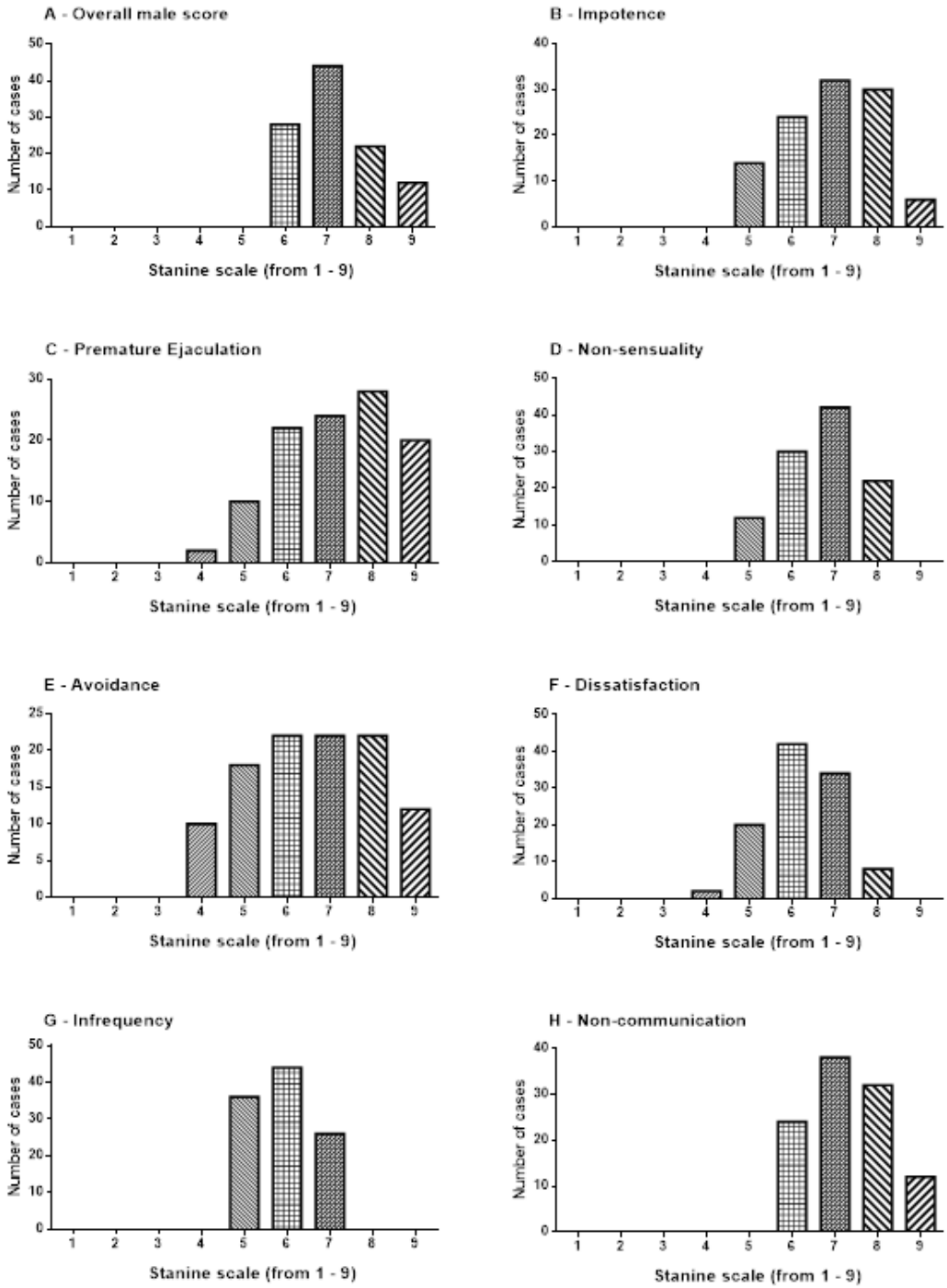

Figure 4.2: Score of sexual dysfunctions among participants according to Golombok Rust Inventory of Sexual Satisfaction (GRISS) questionnaire. Graphs show the number of participants (y-axis) and Stanine scale (from 1 – 9 on the x-axis) for each GRISS subscale. Normal scores range from 1 – 4 and 5 – 9 indicate the abnormal score

4.6.3 Subscale score among participants using GRISS

As shown in **Table 4.4**, all consenting participants had one or more baseline subscale scores reflecting sexual problems as the overall GRISS scale and the score for each subscale was above the upper value of 5. The distribution of the baseline subscale score for each of the groups was almost the same such that there was no significant ($p<0.05$) difference between their means.

After 12 months of follow-up, the overall male scale (3.259 ± 0.9027), impotence (3.037 ± 1.1260), premature ejaculation (3.481 ± 1.2820), non-sensuality (2.667 ± 0.9199), avoidance (3.148 ± 1.4060), dissatisfaction (2.222 ± 0.8006), infrequency (1.963 ± 0.8077) and non-communication (3.407 ± 1.1180) were significantly ($p<0.0001$) lower in the surgery group compared with the observed group (**Table 4.4**).

Table 4.4: Subscale score among participants using GRISS

GRISS SCALE	Observed Group (n=51)	Surgery Group (n=52)	p-value
Baseline (Onset)			
Overall male scale	7.115 ± 1.033	7.222 ± 0.891	0.6882
Impotence	6.769 ± 1.142	7.037 ± 1.126	0.3941
Premature Ejaculation	6.846 ± 1.287	7.519 ± 1.312	0.0654
Non-sensuality	6.615 ± 0.983	6.778 ± 0.892	0.5312
Avoidance	6.231 ± 1.478	6.963 ± 1.480	0.0775
Dissatisfaction	6.308 ± 0.970	6.185 ± 0.879	0.6318
Infrequency	5.846 ± 0.732	5.963 ± 0.808	0.5840
Non-communication	7.269 ± 0.919	7.333 ± 1.000	0.8092
12 Months			
Overall male scale	7.269 ± 0.8744	3.259 ± 0.9027	< 0.0001
Impotence	7.231 ± 0.7646	3.037 ± 1.1260	< 0.0001
Premature Ejaculation	7.077 ± 0.8910	3.481 ± 1.2820	< 0.0001
Non-sensuality	6.692 ± 1.1580	2.667 ± 0.9199	< 0.0001
Avoidance	6.500 ± 1.0680	3.148 ± 1.4060	< 0.0001
Dissatisfaction	6.692 ± 1.0110	2.222 ± 0.8006	< 0.0001
Infrequency	6.846 ± 0.8339	1.963 ± 0.8077	< 0.0001
Non-communication	7.000 ± 0.9381	3.407 ± 1.1180	< 0.0001

Data presented as mean ± standard deviation (SD); quantitative variables compared using Unpaired t-test statistics and p-value < 0.05 considered statistically significant.

4.6.4 Distribution of gonadal function over 48 months of follow-up among study participants

The gonadal function was compared to the baseline measurement for over the 48 months follow-up in each group. From the unpaired t-test analysis, before the operation, there was no difference between the serum testosterone ($p = 0.6078$), FSH ($p = 0.6522$) and LH ($p = 0.2281$). After 48 months of follow-up, the serum testosterone increased in 12 months ($p < 0.0001$), 24 months ($p < 0.0001$), 36 months ($p < 0.0001$), and 48 months ($p < 0.0001$) in patients whom had undergone varicocelectomy compared with the observed group whilst the levels of serum FSH and serum LH values decreased ($p < 0.0001$) respectively (**Table 4.5 and Figure 4.3A and 4.3B**).

According to the paired t-test statistics showing whether the difference between gonadal hormones among each group was significant; in the surgery group, baseline serum testosterone was 2.185 ± 0.730 nmol/L but increased in 12 months ($p < 0.0001$) through to 48 months ($p < 0.0001$) whilst serum FSH (baseline: 24.28 ± 7.001 IU/L) and LH (baseline: 12.46 ± 4.207 IU/L) decreased significantly ($p < 0.0001$) throughout the 48 months of follow-up. On the other hand, the baseline serum testosterone was 2.069 ± 0.899 nmol/L but reduced in 12 months ($p = 0.001$) and 36 months ($p < 0.0001$) whilst serum FSH (baseline: 23.30 ± 8.642 IU/L) and LH (baseline: 11.21 ± 3.168 IU/L) continued to increase ($p<0.0001$) for the 48 months of follow-up in the observed group (**Table 4.5**).

For the one-way ANOVA statistics, serum FSH increased significantly ($p<0.05$) in the observed group for every 12 months of follow-up through to the 48 months, but the group means did not vary statistically ($F (4,125) = 0.7348$, $p = 0.5699$; p-trend = 0.0949). However,

after 48 months of follow-up, the serum FSH values were significantly lowered and the linear trend indicated a significant reduction (F (4,130) = 14.99, p<0.0001; p-trend < 0.001) for each year among participants who had the surgery. The serum lutropin (luteinizing hormones) generally showed a significant difference between the observed group and the varicocelectomy group. Luteinizing hormones (LH) significantly (F (4,125) = 3.823, p = 0.0058) increased in the observed group with the linear trend showing the rise (p-trend = 0.002) but decreased (F (4,130) = 19.80, p < 0.0001) in the operated group with a lowered linear trend (p<0.0001). Furthermore, total testosterone increased significantly (F (4,130) = 32.17, p < 0.0001) in the operated group with a linear trend (p < 0.0001) depicting the increase but was moderately decreased in the observed group although this was not statistically significant (**Table 4.5**).

4.6.5 Comparison of overall percentage change in gonadal function over 48 months follow-up

As shown in **Figure 4.4**, the baseline serum total testosterone (p = 6078), serum FSH (p = 6522) and LH (p=6078) did not vary significantly among the two groups (**Figure 4.4A**). However, a 76.3% increase in testosterone was observed in 12 months, 194.0% in 24 months, 221.0% in 36 months, and 231.9% increase in 48 months among participants who had surgery compared with a percentage reduction in the observed group. The percentage variations between these two groups were statistically significant (**Figure 4.4B**).

A significant (p<0.0001) reduction in FSH was observed in the surgery group over the 48 months with the percentage change of 14.7% in 12 months, 29.9% in 24 months, 33.8% in 36 months, and 40.8% in 48 months. While a steady increase in FSH in the observed group

with a percentage change of 8.6, 13.2, 18.1, and 20.6% in the first year, second, third, and fourth-year respectively (**Figure 4.4C**).

Again, LH reduced significantly ($p<0.0001$) in the surgery group with the change of 21.8% in 12 months, 31.0% in 24 months, 32.4% in 36 months, and 36.4% in 48 months. Whilst among the observed group, LH increased with a change from the baseline values of 8.6% in 12 months, 23.4% in 24 months, 30.2% in 36 months, and 39.8% in 48 months (**Figure 4.4D**).

4.6.6 Comparison of annual percentage change in gonadal function over the 48 months follow-up

A comparison of annual percentage change in the gonadal function between the two groups is shown in Figure 5. Among participants who had undergone varicocelectomy, serum total testosterone increased by 76.3% in the first year from the baseline, reduced to 69.7% in the second year, and 11.2% in the third year and further to 5.2% in the fourth year. While in the observed group, a decrease of 6.3% was observed in year one, an increase to 3.1% annually from the first year, then a decrease to 10.2% in the third year and finally increase to 0.3% in the fourth year. The annual variations in percentage change were statistically significant ($p<0.05$) (**Figure 4.5A**).

As shown in **Figure 4.5B**, serum FSH decreased by 14.7% in the first year from the baseline, decreased further by 17.5% in the second year, an annual decrease of 4.6% in the third year, and in the fourth year, a downward increase by 9.9% in the surgery group. In the observed group, a percentage change of 8.6 was observed in year one, 3.8% in year two, 4.0% in year three, and finally to 2.3% in year four (**Figure 4.5B**).

In the surgery group, serum LH had reduced significantly by 21.8% in the first year, and a year after, changed to 12.5%, further reduced by 1.3% in the third year, and finally to 5.4% in the fourth year. Whilst in the observed group, a percentage change of 8.6% in the first year was observed, then increase to 11.9% the following year, 6.4% in the third year, and further to 8.1% in the fourth year. For each year, the percentage change was statistically significant ($p<0.05$) (**Figure 4.5C)**

Table 4.5: The distribution of gonadal function over 48 months of follow-up among study participants

Variable	Baseline	12 months	24 months	36 months	48 months	One-way ANOVA; p-value	p-trend
FSH (IU/L)							
Observed group	23.30 ± 8.642	24.91 ± 8.446****	25.52 ± 8.339**	26.31 ± 8.257**	27.03 ± 8.799***	F (4,125)=0.7348; p=0.5699	0.0949
Surgery group	24.28 ± 7.001	20.69 ± 6.947****	16.59 ± 5.197****	15.57 ± 4.512****	13.87 ± 4.019****	F (4,130) = 14.99; p<0.0001	<0.0001
p-value	0.6522	0.0518	< 0.0001	<0.0001	<0.0001		
LH (IU/L)							
Observed group	11.21 ± 3.168	12.00 ± 3.335****	13.07 ± 3.850****	13.91 ± 4.368****	14.94 ± 4.439****	F (4,125) = 3.823; p=0.0058	0.0002
Surgery group	12.46 ± 4.207	9.419 ± 2.410****	7.970 ± 1.668****	7.752 ± 1.313****	7.323 ± 1.452****	F (4,130) = 19.80; p<0.0001	<0.0001
p-value	0.2281	0.0021	< 0.0001	<0.0001	<0.0001		
Testosterone (nmol/L)							
Observed group	2.069 ± 0.899	1.931 ± 1.931**	1.977 ± 0.9052	1.773 ± 0.8488***	1.869 ± 1.333	F (4,125)=0.3298; p=0.8575	0.3654
Surgery group	2.185 ± 0.730	3.644 ± 1.239****	5.915 ± 2.101****	6.444 ± 2.211****	6.667 ± 2.202****	F (4,130) = 32.17; p<0.0001	<0.0001
p-value	0.6078	< 0.0001	< 0.0001	< 0.0001	<0.0001		

*The data were presented as mean ± SD. The presence of significant differences among means of the groups was determined by one-way ANOVA followed by Newman-Keul's test as post hoc. Significantly different from baseline (Ctrl): *P<0.05, **P<0.01, ***P<0.001 by Newman-Keuls test*

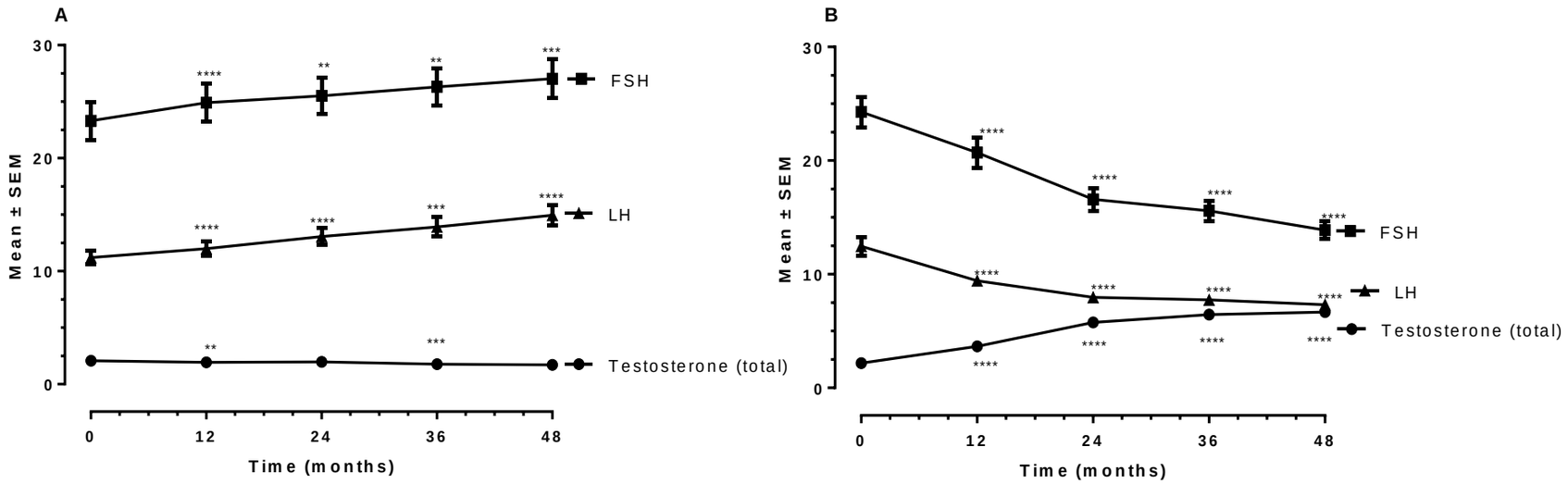

Figure 4.3: Mean difference in the hormones for each of the groups; data presented as means ± standard error of mean A - for the observed group; B - for the surgery group

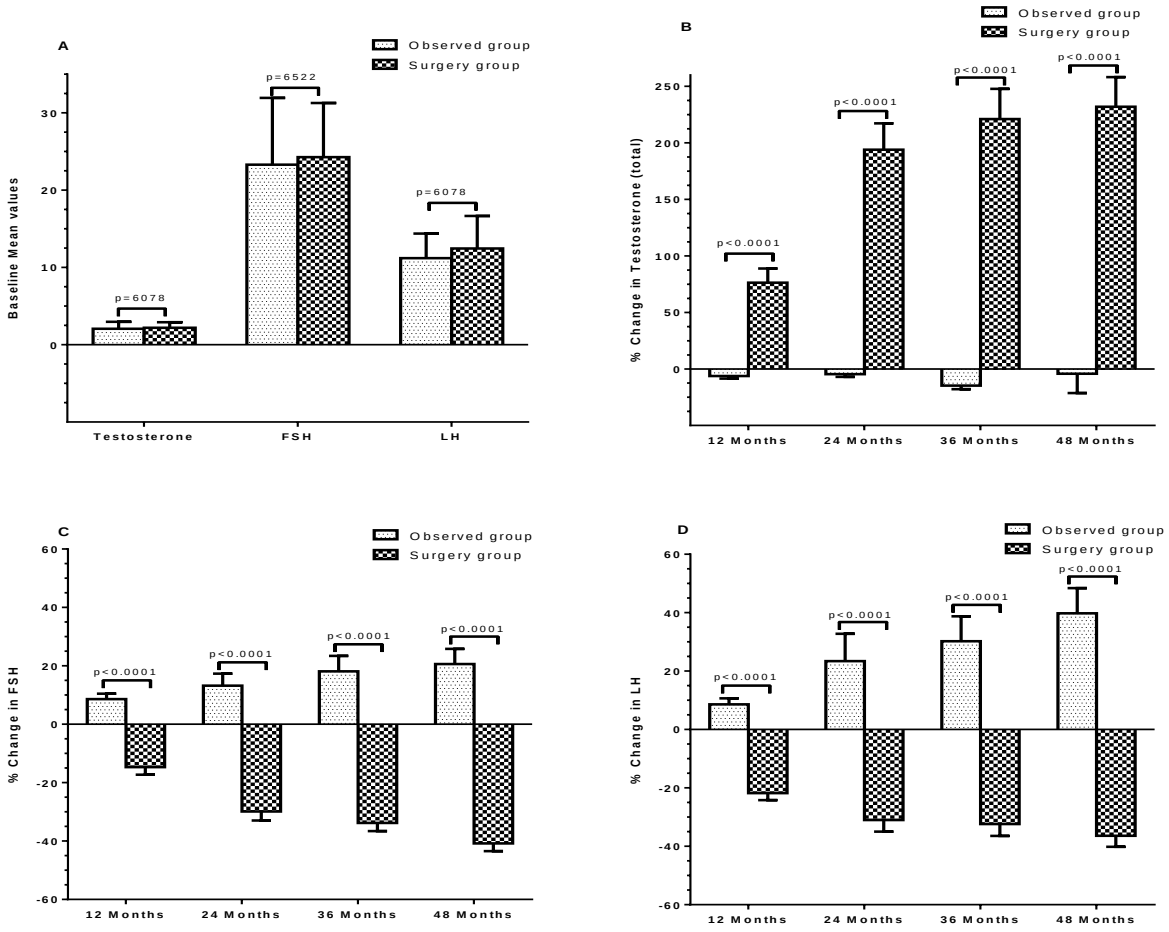

Figure 4.4: Comparison of overall percentage change in gonadal function between the observed group and operated group over 48 months. A = a graph of mean values of total testosterone, FSH, and LH between the two groups; B = a graph % change in Testosterone; C = a graph % change FSH; and D = a graph % change in LH. Data presented as group means (SEM). Significantly different between observed group and the surgery group at: *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001

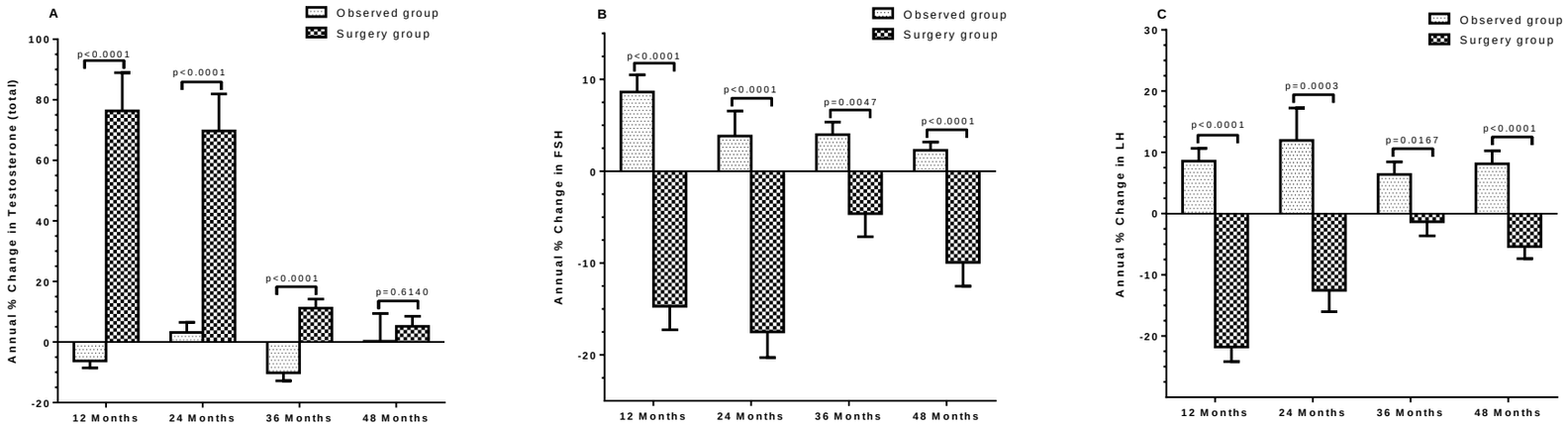

Figure 4.5: Comparison of annual percentage change in gonadal function between the observed group and operated group. A = a graph of Annual % change in Testosterone; B = a graph of Annual % change in FSH; C = a graph of Annual % change in LH respectively. Data presented as group means (SEM). Significantly different between observed group and surgery group at: *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001

4.7 DISCUSSION

To the best of our knowledge, this is the first study on the effect of varicocelectomy on the gonadal function in patients reporting sexual dysfunction among Ghanaians. Erectile dysfunction is commonly reported among mature and aging men (Feldman *et al.*, 1994; Ayta *et al.*, 1999) with prevalence varying from 12% to 71% in other parts of the world (Braun *et al.*, 2000; Lyngdorf and Hemmingsen, 2004) and 66% among the Ghanaian populace (Amidu *et al.*, 2010).

In this study, all the men at baseline reported sexual dysfunction on the GRISS scale and the score for each subscale was above 5 (Rust and Golombok, 1985) and was clinically diagnosed (Dubin and Amelar, 1970) and confirmed with varicocele (Hussein, 2006). In terms of the laterality of varicocele, 94.0% of patients were left-sided, while the others were bilateral. This is consistent with earlier studies reporting that right-sided varicocele is extremely rare and the incidence of bilateral varicocele is 2.5% to 6.5% depending on the diagnosis (Matsuda, 1993; Onozawa *et al.*, 2002). It was noticed that participants who had undergone varicocelectomy noticed improved erectile function 12 months of follow-up post-operation. This is consistent with the findings by Sathya Srini and Belur Veerachari (2011) who reported improved erectile function post varicocelectomy. The possible explanation was due to the improvement of total testosterone levels which play an important role in the male sexual characteristics controlling the timing of the erectile process as a function of sexual desire (Corona and Maggi, 2010).

There is still a debate on the effect of varicocele on Leydig cell function and testosterone biosynthesis. Although some researchers reported no significant effect of varicocelectomy on testosterone levels (Segenreich *et al.*, 1986; Pasqualotto *et al.*, 2008), fewer other studies showed a significant improvement in gonadal function following varicocelectomy (Su *et al.*,

1995; Hurtado de Catalfo *et al.*, 2007; Sathya Srini and Belur Veerachari, 2011). Sathya Srini and Belur Veerachari (2011) reported a significant rise in serum total testosterone from 1.77 ± 0.18 ng/mL before varicocelectomy to 3.01 ± 0.43 ng/mL after 12 months of follow-up post-surgery and this was associated with an insignificant drop in serum FSH and serum LH. Su *et al.* (1995) also found that serum total testosterone but not serum LH and serum FSH levels, increased post microsurgical varicocelectomy. This is consistent with the current study which found a significant increase in serum total testosterone levels after varicocelectomy in the operated group. In this study, men were classified based on their pre-operative hormones and this may explain the variations in serum testosterone improvement as reported. This suggests that the induced Leydig cell dysfunction caused by varicocele can be reversed by varicocelectomy.

Clinically, Sakamoto and Ogawa (2009) reported an association between varicocele and relative testicular hypotrophy. Patients with severe varicocele showed lower testicular volume and increased FSH levels (Lotti *et al.*, 2009), and management of the varicocele may reduce this negative effect (Sakamoto and Ogawa, 2009). A systematic review and meta-analysis of five studies including 312 patients by Tian *et al.* (2018) showed that serum FSH level (95% CI: 0.19-0.77; p=0.001) and serum LH level (95% CI: 0.25-0.91; p=0.0005) were higher before operation than after varicocelectomy. In this present study, serum FSH and serum LH decreased significantly after varicocelectomy among the surgery group over the 48 months of follow-up while there were no significant changes in the observed group. The overall percent decrease was consistent in both FSH and LH over the period in the surgery group. The possible explanation may be due to improvement in Leydig cell function with its effect seen in increased serum total testosterone which gives negative feedback on the hypothalamus-pituitary-gonadal axis causing a decreased FSH and LH.

Some clinical studies have reported the effect of varicoceles on serum testosterone and sexual dysfunction. Comhaire and Vermeulen (1975) earlier reported decreased testosterone level and erectile dysfunction in 30% of men (10 out of 33) with varicoceles and both symptoms improved after varicocelectomy. In 2011, a study conducted by Tanrikut *et al.* (2011) demonstrated that men with varicocele had lower serum total testosterone levels compared with controls, and about 79% of cases post-varicocelectomy reported with normal serum total testosterone levels. Cayan *et al.* (1999) found that men with varicoceles exhibit decreased free testosterone levels and increased plasma FSH levels; after microsurgical varicocelectomy, the total plasma and free testosterone levels significantly increased and FSH level decreased. The finding in this current study is consistent with Cayan *et al.* (1999).

In a meta-analysis of seven studies involving 712 patients to compare pre-and post-surgical serum testosterone levels, Chen *et al.* (2017) found that the mean postoperative serum testosterone improved by 34.3 ng/dL compared with pre-treatment levels; an increase by 105.65 ng/dL in the hypogonadal men, favoring those who had undergone varicocele repair. After the microsurgical inguinal varicocelectomy, this study observed a sharp increase in serum total testosterone by 76.3% in the first year and subsequently, a reduction to 69.7% in the second year, 11.2% in the third year, and 5.2% in the fourth year. Whilst a sharp decrease in serum FSH and LH (annual percentage change) was observed post-operation. The possible explanation may be that, within the first two years post varicocele repair by microsurgical sub-inguinal varicocelectomy, serum testosterone will observe a sharp increase whilst serum FSH and LH will also reduce significantly. But from the third year onwards, the variation in the annual percent changes in these gonadotropins will be marginal and this may be due to hormonal down regulations.

The following postoperative complications were observed in the surgery group: (i) Postoperative pain requiring strong opioids 5/52 (9.6%); After the surgery, all patients received paracetamol 1000 mg TID for 24 hours with the majority not experiencing pain. However, three patients experienced severe postoperative pain that was not relieved by paracetamol. They, therefore, were given IM Pethidine 50 mg TID to control the pain. (ii) Postoperative erythema 2/52 (3.8%); a mild form of surgical site infection which was noticed on postoperative days 3 and 4. Wound swab for culture and sensitivity yielded negative cultures. The wound healed spontaneously without the need for antibiotics.

One of the shortcomings of this study was the drop-out during follow-up, especially among the observed group. This was so because participants were followed for 48 months (4 years). It is recommended to use this choice (microsurgical sub-inguinal varicocelectomy) for similar patients, however, further studies with large sample sizes and possible placebo are needed to provide more evidence to recommend such therapy. Also, varicocelectomy plus supraphysiologic dosages of human chorionic gonadotropin (hCG) therapy have found significant superiority in infertile men with varicocele and could be a topic of further studies.

4.8 CONCLUSION

Long-standing varicocele may cause Leydig cell damage and this may be seen by causing a further decrease in total testosterone and a concomitant rise in follicle-stimulating hormone (FSH) and luteinizing hormone (LH). This study found that serum total testosterone, FSH, and LH observed spike changes within the first- and second-year in the surgery group but changes were marginal from the third year onwards. Microsurgical sub-inguinal varicocelectomy improved serum total testosterone, decrease both serum FSH and LH levels, and improved sexual dysfunction in patients reporting with varicocele.

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Chapter 5

PAPER III

5.1 FACTORS ASSOCIATED WITH IMPROVED SEMEN CHARACTERISTICS FOLLOWING MICROSURGICAL SUB-INGUINAL VARICOCELECTOMY IN INFERTILE PATIENTS

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Author Statement

Authors **Yussif Adams (Candidate)**, **Akisibadek Alekz Afoko (Supervisor)** and **Nafiu Amidu (Supervisor)** designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author **Akisibadek Alekz Afoko** performed the surgery. Authors **Yussif Adams**, **Lawrence Quaye**, **Simon Bannison Bani, and Peter Paul M. Dapare** managed the analysis of the study, software and did the validation of the results. Authors **Yussif Adams**, **Akisibadek Alekz Afoko**, and **Vivian Afoko** did field sampling and part of the laboratory analysis, analysed the results, and manged the literature searches.

5.2 ABSTRACT

Background: *Varicocelectomy is widely used for the treatment of patients with male fertility factors reporting varicocele. However, factors to predict which of the varicocele patients are likely to benefit from the surgery in terms of improved semen quality and characteristics*

have proven to be very difficult. The aim of the study is to determine factors associated with improved semen characteristics post microsurgical sub-inguinal varicocelectomy. ***Methods****: A total of 127 oligozoospermic patients with varicoceles requiring varicocelectomy referred to Tamale Teaching Hospital, Ghana from September 2017 to August 2021 were recruited. Patients were categorized into two groups; 'responders' and 'non-responders'. Patients who showed significant improvement in semen characteristics (sperm count, concentration, motility, and morphology) 12 months after varicocelectomy were grouped as responders, whereas those who showed no improvement 12 months after surgery were considered non-responders. The predictive factors considered were; age, body mass index, varicocele grade, testicular hemodynamic, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and semen characteristics. These factors were assessed using logistic regression analysis at an alpha value of 0.05.* ***Results****: The men were aged between 31.0 and 67.0 years old. Among the 127 patients, sperm concentration significantly ($p < 0.0001$) improved from 7.86 ± 3.876 to $32.87 \pm 15.57 \times 10^6$/mL and sperm motility increased from 34.40 ± 5.134 to $62.41 \pm 12.93 \times 10^6$/mL in 69 patients (54.3%). In the logistic regression analysis, pre-operative serum FSH (aOR = 0.494; 95% CI: 0.267-0.913; p = 0.024), total testosterone (aOR = 3.618; 95% CI: 1.325 - 9.879; p = 0.012) and resistive index (L_RI cap) on the left capsular arteries (aOR = 0.452; 95% CI: 0.211 – 0.969; p = 0.045) were predictors of improved sperm concentration.* ***Conclusion****: Microsurgical sub-inguinal varicocelectomy improved sperm characteristics. The predicting factors associated with improved semen characteristics post varicocelectomy are high testosterone, low serum FSH, and low left capsular resistive index.*

Keywords: varicocele, varicocele repair, infertility, hypogonadism, testicular hemodynamic

5.3 ABBREVIATIONS AND ACRONYMS

aOR adjusted odds ratio

BMI body mass index

DBP diastolic blood pressure

FSH Follicle-stimulating hormone

LH Luteinizing hormone

L_PSVcap peak systolic velocity for left capsular arteries

L_EDVcap end-diastolic velocity for left capsular arteries

L_RIcap resistive index for left capsular and centripetal arteries

OR odds ratio

SBP systolic blood pressure

SUE Scrotal ultrasound evaluation

TTH Tamale Teaching Hospital

WHO World Health Organization

5.4 INTRODUCTION

Varicocele is a disorder of venous return caused by abnormal dilatation of pampiniform plexus draining the testicles (Afoko *et al.*, 2010; Clavijo *et al.*, 2017). This condition is commonly found in men with both primary and secondary male factor fertility (Nallella *et al.*, 2006; Samplaski *et al.*, 2014) with an incidence of 21% to 45% in men with primary infertility, and 75% to 81% with secondary infertility (Gorelick and Goldstein, 1993; Jarow *et al.*, 1996; Miyaoka and Esteves, 2011). Due to anatomical position, the majority (> 90%) of varicoceles are left-sided with about 1.1% being bilateral, and 0.2% isolated right varicoceles (Damsgaard *et al.*, 2016).

Varicocele affects fertility but the etiology remains debatable. A few studies have reported no effect (Rageth *et al.*, 1992; Breznik *et al.*, 1993; Krause *et al.*, 2002) but recent studies

have shown that varicocele causes decrease testicular function, leading to altered spermatogenesis and diminished testosterone levels (Agarwal *et al.*, 2016; Kang *et al.*, 2021). Sertoli and Leydig cells are responsible for spermatogenesis and testosterone production and both are located nearby in the testis. These cells may be affected by changes within the testicular environment such as; hypoxia in the testes as a result of venous stasis (Hendin *et al.*, 1999), sperm DNA damage caused by increased production of reactive oxygen species (Agarwal *et al.*, 2014), increased scrotal temperature (Shiraishi *et al.*, 2012), and reflux from the adrenal vein into the spermatic vein (Comhaire and Vermeulen, 1974).

Long-standing varicocele is associated with Leydig cell dysfunction and hypogonadism (Sathya Srini and Belur Veerachari, 2011). Among patients who are affected, there is evidence of raised serum FSH and LH levels and reduced serum total testosterone concentrations (Lotti *et al.*, 2009; Tian *et al.*, 2018). This suggests that varicocele might lead to hormonal dysfunction through the hypothalamic-pituitary-gonadal axis (Li *et al.*, 2012).

Varicocelectomy is widely used for the treatment of patients with male fertility factors reporting varicocele. The ultimate aim of this surgical procedure is to improve couples' chances of achieving a pregnancy and live birth. Several ligation methods are used but the goal standard for treatment is microsurgical sub-inguinal (lymphatic- and artery sparing) varicocelectomy (Silveri *et al.*, 2003). Based on a long-term study, Cayan *et al.* (1999) proposed that this method improves semen quality and is associated with low post-surgery recurrence and complication rates.

In most men presenting with varicocele, varicocelectomy results in improved semen parameters but not all published data agrees to this (Redmon *et al.*, 2002; Baazeem *et al.*, 2011). Based on current evidence, the guidelines and the protocol by the American Urological Association (AUA), the American Society for Reproductive Medicine (ASRM), and the European Association of Urology (EAU) recommend varicocele repair for patients

with palpable varicocele with one or more semen parameter abnormalities' whether or not they are attempting to conceive a child (Shridharani *et al.*, 2012; Kang *et al.*, 2021). However, factors to predict which of the varicocele patients are likely to benefit from the varicocele repair in terms of improved semen quality and characteristics have proven to be very difficult. Hence, the study aims to determine the predictive factors of improving semen quality post microsurgical sub-inguinal varicocelectomy.

5.5 MATERIALS AND METHODS

The study included 127 oligozoospermic patients with varicoceles referred to Tamale Teaching Hospital for assessment of fertility problems. The study was conducted between September 2017 to August 2021 and was approved by the Ethics and Review Board of the Department of Research and Development, Tamale Teaching Hospital (Number: TTH/R&D/SR/119). Thus, has been performed following the standard laid down protocol in the 1964 Declaration of Helsinki. Informed consent was obtained from all participants before the study.

Participants eligible for the study were sexually active men who had maintained a stable heterosexual relationship for at least 2 years and reported male factor fertility. Male factor fertility was defined as the inability of a couple to conceive a child after one year of unprotected sexual intercourse with a normal female partner or spouse (i.e., normal reproductive history, normal ovulation, and tubal patency) (Nallella *et al.*, 2006). However, participants with a history of mumps orchitis, orchidectomy, undescended testis, uncontrolled hypertension (blood pressure of ≥ 140/90 mmHg), and uncontrolled diabetes (glycated hemoglobin of > 7%) were excluded from the study.

The men were aged between 31.0 and 67.0 years old. Pre-operative evaluation included; a complete demographic history using a semi-structured questionnaire, physical examination and confirmation of varicocele by ultrasound scan examination, semen analysis, and

measurement of serum hormones. Dubin and Amelar (1970) approach was used to detect, confirm, and clinical-grade varicocele. Varicocele was graded as grade I (palpable only during the Valsalva maneuver), grade II (palpable without the Valsalva maneuver), or grade III (visible without palpation) (WHO, 1992). A duplex Doppler ultrasound of the testes (Samsung Medison Accuvix V20 scan, Samsung Electronics, South Korea) with measurement of PSV (peak systolic velocity), EDV (end-diastolic velocity), and RI (resistive index) for capsular arteries was done to evaluate testicular malposition, blood reflux along the pampiniform plexus, or the extent of any fluid collections.

Semen analysis was performed using two different semen specimens (mean values adopted), each obtained by masturbation after 3 to 5 days of sexual abstinence. The pre-operative semen specimens were collected at least 2 weeks before the surgery while the post-surgery specimens were collected at 9 months and 12 months intervals respectively. The semen samples were analyzed according to WHO criteria (Cooper *et al.,* 2010). From the sperm concentration, participants were categorized into two groups; responders and non-responders. Responders were participants who showed significant improvement (more than 50% rise was recognized at least two times post-operatively in comparison with such counts before operation) in semen characteristics (sperm count, sperm concentration, motility, and morphology) 12 months post varicocelectomy, whereas those who showed no improvement 12 months after surgery were considered non-responders (Kondo *et al.*, 2009).

Blood samples were collected before and after surgery for the fertility hormones assay. Serum FSH (follicle-stimulating hormone) and LH (luteinizing hormone) were measured by electrochemiluminescence with a Hitachi-Roche analyzer (Cobas 6000, Roche Diagnostics, IN, USA). Serum total testosterone was analyzed by radioimmunoassay.

5.5.1 Statistical Analysis

Data were entered into Microsoft Excel version 10 (www.microsoft.com) and exported to SPSS version 23 (SPSS Inc., Chicago, IL, USA) for analysis. Categorical variables are presented as frequency (percent) and continuous variables are presented as mean ± SD. Statistical analyses were performed using the Mann-Whitney test and Chi-square test. Predictors of improved semen characteristics were assessed using univariate and multivariate logistic regression analysis. A two-tailed p-value less than 0.05 was considered statistically significant.

5.6 RESULTS

5.6.1 Background of oligozoospermic patients

Among 127 patients, improved sperm concentration was observed in 69 participants (responders, 54.3%). After microsurgical sub-inguinal varicocelectomy, sperm concentration significantly increased from 7.86 ± 3.876 to 32.87 ± 15.57 million per millilitre ($p < 0.0001$) (**Table 5.1**). Significant differences were seen in post-operative L_EDVcap ($p = 0.049$), L_RIcap ($p = 0.024$), serum FSH ($p = 0.014$), LH ($p = 0.041$), total testosterone ($p = 0.038$), sperm concentration ($p < 0.0001$), sperm count ($p < 0.0001$), sperm motility ($p < 0.0001$), and normal sperm morphology ($p < 0.0001$) between responders and non-responders.

In the responders' group, sperm count significantly improved from 29.40 ± 8.154 to 136.0 ± 64.50 ($p < 0.0001$) after microsurgical varicocelectomy. Also, sperm motility and normal sperm morphology values significantly ($p < 0.0001$) increased post-surgery (**Table 5.1**).

Table 5.1: Background of oligozoospermic patients

	Oligozoospermic patients (n = 127)		
Variable	**Responders (n = 69)**	**Non-responders (n = 58)**	**p-value**
Age (years)	49.50 ± 2.677	51.00 ± 2.554	0.103
Anthropometry measurements			
BMI (kg/m^2)	25.34 ± 1.452	23.90 ± 2.944	0.140
Body fat (%)	16.25 ± 3.545	18.94 ± 8.066	0.310
Muscle mass (%)	34.10 ± 4.630	35.76 ± 4.357	0.822
visceral fat (%)	7.500 ± 2.014	7.860 ± 3.583	0.761
Blood pressures			
SBP (mmHg)	130.5 ± 6.932	129.5 ± 6.152	0.871
DBP (mmHg)	83.70 ± 5.417	82.21 ± 5.506	0.721
Varicocele grade			
Grade II	40 (58.0%)	37 (63.8%)	0.648
Grade III	19 (42.0%)	21 (36.2%)	
Hemodynamics			
Pre-operative L_PSVcap	11.05 ± 0.3629	11.11 ± 1.624	0.903
Post-operative L_PSVcap	10.37 ± 0.2983	10.57 ± 1.347	0.637
Pre-operative L_EDVcap	4.220 ± 0.2573	4.695 ± 0.8454	0.086
Post-operative L_EDVcap	4.180 ± 0.1476	4.707 ± 0.8178	0.049
Pre-operative L_RIcap	0.6190 ± 0.01197	0.6465 ± 0.04660	0.096
Post-operative L_RIcap	0.5558 ± 0.03692	0.5840 ± 0.02171	0.024
Hormones			
Pre-operative FSH (IU/L)	24.15 ± 2.398	23.70 ± 2.905	0.680
Post-operative FSH (IU/L)	15.58 ± 2.744	18.81 ± 3.806	0.014
Pre-operative LH (IU/L)	10.350 ± 3.261	11.92 ± 3.827	0.362
Post-operative LH (IU/L)	7.790 ± 1.264	9.558 ± 2.482	0.041
Pre-operative Total Testosterone (nmol/L)	2.100 ± 0.4110	2.121 ± 0.7906	0.936
Post-operative Total Testosterone (nmol/L)	5.400 ± 1.299	4.579 ± 1.219	0.038
Semen parameters			
Pre-operative sperm concentration (x 10^6 mL^{-1})	7.86 ± 3.876	7.898 ± 3.704	0.362
Post-operative sperm concentration (x 10^6 mL^{-1})	32.87 ± 15.57	8.550 ± 4.039	< 0.0001
Pre-operative sperm count (x 10^6 mL^{-1})	29.40 ± 8.154	28.47 ± 12.45	0.475
Post-operative sperm count (x 10^6 mL^{-1})	136.0 ± 64.50	29.76 ± 9.462	< 0.0001
Pre-operative sperm motility (%)	34.40 ± 5.134	33.40 ± 7.634	0.768
Post-operative sperm motility (%)	62.41 ± 12.93	42.40 ± 14.37	< 0.0001
Pre-operative normal sperm morphology (%)	4.60 ± 2.459	3.19 ± 1.692	0.903
Post-operative normal sperm morphology (%)	7.07 ± 1.184	4.20 ± 1.269	< 0.0001

Statistical analyses were performed using the Mann-Whitney test and Chi-square test. BMI – body mass index; SBP – systolic blood pressure; DBP – diastolic blood pressure; L_PSVcap – Left peak systolic velocity for capsular and centripetal arteries; L_EDVcap – Left_end diastolic velocity for capsular and centripetal arteries, L_RIcap – Left resistive index for capsular and centripetal arteries, FSH – a follicle-stimulating hormone, LH – luteinizing hormone

5.6.2 Factors associated with improvement of sperm concentration

In the univariate binary logistic regression analysis, resistive index (L_RIcap) [odds ratio (OR) = 0.528; 95% confidence interval (95% CI): 0.148 - 0.989; p = 0.049], serum FSH (OR = 0.645; 95% CI: 0.445 - 0.937; p = 0.021), total testosterone (OR = 2.301; 95% CI: 1.274 - 4.156; p = 0.006), and sperm count (OR = 1.082; 95% CI: 1.012 - 1.157; p = 0.021) were the factors associated with improvement of sperm concentration. In the multivariate analysis, after adjusting for resistive index, serum FSH, serum total testosterone, and sperm count; a positive correlation with serum total testosterone (aOR = 3.618; 95% CI: 1.325 - 9.879; p = 0.012) and negative correlations with both serum FSH (aOR = 0.494; 95% CI: 0.267-0.913; p = 0.024) and L_RIcap (aOR = 0.452; 95% CI: 0.211 – 0.969; p = 0.045) were observed. In summary, pre-operative high testosterone, low serum FSH, and low resistive index were factors associated with improved sperm concentration (**Table 5.2**).

Table 5.2: Predictors of sperm concentration improvement

	Univariate		Multivariate	
Variable	**Odds ratio (95% CI)**	**p-value**	**Odds ratio (95% CI)**	**p-value**
Age (years)	0.467 (0.284 - 1.766)	0.683	0.452 (0.183 - 1.115)	0.085
BMI (kg/m^2)	1.197 (0.939 - 1.527)	0.147	1.654 (0.901 - 2.708)	0.065
Varicocel grade	0.966 (0.778 - 1.200)	0.756	0.942 (0.840 - 1.056)	0.306
Blood pressures				
SBP (mmHg)	1.003 (0.963 - 1.045)	0.868	1.023 (0.939 - 1.114)	0.609
DBP (mmHg)	1.099 (0.710-1.196)	0.518	1.207 (0.825 - 1.421)	0.314
Pre-operation Hemodynamic				
L_PSVcap	0.864 (0.475 - 1.570)	0.631	0.737 (0.338 - 1.609)	0.444
L_EDVcap	0.308 (0.077 - 1.234)	0.096	0.305 (0.071 - 1.314)	0.111
L_RIcap	0.528 (0.148 - 0.989)	0.049	0.452 (0.211 – 0.969)	0.045
Hormones				
FSH (IU/L)	0.645 (0.445 - 0.937)	0.021	0.494 (0.267-0.913)	0.024
LH (IU/L)	0.811 (0.648 - 1.016)	0.068	0.803 (0.467 - 1.380)	0.427
Total Testosterone (nmol/L)	2.301 (1.274 - 4.156)	0.006	3.618 (1.325 - 9.879)	0.012
Semen parameters				
Pre-operative sperm concentration (x 10^6 mL^{-1})	1.798 (1.234-2.619)	0.002	2.176 (1.108 - 4.273)	0.024
Pre-operative sperm count (x 10^6 mL^{-1})	1.082 (1.012 - 1.157)	0.021	1.059 (0.975 - 1.149)	0.173
Pre-operative sperm motility (%)	1.052 (0.981 - 1.127)	0.153	1.058 (0.951 - 1.178)	0.295
Pre-operative morphology (%)	0.997 (0.947 - 1.049)	0.900	0.980 (0.913 - 1.052)	0.574

Predictors of improved semen characteristics were assessed using univariate and multivariate logistic regression analysis; BMI – body mass index; SBP – systolic blood pressure; DBP – diastolic blood pressure; L_PSVcap – Left peak systolic velocity for capsular and centripetal arteries; L_EDVcap – Left_end diastolic velocity for capsular and centripetal arteries; L_RIcap – Left resistive index for capsular and centripetal arteries; FSH – a follicle-stimulating hormone, LH – luteinizing hormone

5.7 DISCUSSION

Varicocele affects spermatogenesis (Scott and Young, 1962) and many studies have reported alterations in semen characteristics in patients with varicocele (Shiraishi *et al.*, 2012; Xue *et al.*, 2012; Agarwal *et al.*, 2016; Adams *et al.*, 2022a). These had led to the conclusions that varicocele is responsible for 45 – 80% of infertility in men (Nagler *et al.*, 1993; Witt and Lipshultz, 1993; Agarwal *et al.*, 2007).

Though several studies concluded that varicocele repair results in improvement of semen characteristics, not all findings support this claim (Redmon *et al.*, 2002; Baazeem *et al.*, 2011). We initially reported significant improvement of semen characteristics following microsurgical sub-inguinal varicocelectomy (Adams *et al.*, 2022a). In this study, however, the aim is to retrospectively analyze the factors potentially related to the surgery outcome. Among the 127 participants studied, 54.3% had improved sperm concentrations post varicocelectomy. This is in line with the findings by Kondo *et al.* (2009) in which 57% of patients were reported to have improved sperm parameters following a varicocele repair. Again, Chen and Chen (2011) reported a much higher (71.4%) improvement of semen characteristics in infertile patients 6-months after the varicocele repair.

Dubin and Amelar (1977) performed surgical correction of varicocele on 986 cases over twelve years and found that 70 percent of patients had improved semen quality, and 53 percent of wives became pregnant. The study concluded that the results were better for patients who had pre-operative sperm counts greater than 10^6 mL^{-1} than for patients who had pre-operative sperm counts of less than 10^6 mL^{-1} (Dubin and Amelar, 1977). Segenreich *et al.* (1986) later reported a better pregnancy outcome despite counts below these values ($< 10^6$ mL^{-1}). In this study, patients with sperm count greater than 10 million per milliliters before varicocele repair had improved semen characteristics (OR = 1.082; p = 0.021) compared

with those with sperm count of lesser values. However, sperm count was not a better predictor when adjusted for by confounders. This result contradict the findings by Kondo *et al.* (2009) who reported that sperm count before ligation was not a predictor of improved seminal parameters.

The absence of universally standardized criteria for improvement of semen parameters has made prediction of patients who are likely to benefit from varicocele repair very difficult. Fewer studies have considered factors associated with surgical outcome in varicocele. For instance, Giannakis *et al.* (2004) found that testicular telomerase activity was the main parameter predicting the effect of varicocelectomy on spermatogenesis but Ishikawa and Fujisawa (2005) and Kondo *et al.* (2009) found that age (i.e., as one grows older, telomerase activity decreases) was not a significant predictor in both univariate and multivariate analysis.

Huang *et al.* (2014) in a more recent study grouped men who had varicocele into "responders" and "non-responders" based on semen analyses at 3, 6, and 12 months post-operatively. They found that patient age (OR = 0.56; $p < 0.0001$) and pre-operative sperm density (OR: 1.22; $p = 0.0001$) were significantly associated with the likelihood of successful varicocele repair (Huang *et al.*, 2014). Similarly, Samplaski *et al.* (2014) also found that patient age, varicocele grade, and pre-operative semen parameters (ejaculate volume, sperm concentration, total motile sperm count, motility, and normal sperm morphology) were associated with improved semen parameters post varicocelectomy. In this study, pre-operative sperm count predicts improved sperm concentration at the univariate analysis but patient age and varicocele grade were not predictors of sperm concentrations.

The effect of varicocele on Leydig cell function and testosterone biosynthesis is still a subject of debate. Many of the existing findings have conflicting results. Studies by Pasqualotto *et al.* (2008) and Segenreich *et al.* (1986) found no significant effects of varicocele repair on testosterone levels. However, other studies reported a significant improvement of gonadal function following varicocelectomy (Su *et al.*, 1995; Hurtado de Catalfo *et al.*, 2007; Sathya Srini and Belur Veerachari, 2011). According to WHO (1999), a varicocele may be a factor in the progressive worsening of testicular function (both steroidogenesis and spermatogenesis) over time and this may result in high serum FSH and low testosterone levels in patients. In this study, pre-operative low serum FSH and high testosterone were predictors of improved sperm concentration. This is consistent with the findings of Kondo *et al.* (2009) who reported similar results as good prognostic factors for varicocelectomy. Similarly, studies by Chen and Chen (2011) found that among other factors, low serum FSH (<11.3 mIU/Ml) was a predictor of improved semen characteristics after varicocele repair. The possible explanation may be that patient with normal Sertoli and Leydig cell functions benefits from the microsurgical sub-inguinal varicocelectomy.

An inverse correlation between body mass index (BMI) and incidence of varicocele has been reported (Gokce *et al.*, 2013; Rais *et al.*, 2013). However, there is limited available published data on the correlation of BMI with the improvement of semen characteristics post-varicocelectomy. In this study, BMI was not a predictor of improved semen concentration and this agrees with findings by Chen and Chen (2011) who reported similar findings among 35 men who had undergone varicocelectomy.

Varicocele is a disorder in which the pampiniform plexus draining the testicle is enlarged, with reflux of venous blood (Clavijo *et al.*, 2017; Bertolotto *et al.*, 2020). The consequence

of this venous abnormality is often arrest of ipsilateral testicular growth, thus arterial 'insufficiency' and hypoperfusion of testicular tissues. Afoko *et al.* (2010) reported a significant reduction in arterial perfusion of testicular tissues evidenced by the increase in the resistive index (RI) in an observed group compared with improved testicular perfusion evidenced of decreased RI in the surgery group among adolescents with left-sided varicocele. Resistive index (RI) is an ultrasonic parameter showing microcirculation function and testicular parenchymal perfusion (Zolfaghar-Khani *et al.*, 2020) The S-D/S formula, where S represents peak systolic velocity (PSV) and D stand for end-diastolic velocity (EDV), is used to measure the index (RI). Increased RI in the testes is associated with disruptions in microcirculation as a result of a significant reduction in testicular blood flow (Al-Naffakh, 2012; Gloria *et al.*, 2018; Zolfaghar-Khani *et al.*, 2020). In this study, pre-operative low resistive index (RI) on the left capsular artery was a factor associated with improved sperm concentration in logistic regression analyses. Afoko *et al.* (2010) concluded that the main hemodynamic indicator that strongly correlates with semen quality characteristics among adolescents with varicocele was resistive index (RI) especially in centripetal arteries and this may be true for adults as well.

Given the predictive factors associated with improved semen parameters in patients with varicocele, it is worth recognizing that improvement on the semen quality post varicocele repair does not guarantee patients to father children. Further studies on a larger population of varicocele patients with pregnancy rate as the primary outcome will help to conclusively determine the effectiveness of microsurgical sub-inguinal varicocelectomy.

5.8 CONCLUSION

These findings suggest that the significant predictive factors associated with improved semen characteristics following microsurgical sub-inguinal varicocelectomy in infertile men are; pre-operative low serum FSH, high testosterone, and low left capsular resistive index (L_RI cap).

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Chapter 6

PAPER IV

6.1 CHANGES IN TESTICULAR ARTERIAL HAEMODYNAMIC, GONADOTROPIN LEVELS AND SEMEN PARAMETERS AMONG VARICOCELE PATIENTS RANDOMIZED TO VARICOCELECTOMY OR OBSERVED IN TAMALE, GHANA

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Author Statement

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Conceived and designed the research, did field sampling and part of the laboratory analysis, analysed and validated the results, drafted and reviewed the manuscript.

Akisibadek Alekz Afoko (Co-supervisor)

Performed the surgery, supervised the work, provided resource, validated the results, and reviewed the manuscript.

Nafiu Amidu (Supervisor)

Supervised the work, validated the results, and reviewed the manuscript.

6.2 ABSTRACT

***Background**: Spermatogenesis is a sensitive and precise process where blood demand and supply are of significant importance. This is a randomized trial to compare testicular blood supply, gonadal hormones, and semen characteristics among three groups: Surgery group, varicocele patients who had microsurgical sub-inguinal varicocelectomy (n = 127); Observed group, varicocele patients whose spermatic veins were preserved (n = 114); and healthy controls (n = 33). **Methods**: The blood flow parameter selected was resistive index (RI) measured using Color Doppler Ultrasonography (CDUS). Serum total testosterone, FSH, LH were measured, and semen analysis performed at baseline and after 12 months of follow-up. The data was computed using GraphPad Prism (v8.0) at an alpha of 0.05. **Results**: In the observed group, increased +0.0060 in the right (R_RI) and in the left (L_RI) +0.0026 capsular arteries from baseline measuremt to 12 months follow-up. The surgery group, reduced -0.079 in the right (R_RIcap) and -0.0731 in the left the (L_RIcap) capsular arteries ($p < 0.0001$). At 12 months, the changes in the RI for both left and right capsular arteries in the surgery group did not reach the values of the controls (R_RIcap = 0.4894 ± 0.055 and L_RIcap = 0.485 ± 0.064). The postoperative semen characteristics, serum total testosterone, FSH, and LH levels changed significantly after the 12 months follow-up but no changes were detected in the observed group. In the surgery group, resistive index on the left ($r = -0.63$; $p<0.0001$) and right ($r = -0.49$; $p = 0.004$) correlated with total testosterone, FSH ($r = 0.57$; $p = 0.001$ for left; $r = 0.52$; $p = 0.002$ for right) and LH ($r = 0.61$; $p = 0.0002$ for left; $r = 0.41$; $p = 0.020$ for right). Furthermore, resistive index on the left capsular arteries correlated with changes in sperm count ($r = -0.46$; $p = 0.008$) and sperm concentration ($r = -0.35$; $p = 0.011$) in the surgery group but no significant correlation was found in the observed group. **Conclusion**: Varicocelectomy improve blood supply to the*

testicular tissues evidenced by reduced resistive index in the surgery group. These Doppler indices especially the RI in the left capsular artery, can be used to evaluate the success of surgery because it correlates with total testosterone, FSH, LH, and semen quality.

Keywords: varicocele, fertility, color duplex Doppler ultrasound, resistive index, microsurgical sub-inguinal varicocelectomy, gonadotropins, semen analysis

6.3 ABBREVIATIONS AND ACRONYMS

aOR	adjusted odds ratio
BMI	body mass index
DBP	diastolic blood pressure
FSH	Follicle-stimulating hormone
LH	Luteinizing hormone
L_PSVcap	peak systolic velocity for left capsular and centripetal arteries
L_EDVcap	end-diastolic velocity for left capsular and centripetal arteries
L_RIcap	resistive index for left capsular and centripetal arteries
OR	odds ratio
SBP	systolic blood pressure
SUE	Scrotal ultrasound evaluation
TTH	Tamale Teaching Hospital
WHO	World Health Organization

6.4 INTRODUCTION

Varicocele is a disorder of venous returns caused by abnormal enlargement of the internal spermatic veins draining the testicles (Clavijo *et al.*, 2017; Kang *et al.*, 2021). This treatable medical condition is found in 15% of the general population, 21% to 45% in men with primary infertility, and 75% to 81% of men with secondary infertility (Gorelick and Goldstein, 1993; Jarow *et al.*, 1996; Tarhan *et al.*, 2011).

Varicocele affects fertility but the pathophysiology remains debatable. Studies have shown that varicocele plays a role in decreased testicular function by altering spermatogenesis and reducing testosterone production (Agarwal *et al.*, 2016; Kang *et al.*, 2021). The Sertoli cells act to regulate the development of the germinal cells along with the maturation and release of spermatozoa into the central lumen of the seminiferous tubule. They are joined to each other by tight junctions forming the so-called blood-testis barrier. This barrier prevents the passage of molecules between the basal and luminal compartment of the seminiferous tubules and prevents the initiation of an immune response to spermatozoa by the human immune system. Leydig cells are responsible for spermatogenesis and testosterone productions under the influence of Luteinizing hormone. Both Sertoli cells and Leydig cells may be affected by changes within the testicular environment (Kang *et al.*, 2021).

Spermatogenesis is a sensitive and precise process where blood demand and supply are of significant importance. Blood supply to the testes is derived from testicular arteries which arise from the aorta, although, other sources include; the cremasteric artery which supplies the peri-testicular tissues, and the deferential artery which supplies the epididymis and vas deferens (Al-Naffakh, 2012; Kang *et al.*, 2021). Adequate blood supply to the testes is needed for spermatogenesis and decreased blood supply may cause ischemia.

In most studies, Doppler indices are used to obtain information about blood flow within the testicles with a recently introduced ultrasonic parameter, resistive index (RI), which shows testicular parenchymal perfusion and microcirculation function. This index is calculated using the S-D/S formula, where 'S' stands for Peak Systolic Velocity (PSV), and 'D' represents End Diastolic Velocity (EDV). In the testicles, high values of RI (> 0.6) imply disruption in microcirculation and thus hypoxia in the testicles (Al-Naffakh, 2012; Halpern *et al.*, 2016).

Earlier studies reported no significant difference between the mean Doppler indices before and after surgical ligation of the spermatic veins (Študent *et al.*, 1998; Cocuzza *et al.*, 2010). In recent times, however, Tarhan *et al.* (2011) found a significant improvement in testicular blood supply and sperm parameters after microsurgical sub-inguinal varicocelectomy. In this study, though there was no significant difference in the Doppler indices on the right artery, the mean values of blood flow velocities (peak systolic and end-diastolic) on the left artery increased while the resistive and pulsatility index decreased significantly after surgery (Tarhan *et al.*, 2011). In another study, Rehman *et al.* (2019) reported a significant negative correlation ($r = -0.28$; $p < 0.05$) between progressive motility of spermatozoa and RI of the capsular arteries in sixty prospective patients undergoing microsurgical varicocelectomy.

The microsurgical repair approach has become popular among other varicocele treatment methods because it is associated with improved Leydig cell function (Çayan *et al.*, 2020; Kang *et al.*, 2021; Adams *et al.*, 2022a) and reduced complications and/or recurrence of varicocele (Cayan *et al.*, 1999). The two approaches are microsurgical sub-inguinal and inguinal varicocelectomy but vascular anatomy gets more complicated as we get through the sub-inguinal region to the inguinal region, thus leaving the microsurgical sub-inguinal

(lymphatic- and artery sparing) varicocelectomy the most appropriate choice (Tarhan *et al.*, 2011). Hence, the study aims to determine the effect of microsurgical sub-inguinal varicocelectomy in modulating testicular tissue perfusion among varicocele patients using Color Doppler ultrasonography (CDUS) indices.

6.5 MATERIALS AND METHODS

6.5.1 Study design and population

This was an interventional study conducted at the Urology Unit of a tertiary hospital in Tamale, Ghana which span from September 2017 to August 2021. The Ethics and Review Board of the Department of Research and Development, Tamale Teaching Hospital (Number: TTH/R&D/SR/119) approved this study. A total of 241 varicocele patients and 33 healthy controls were included in the study. Varicocele patients who were eligible for inclusion were given the option of immediately undergoing microsurgical sub-inguinal varicocelectomy or observed for 12 months with a subsequent reassessment of the management plan and possibly delayed operation. Based on the willingness to accept either option, patients were randomized to the surgery group (n = 127) and the observed group (n = 114). Patients were eligible for inclusion if they reported male factor fertility and or had complained of any form of sexual dysfunction including weak sex drive, premature ejaculation, avoidance of sexual intercourse. Male factor fertility was defined as the failure of a couple to conceive a child after one year of unprotected sexual intercourse with a normal female partner or spouse (i.e. normal ovulation, normal reproductive history, and tubal patency) (Nallella *et al.*, 2006). However, patients with history of undescended testis, orchidectomy, mumps orchitis, uncontrolled diabetes (glycated hemoglobin of > 7%), and

uncontrolled hypertension (blood pressure of ≥ 140/90 mmHg) as discussed elsewhere (Adams *et al.*, 2022a) were excluded from the study.

6.5.2 Sociodemographic characteristics, Clinical examination, and Scrotal Ultrasound Evaluation

Structured pre-tested questionnaires were administered to participants to collect sociodemographic data, cigarette smoking, medical history, sexual function, and results of the gynecological evaluation of their spouse.

All patients were examined by a urologist. Varicocele diagnosis was based on physical examination and confirmed with color duplex Doppler ultrasonography examination. Dubin and Amelar (1970) approach was used to detect, confirm, and clinical-grade varicocele. Palpation of the spermatic cord was carefully done with patients standing erect and performing the Valsalva manoeuvre. Pampiniform plexus veins palpable only during the Valsalva manoeuvre was assigned grade I, palpable without the Valsalva manoeuvre was given grade II, and visible without palpation was considered grade III.

Participants were examined using two phases of scrotal Doppler ultrasound scan; the first phase was with participants in the supine position (with penis resting on suprapubic region) and the second in an upright position. A color Doppler ultrasonography (CDUS) of the testes (Samsung Medison Accuvix V20 scan, Samsung Electronics, South Korea) with measurement of PSV (peak systolic velocity), EDV (end-diastolic velocity), and RI (resistive index) for capsular arteries were calculated and recorded for both testes and to evaluate blood reflux along the pampiniform plexus, the extent of any fluid collections or testicular malposition. This CDUS has 83% to 95% sensitivity and 94% specificity for identifying subclinical varicocele compared with the physical examination which has only 70%

specificity (Trum *et al.*, 1996; Rehman *et al.*, 2019). Images obtained from the region of interest were delineated over both the left and right hemiscrota, femoral artery, and femoral muscles as shown in **Figure 6.1**. In the surgery group, scrotal scans were performed 2 weeks pre-varicocelectomy, and repeated 6- and 12-months post-surgery in each participant.

6.5.3 Laboratory Analysis

6.5.3.1 Hormonal analysis

Using the standard venepuncture method, a venous blood sample (4mls) was collected from each participant at the onset of the study (baseline) and after 12 months of follow-up for hormone assay. The hormonal analysis included measurement of serum concentration of total testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) as described previously (Adams *et al.*, 2022b).

6.5.3.2 Semen analysis

Semen analysis was performed in duplicates (mean values adopted) following WHO 2010 guidelines (Organization, 2010). Participants were advised to abstain from sexual intercourse for 3 to 5 days before producing samples. The specimens were collected into a clean sterile container confirmed to be non-toxic for spermatozoa by masturbation, and transported within 30 minutes to the laboratory. Each participant had baseline, 9- and 12-months semen samples respectively. For the surgery group, the baseline semen samples were collected at least 2 weeks before the surgery. The semen specimens were analyzed after complete liquefaction. Macroscopic and microscopic analysis of semen was performed following WHO guidelines. Sperm vitality was measured using Eosin Y 0.5% dye (Eosin Gelblich, Darmstadt, Germany) and the Kruger criteria using Nigrosin 8% staining technique (Nigrosin, Water Soluble, Darmstadt, Germany) was used to determine the sperm morphology (Elder and Dale, 2020).

6.5.4 *Microsurgical sub-inguinal varicocelectomy for the surgery group*

Participants were informed about their condition, the rationale of the varicocelectomy, risks, and benefits of the surgery by a urologist. The surgical procedure was carried out as described by Marmar *et al.* (1985). A microsurgical open sub-inguinal varicocelectomy procedure was performed under spinal anaesthesia using microsurgical instruments and magnification with an operating microscope KARL CAPS SOM 82 (Germany) for the surgery group. The lymphatic vessels and testicular arteries were spared, and both the internal and external spermatic fasciae were closed using PGA 3/0 running sutures. The wound was closed in layers and a subcuticular skin stitch was applied using 4/0 PGA sutures as described elsewhere (Adams *et al.*, 2022a).

6.5.5 Twelfth-months (12 months) follow-up

All three (3) groups were followed for 12 months after the day of the last baseline semen analysis. Participants in the surgery group were reassessed every 90 days to confirm the absence of genital infection, formation of hydrocele, recurrence of varicocele, and change in testicular size. Hormonal measurements (FSH, LH, and Total testosterone), seminal fluid analysis, and testicular hemodynamic indices (PSV, EDV, and RI) were repeated at 12 months of the follow-up.

6.5.6 Statistical analysis

The data were entered into a Microsoft Excel spreadsheet and then exported to Graphpad Prism version 6.2 (www.graphpad.com) software was used for data analysis. The Kolgoromo-Smirnov test was used to check for normality and outliers. The parametric data were presented as mean ± standard deviation (SD). Statistical t-test (paired and unpaired) was used to compare between two groups while the presence of significant differences

among means of the groups was determined by one-way ANOVA followed by Newman-Keul's test as post hoc. The correlation between resistive index (RI) and age, gonadal hormones, and semen parameters was performed using Pearson correlation analysis. Statistical significance was considered at an alpha value of $p < 0.05$.

6.6 6.5 RESULTS

A total of 241 infertile men with grade II/III varicocele, aged between 31 to 67 years, were randomized into 2 groups; surgery group (n = 127) and observed group (n = 114). The surgery group, with mean (SD) age 40.52 (4.666) years, underwent microsurgical sub-inguinal varicocelectomy while the observed group, with a mean age of 41.35 (5.262) years, had their spermatic veins preserved but were followed over 12 months. A total of 33 healthy men, mean age 42.59 (5.073) years, served as controls. All participants underwent duplex Doppler ultrasound of testes with measurement of PSV, EDV, and RI for capsular arteries. Again, gonadal hormones (serum total testosterone, FSH, and LH) and semen analysis were measured at baseline (onset) and repeated after 12 months of follow-up.

As shown in **Table 6.1**, in the observed group, the R_RI in capsular arteries increased from 0.613 ± 0.056 baseline to 0.619 ± 0.0571 at 12 months while the L_RI in capsular arteries increased from 0.6052 ± 0.0301 to 0.6078 ± 0.031. On the other hand, in the surgery group, the R_RIcap reduced significantly ($p < 0.0001$) from 0.623 ± 0.0562 baseline to 0.544 ± 0.0574 at 12 months while the L_RIcap reduced from 0.6127 ± 0.0381 to 0.5396 ± 0.036 at 12 months (**Table 6.1**).

The in-between group comparison shows that there was no significant difference ($p > 0.05$) in the baseline PSV, EDV, and RI between the observed and surgery group. However, both resistive index (RI) of capsular arteries in the left ($p < 0.0001$) and right ($p < 0.0001$) were

significantly reduced in the surgery group compared with the observed group. At 12 months, the changes in the RI for both left and right in the surgery group did not reach the values of the controls (R_RIcap = 0.4894 ± 0.0545 and L_RIcap = 0.485 ± 0.064) (**Table 6.1**).

The postoperative serum total testosterone, FSH, and LH concentrations changed significantly in the surgery group while no change was detected in the observed group (**Figure 6.2**).

Furthermore, the postoperative sperm count, sperm concentration, sperm motility, AFLP, and percentage of normal sperm morphology increased significantly in the surgery group compared with the observed group. Post-surgery sluggish motile sperms and immotile sperm were significantly reduced in the surgery group but no significant improvement was achieved in the observed group (**Figure 6.3**).

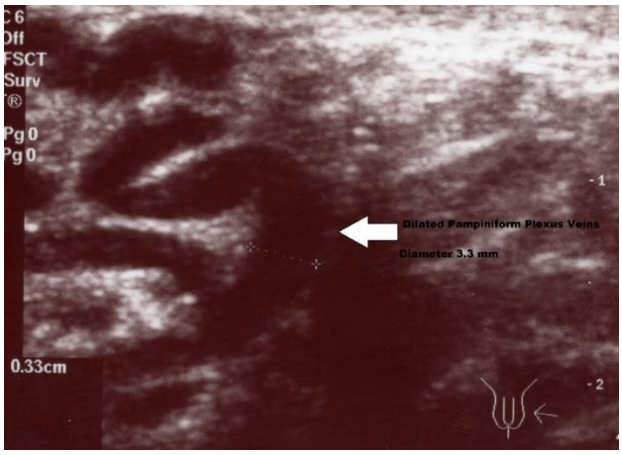
Pearson correlation was performed between resistive index (left and right capsular artery) and age, gonadal hormones and semen analysis among the participants (**Table 6.2**). Among those who had undergone the surgery, there was a significant inverse correlation between serum total testosterone and resistive index on the left ($r = -0.63$; $p<0.0001$) and right ($r = -0.49$; $p = 0.004$) capsular arteries. Also, on both capsular arteries, resistive index correlated with serum FSH ($r = 0.57$; $p = 0.001$ for left and $r = 0.52$; $p = 0.002$ for right) and LH ($r = 0.61$; $p = 0.0002$ for left and $r = 0.41$; $p = 0.020$ for right) in the surgery group. Furthermore, the resistive index on left capsular arteries correlated with changes in sperm count ($r = -0.46$; $p = 0.008$) and sperm concentration ($r = -0.35$; $p = 0.011$) but no significant correlation was observed in the right capsular arteries (**Table 6.2**).

Table 6.1: Pre- and Post-hemodynamic measurements over 12 months follow-up

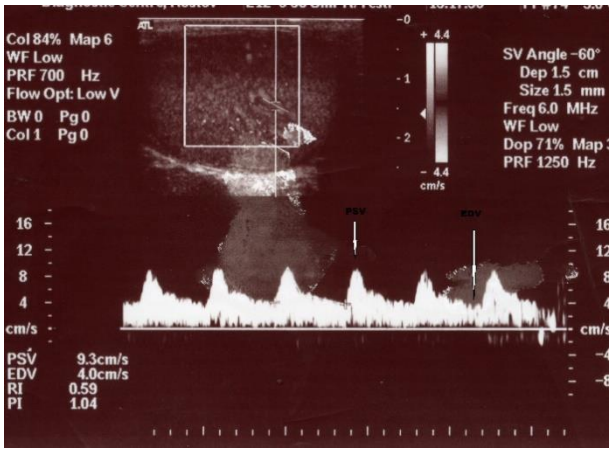
	Varicocele group		No Varicocele	One-way ANOVA	
Variable	**Observed (n=114)**	**Surgery (n=127)**	**Control group (n=33)**	**F-test**	**p-value**
Age (years)	41.35 ± 5.262	40.52 ± 4.666	42.59 ± 5.073	0.3416	0.5130
Baseline (onset)					
Right capsular artery					
R_PSVcap	11.66 ± 1.918	11.25 ± 2.002	11.32 ± 1.347	0.4976	0.6098
R_EDVcap	4.504 ± 0.917	5.520 ± 1.005^{++}	5.794 ± 0.623$^{££££}$	17.02	< 0.0001
R_RIcap	0.613 ± 0.056	0.623 ± 0.0562^{+++}	0.4856 ± 0.0511$^{££££}$	40.08	< 0.0001
Left capsular artery					
L_PSVcap	11.52 ± 1.531	10.85 ± 1.767	11.45 ± 1.281	1.586	0.2111
L_EDVcap	4.556 ± 0.8178	4.954 ± 0.8552^{++++}	5.956 ± 0.6881$^{££££}$	25.32	< 0.0001
L_RIcap	0.6052 ± 0.0301	0.6127 ± 0.0381^{++++}	0.4756 ± 0.0751$^{££££}$	43.12	< 0.0001
12 months follow-up					
Right capsular artery					
R_PSVcap	11.65 ± 1.865	11.23 ± 1.974	11.38 ± 1.520	0.3794	0.6855
R_EDVcap	4.504 ± 0.917*	5.104 ± 0.986^{++}	5.772 ± 0.5772$^{££££}$	16.99	< 0.0001
R_RIcap	0.619 ± 0.0571****	0.544 ± 0.0574^{+++}	0.4894 ± 0.0545$^{££££}$	34.68	< 0.0001
Left capsular artery					
L_PSVcap	10.61 ± 1.218	10.84 ± 1.736	11.41 ± 1.063$^{££}$	2.747	0.0700
L_EDVcap	4.552 ± 0.781	4.985 ± 0.839^{++++}	5.831 ± 0.496$^{££££}$	25.13	< 0.0001
L_RIcap	0.6078 ± 0.031****	0.5396 ± 0.036^{++++}	0.485 ± 0.064$^{££££}$	25.92	< 0.0001

*The data were presented as mean ± SD. The presence of significant differences among means of the groups was determined by one-way ANOVA followed by Newman-Keul's test as post hoc. (*) = Comparison between the observed group and surgery group; (+) = Comparison between surgery group and control group; (£) = Comparison between observed group and control group. Significantly different from baseline (Ctrl): *P<0.05, **P<0.01, ***P<0.001 by Newman-Keuls test. [R_PSVcap – right – peak systolic velocity capsular artery, R_EDVcap – right-end diastolic velocity capsular artery, R_RIcap – right-resistive index capsular artery, L_PSVcap – left– peak systolic velocity capsular artery, L_EDVcap – left-end diastolic velocity capsular artery, L_RIcap – left-resistive index capsular artery]*

A



B



C

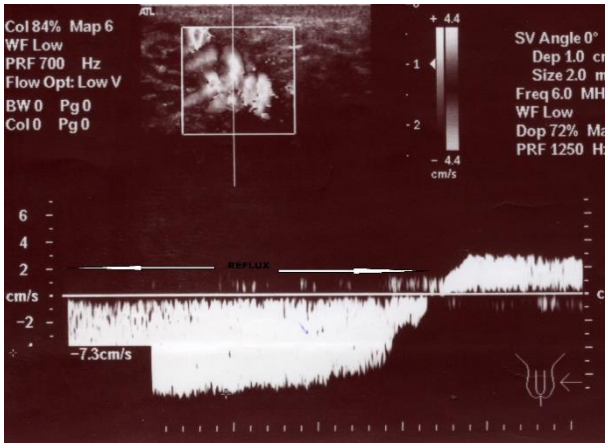


Figure 6.1: Images obtained from the region of interest; (A) Pampiniform plexus veins, (B) Duplex indices; (C) Reflux in testicular vein (right)

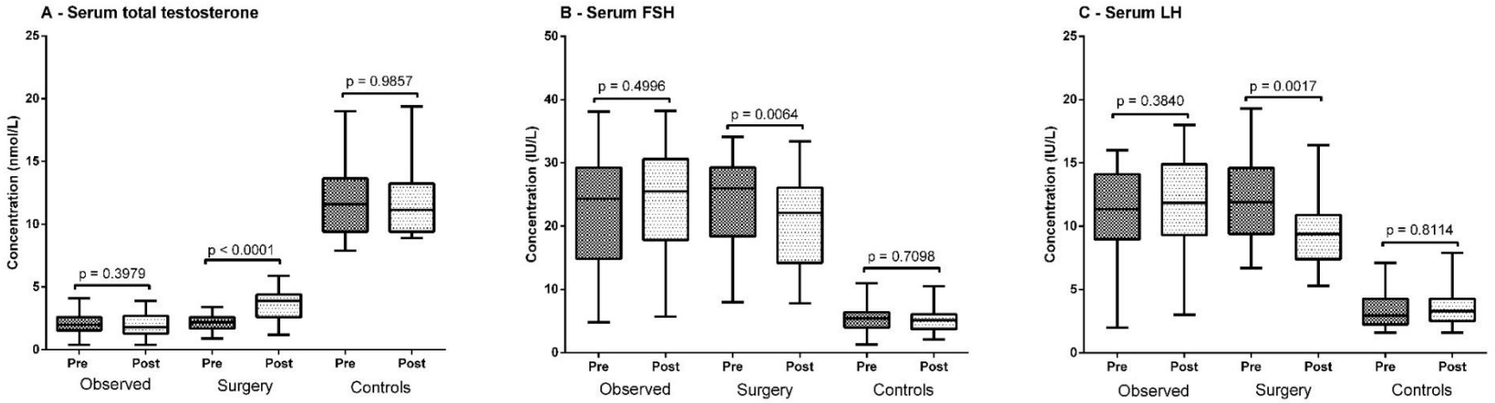


Figure 6.2: Pre- and Post-hormonal analyses over the 12 months follow-up (a) Serum total testosterone (TT), (b) Serum follicle-stimulating hormone (FSH), and (c) Serum luteinizing hormone (LH)

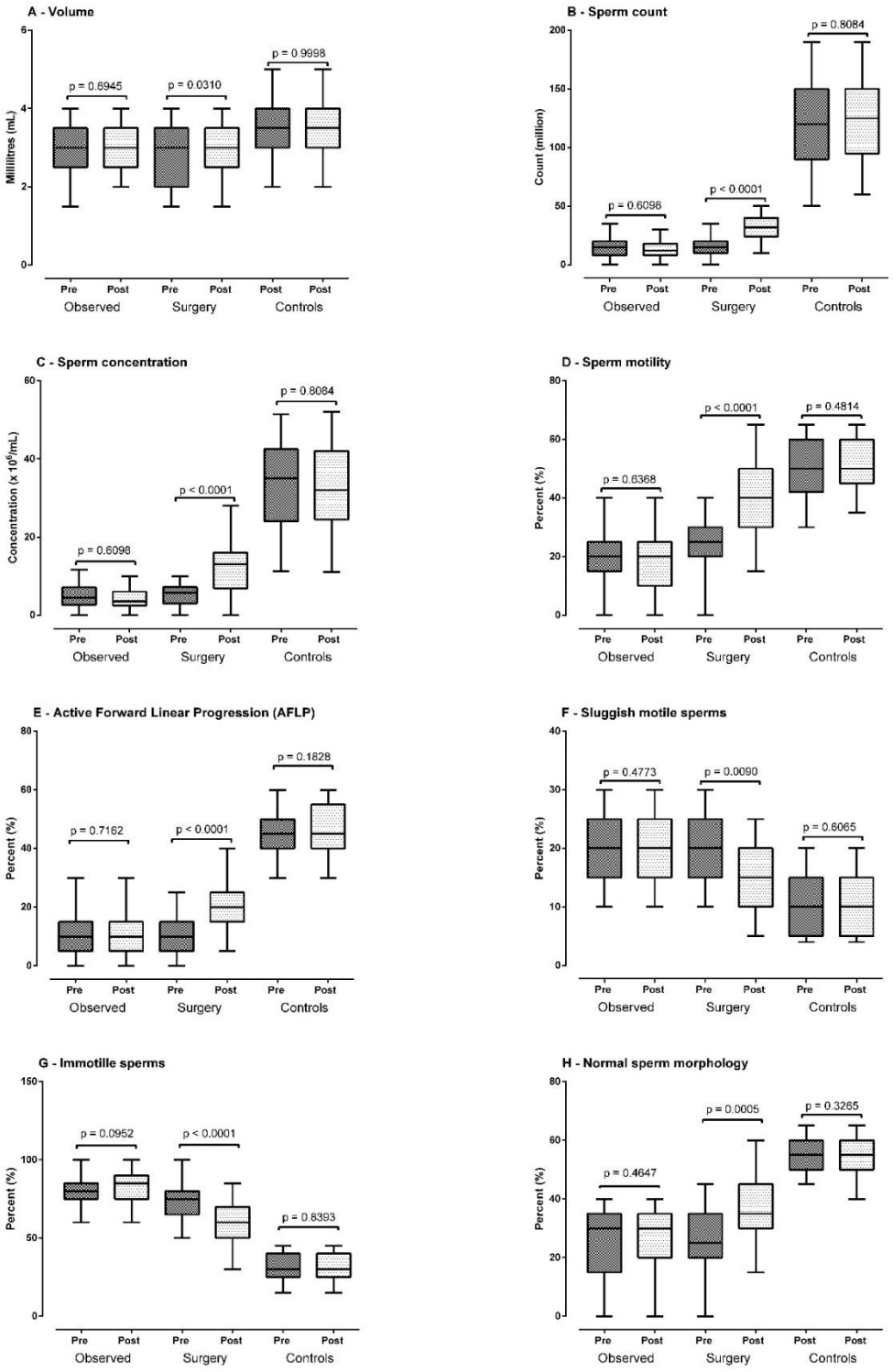


Figure 6.3: Pre- and Post-semen analysis over 12 months follow-up (a) Volume, (b) sperm count, (c) sperm concentration, (d) sperm motility, (e) Active forward linear progression (f) Sluggish motile sperms, (g) Immotile sperms, and (h) Normal sperm morphology

Table 6.2: Pearson correlation coefficients between resistive index (RI) and age, gonadal hormones, and semen analysis

	Observed group (n=114)				Surgery group (n=127)				Control group (n=33)			
	L_RIcap		R_RIcap		L_RIcap		R_RIcap		L_RIcap		R_RIcap	
Variable	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value
Age (years)	0.03	0.874	-0.03	0.865	0.29	0.110	0.21	0.255	-0.02	0.911	0.22	0.221
Total Testosterone	-0.35	0.074	-0.10	0.614	-0.63	<0.0001	-0.49	0.004	0.10	0.584	-0.09	0.634
FSH (IU/L)	0.41	0.033	0.36	0.068	0.57	0.001	0.52	0.002	0.03	0.874	-0.01	0.953
LH (IU/L)	-0.20	0.329	-0.02	0.867	0.61	0.0002	0.41	0.020	-0.15	0.408	0.02	0.904
pH	-0.05	0.791	0.01	0.958	0.11	0.645	-0.01	0.984	0.23	0.244	0.05	0.807
volume (ml)	0.06	0.781	-0.37	0.054	-0.01	0.981	-0.09	0.728	-0.17	0.404	0.02	0.920
Sperm Count (Millions/ml)	-0.25	0.212	0.04	0.831	-0.46	0.008	0.11	0.669	-0.24	0.227	0.33	0.097
Sperm Concentration (x 10^6/ml)	-0.24	0.225	0.22	0.281	-0.35	0.011	0.13	0.588	-0.17	0.399	0.25	0.208
Motility (%)	-0.01	0.963	-0.14	0.477	0.02	0.920	-0.38	0.105	-0.15	0.462	0.21	0.299
AFLP (%)	-0.18	0.360	-0.24	0.227	0.01	0.988	-0.25	0.300	-0.12	0.540	0.11	0.571
Sluggish sperms (%)	0.21	0.295	0.05	0.799	0.05	0.847	-0.41	0.083	-0.06	0.778	0.14	0.491
Immotile sperms (%)	0.01	0.963	0.14	0.477	-0.18	0.464	-0.16	0.503	0.15	0.462	-0.21	0.299
Normal sperm morphology (%)	0.14	0.488	-0.20	0.312	-0.08	0.752	-0.29	0.222	0.01	0.985	-0.01	0.970

The data presented as "r (p-value)" where r = Pearson correlation coefficient. P < 0.05 considered statistically significant. FSH – a follicle-stimulating hormone, LH – Luteinizing hormone, AFLP – active forward linear progression, R_RIcap – right-resistive index capsular artery, L_RIcap – left-resistive index capsular artery

6.7 DISCUSSION

The results of this study showed that the resistive index (RI) varied significantly between the three groups at the onset of the study. The RI was higher (> 0.6) among patients reporting with varicocele compared with healthy controls. This finding is consistent with several studies (French *et al.*, 2008; Al-Naffakh, 2012; Chen, 2017) who reported increased resistive index in varicocele patients compared with controls. The resistive index is related to vascular resistance and high values (> 0.6) imply disruption in microcirculation, thus hypoxia in the testes as a result of decreased blood supply to the testicles (Al-Naffakh, 2012; Halpern *et al.*, 2016). Again, Gandhi *et al.* (2016) concluded that higher levels of resistive index suggest ischemia in the testes.

At the baseline measurements, the resistive index between the observed group and the surgery group did not vary but a significant decrease in both left and right capsular arteries was observed in patients who had undergone the surgery after the 12 months follow-up. These findings agreed with Tarhan *et al.* (2011) who reported that blood flow velocities (RIcap) in the left testicular artery decreased significantly after surgery. A study conducted by Afoko *et al.* (2010) among adolescents reporting varicocele found similar findings. However, this contradicts the findings of Študent *et al.* (1998) who reported no significant changes between the mean RI in patients with spermatic vein ligation and spermatic vein preservation before and after surgery. In Tarhan *et al.* (2011) study, RI in the right testicular artery before and after the surgery did not vary but the current study found otherwise.

Spermatogenesis is affected by the demand and supply of blood to the testicles and changes in the blood flow as a result of varicocele may lead to impaired sperm production (Zolfaghar-Khani *et al.*, 2020). In this study, the pre-and post-surgery semen parameters (sperm count, sperm concentration, sperm motility, AFLP, sluggish, immotile sperms, and

normal sperm morphology) varied significantly in the surgery group compared with the observed group after the surgery. In a varicocele, there is retrograde blood flow as a result of dilatation of the pampiniform plexus resulting in increased scrotal temperature (Shiraishi *et al.*, 2012), ischemia and or hypoxia caused by venous stasis (Hendin *et al.*, 1999), and damage sperm DNA as a result of increased reactive oxygen species (ROS) production (Agarwal *et al.*, 2014). Improved semen parameters in the surgery group post operation may suggest improved perfusion in the testes which manifest as reduced resistive index in the surgery group.

The Leydig and Sertoli cell functions are worsened in long-standing varicocele, a significant risk factor for hypogonadism. Lotti *et al.* (2009) found a significant decreased in total testosterone and an increase in both follicle-stimulating hormones (FSH) and luteinizing hormone (LH) in severe varicocele patients. In this current study, the varicocele patients had significantly reduced serum total testosterone levels and elevated serum FSH and LH concentrations compared with the healthy controls at the baseline measurements. After 12 months of follow-up, these hormones improved significantly in patients who had the surgery compared with the observed group. This supports studies (Su *et al.*, 1995; Hurtado de Catalfo *et al.*, 2007; Sathya Srini and Belur Veerachari, 2011; Li *et al.*, 2012; Çayan *et al.*, 2020) that reported significant improvement in gonadal function following microsurgical sub-inguinal varicocelectomy. The possible explanation may be that varicocelectomy reversed the induced Leydig cell dysfunction caused by varicocele.

One characteristic of varicocele is the poor venous hemodynamic changes which causes retrograde flow in testicular veins losing the cooling effects that maintain the low intra-scrotal temperature in the spermatic cord (Rehman *et al.*, 2019). This may affect spermatogenesis and can lead to decrease testosterone synthesis although the exact

mechanism remains unclear. In this study, arterial perfusion of testicular tissue was significantly reduced evidenced by increased resistive index in the observed group. Varicocele repair appears to reverse this trend as observed by reduced RI which correlate with total testosterone ($r = -0.46$; $p = 0.008$), FSH ($r = 0.57$; $p = 0.001$), and LH ($r = 0.61$; $p = 0.0002$) in the surgery group. These findings are consistent with other studies (Schurich *et al.*, 2009; Sathya Srini and Belur Veerachari, 2011) who hypothesized that adequate blood supply to the testicular tissues improves gonadal function.

The debate on the correlation between testicular hemodynamic parameters and improved semen quality characteristics post-varicocelectomy is still ongoing. A study by Ur Rehman *et al.* (2018) found that internal spermatic vein (ISV) correlates with semen quality. In the study, ISV correlated with progressive motility ($r = -0.759$; $p = 0.029$) and nonprogressive motility ($r = -0.738$; $p = 0.037$) (Ur Rehman *et al.*, 2018). In another study, Rehman *et al.* (2019) concluded that progressive motility of sperms has a correlation with the intraparenchymal blood flow of testes and could be associated with the resistive index of testicular blood flow. Among adolescents with left-sided varicocele, Afoko *et al.* (2010) found that resistive index especially in centripetal arteries correlates strongly with semen quality parameters in the surgery group. However, Semiz *et al.* (2014) found no significant relationship between the hemodynamic pattern of blood flow and semen analysis parameters. In this study, the resistive index on the left capsular arteries correlated with sperm concentration and sperm count which agrees with previous literature (Afoko *et al.*, 2010; Akand *et al.*, 2017; Rehman *et al.*, 2019).

The post-surgery complications are discussed elsewhere (Adams *et al.*, 2022a). the hypothesis of improvement in vascular supply is limited to mean values and could be a limitation. Omission of resistive index measurements for centripetal arteries, pulsatility index

(PI), as well as the absence of genetic testing, could be some of the limitations of this study. Again, this is a dingle-center study in a tertiary hospital, hence require further investigation in future studies.

6.8 CONCLUSION

In varicocele patients, the blood supply to the testicular tissues is significantly reduced evidenced by the increase in a resistive index (RI) in capsular arteries of the observed group and this appears to improve after surgery as observed by reduced RIcap in the surgery group. Hence, testicular blood flow parameters can be used to evaluate the success of surgery because, improved testicular hemodynamic (RI in the left capsular artery) correlated with total testosterone, FSH, LH, and semen quality parameters.

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Chapter 7

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

7.1 GENERAL DISCUSSION

Varicocele is an abnormal enlargement of the pampiniform plexus draining the testicles with reflux of venous blood (Clavijo *et al.*, 2017; Bertolotto *et al.*, 2020). This treatable condition is common among men seeking medical attention for fertility problems, sexual dysfunction, or complaints of continuing scrotal discomfort (Paick and Choi, 2019). Varicocele has been identified in 15% of the general population, 21% to 45% in men with primary infertility, and 75% to 81% of men with secondary infertility (Gorelick and Goldstein, 1993; Jarow *et al.*, 1996; Tarhan *et al.*, 2011).

Varicocele affects fertility but the pathophysiology remains debatable. A few studies have reported no effect (Rageth *et al.*, 1992; Breznik *et al.*, 1993; Krause *et al.*, 2002) but recent studies have shown that varicocele causes decrease testicular function, leading to altered spermatogenesis and diminished testosterone levels (Agarwal *et al.*, 2016; Kang *et al.*, 2021). Some studies have linked the low or poor quality of spermatogenesis to; anatomical anomaly of varicocele (Xue *et al.*, 2012; Kadioglu *et al.*, 2014), increased scrotal temperature (Shiraishi *et al.*, 2012), an adrenal hormone, and gonadotoxic metabolite refluxes (Inci and Gunay, 2013), epigenetics changes (Seidel, 2015), and increased production of reactive oxygen species (ROS) in the scrotum which results in sperm DNA damage (Agarwal *et al.*, 2014). These related factors may act independently or synergistically affecting spermatogenesis and Leydig cell function.

Varicocele repair has proven to reverse the testicular damage but several repair mechanisms have been reported. The microsurgical repair approach has become popular among other varicocele treatment methods because it is associated with improved Leydig cell function

(Çayan *et al.*, 2020; Kang *et al.*, 2021; Adams *et al.*, 2022) and reduced complications and/or recurrence of varicocele (Cayan *et al.*, 1999). The two approaches are; microsurgical sub-inguinal and inguinal varicocelectomy but vascular anatomy gets more complicated as we get through the sub-inguinal region to the inguinal region, thus leaving the microsurgical sub-inguinal (lymphatic- and artery sparing) varicocelectomy the most appropriate choice (Tarhan *et al.*, 2011). Based on current evidence, the guidelines and the protocol by the American Urological Association (AUA), the American Society for Reproductive Medicine (ASRM), and the European Association of Urology (EAU) recommend varicocele repair for patients with palpable varicocele with one or more semen parameter abnormalities' whether or not they are attempting to conceive a child (Shridharani *et al.*, 2012; Kang *et al.*, 2021).

7.2 CONCLUSION

In paper I, it was found that long-standing varicocele may affect semen parameters and this may be seen by causing a further decrease in semen volume, total sperm count, concentration of spermatozoa, motility, or normal sperm morphology (Adams *et al.*, 2022). Furthermore, varicocele may cause Leydig cell damage and this may be seen by causing a further decrease in total testosterone and a concomitant rise in follicle-stimulating hormone (FSH) and luteinizing hormone (LH). This study found that serum total testosterone, FSH, and LH observed spike changes within the first- and second-year in the surgery group but changes were marginal from the third year onwards (Paper II). Paper III found that the significant predictive factors associated with improved semen characteristics following microsurgical sub-inguinal varicocelectomy in infertile men were; pre-operative low serum FSH, high testosterone, and low left capsular resistive index (L_RIcap). In varicocele patients, blood supply to the testicular tissues is significantly reduced evidenced by the increase in a resistive index (RI) in capsular arteries of the observed group and this appears to improve after surgery as observed by reduced RIcap in the surgery group (Paper IV).

In conclusion, microsurgical sub-inguinal varicocelectomy improves gonadotropins (increase serum total testosterone and decrease both serum FSH and LH levels), semen parameters and pregnancy rate, hence, effective treatment of infertile men with a clinically palpable varicocele. Again, testicular blood flow parameters (especially resistive index in the left capsular artery) can be used to evaluate the success of surgery because it correlates with total testosterone, FSH, LH, and semen quality parameters.

7.3 LIMITATIONS, RECOMMENDATIONS AND FUTURE PERSPECTIVES

The ultimate aim of this surgical (varicocele repair) procedure is to improve couples' chances of achieving a pregnancy and live birth. To determine whether or not infertility-related treatment following varicocele repair is successful, the endpoints commonly analyzed are; semen parameters (that is; semen volume, sperm count, sperm concentration, motility, and/ or morphology), pregnancy rate (PR), and/ or integrity of sperm DNA. But most studies consider semen parameters to be the primary outcome parameter of varicocele therapy. Given the predictive factors associated with improved semen parameters in patients with varicocele, it is worth recognizing that improvement on the semen quality post varicocele repair does not guarantee patients to father children. Further studies on a larger population of varicocele patients with pregnancy rate as the primary outcome will help to conclusively determine the effectiveness of microsurgical sub-inguinal varicocelectomy.

One of the shortcomings of this study (Paper II) was the drop-out during follow-up, especially among the observed group. This was so because participants were followed for 48 months (4 years). It is recommended to use microsurgical sub-inguinal varicocelectomy for similar patients, however, further studies with a larger sample size are needed to provide more evidence to recommend this therapy.

Also, varicocelectomy plus supraphysiologic dosages of human chorionic gonadotropin (hCG) therapy have found significant superiority in infertile men with varicocele and could be a topic of further studies.

Furthermore, measurements of the color Doppler indices (such as a resistive index for centripetal arteries and pulsatility index) as well as the absence of genetic testing were some of the limitations in this study. These require further investigation in future studies.

Molecular testing should done to look out of possible mechanisms associated with varicocele in the Ghanaian population.

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APPENDICES

APPENDIX I: DATA COLLECTION QUESTIONNAIRE

To be completed by each subject participating in the study

Tel.: ……..... Code: ……..

Please tick [√] the appropriate box where applicable

Pre-assessment check for exclusion in the study

i. *Are you a known hypertensive patient? [ ] Yes [ ] No*

ii. *Are you a known diabetic patient? [ ] Yes [ ] No*

iii. *Have you ever been diagnosed of tuberculosis? [ ] Yes[ ] No*

iv. *Do you have past history of any of this; mumps orchitis, undescended testis, or orchidectomy? [ ] Yes[ ] No*

v. *Are you on/ or ever been administered with anti-estrogen and/or testosterone replacement therapy?*

[ ] Yes [ ] No

Sociodemographic characteristics of study participants

1. Age: ……
2. Sex:

   [ ] male [ ] female
3. Marital status

   [ ] single [ ] married [ ] divorced [ ] widowed
4. Highest Education level

   [ ] none [ ] primary [ ] secondary [ ] tertiary
5. Occupation**:**

   [ ] unemployed [ ] trader/self-employed [ ] government worker

   [ ] private worker [ ] others (please specify)……

6. Ethnicity:

   [ ] Dagomba [ ] Dagarba [ ] Gonja [ ] Frafra [ ] Mumprusi [ ] Ewe [ ] Akan [ ] Ga [ ] others (please specify)……

Lifestyle

7. Do you consume alcoholic beverages? [ ] yes [ ] no

   If yes to question 6, how many alcoholic beverages do you consume on an average per day? [ ] 1bottle [ ] 2 -3 bottles [ ] >3 bottles

8. Do you smoke cigarette? [ ] yes [ ] no

   If yes, [ ] 1 pack/day [ ] 2 pack/day [ ] >2 pack/day

9. Number of sexual partners? [ ] one [ ] two [ ] three [ ] four [ ] more than four

Brief Medical history

10. How long have you and partner been trying to conceive with unprotected sexual intercourse?

    Months: …….. Years: ……

11. Have you ever had a pregnancy with your current partner? [ ] Yes [ ] No

    If yes to question 10, how many pregnancies? …… How many did your partner successfully give birth to? …...

12. Have you ever had a pregnancy with another partner? [ ] Yes [ ] No

    If yes to question 11, how many pregnancies? …… How many did your partner successfully give birth to? …...

13. If you have children, how many are boy …… and how many are girls? ……..

14. Has your current partner ever been pregnant with another partner? [ ] Yes [ ] No

    If yes to question 13, how many pregnancies? …… How many did your partner successfully give birth to? …...

15. Have you had any problems with erection? [ ] Yes [ ] No
16. How often do you have sex with your partner? per/day …….. per/week ……..per/month …….
17. Have you ever been treated for a sexually transmitted infection? [ ] Yes [ ] No

    If yes, what infection? …… when?.....
18. Did you ever had a surgery where your testes' was brought into the scrotum when you were a child? [ ] Yes [ ] No

    If so, did it affect your testes? …….. which sides(s)? …….
19. Did you ever had a surgery of your testes? [ ] Yes [ ] No

    If so, did it affect your testes? …….. which sides(s)? …….

Anthropometric measurement

20. Height (cm): i.……ii……average (i & ii)…….
21. Weight (kg): i.……ii……average (i & ii)…….
22. BMI (kg/m^2): i.……ii……average (i & ii)…….
23. body fat (%): i.……ii……average (i & ii)…….
24. Muscle mass (%): i.……ii……average (i & ii)…….
25. Visceral fat: i.……ii……average (i & ii)…….

Blood pressure measurement

26. SBP (mmHg): i.……ii……average (i & ii)…….
27. DBP (mmHg): i.……ii……average (i & ii)…….
28. Pulse (beat/minutes): i.……ii……average (i & ii)…….

Post-surgery questions (Please tick [√] the appropriate box where applicable)

29. Have you had any problems with erection post-surgery? [ ] Yes [ ] No
30. Have you started having sexual intercourse with your partner? [ ] Yes [ ] No

31. How often do you have sex with your partner? per/day …….. per/week ……..per/month ……..

32. Do you use lubricant(s) during sexual activity? [ ] Yes [ ] No

If so, what type/brand? ……..

33. Are you currently taking any medications on a regular basis? [ ] Yes [ ] No

If so, what medication? ……..

34. Has your current partner complained of not seeing her menses (monthly period)?

[ ] Yes [ ] No

If so, when? ……..

35. Is your partner pregnant? [ ] Yes [ ] No

If so, when did she disclose this information to you? ……..

36. Any other complication(s) after your surgery? ……

APPENDIX II: GOLOMBOK-RUST INVENTORY OF SEXUAL SATISFACTION – MALE (GRISS-M) QUESTIONAIRE

Date: ……

Instructions: Each question is followed by a series of possible answers:

N – NEVER

H – HARDLY EVER

O – OCCASIONALLY

U – USUALLY

A – ALWAYS

Read each question carefully and decide which answer best describes the way things have been for you recently; then circle the corresponding letter.

PLEASE ANSWER EVERY QUESTION.

If you are not completely sure which answer is most appropriate, circle the answer which you feel is most appropriate.

Please answer this questionnaire without discussing any of the questions with your partner. In order for us to obtain valid information it is important for you to answer each question as honestly and as accurately.

1	Do you have sexual intercourse more than twice a week?	N	H	O	U	A
2	Do you find it hard to tell your partner what you like and dislike about your sexual relationship?	N	H	O	U	A
3	Do you become easily sexually aroused?	N	H	O	U	A
4	Are you able to delay ejaculation during intercourse if you think you may be "coming" too quickly	N	H	O	U	A
5	Are you dissatisfied with the amount of variety in your sex life with your partner?	N	H	O	U	A
6	Do you dislike stroking and caressing your partner's genitals?	N	H	O	U	A
7	Do you become tense and anxious when your partner wants to have sex?	N	H	O	U	A

8	Do you enjoy having sexual intercourse with your partner?	N	H	O	U	A
9	Do you ask your partner what she likes and dislikes about your sexual relationship?	N	H	O	U	A
10	Do you fail to get an erection?	N	H	O	U	A
11	Do you feel there is a lack of love and affection in your sexual relationship with your partner?	N	H	O	U	A
12	Do you enjoy having your penis stroked and caressed by your partner?	N	H	O	U	A
13	Can you avoid ejaculating too quickly during intercourse?	N	H	O	U	A
14	Do you try to avoid having sex with your partner?	N	H	O	U	A
15	Do you find your sexual relationship with your partner satisfactory?	N	H	O	U	A
16	Do you get an erection during foreplay with your partner?	N	H	O	U	A
17	Are there weeks in which you don't have sex at all?	N	H	O	U	A
18	Do you enjoy mutual masturbation with your partner?	N	H	O	U	A
19	If you want sex with your partner, do you take the initiative?	N	H	O	U	A
20	Do you dislike being cuddled and caressed by your partner?	N	H	O	U	A
21	Do you have sexual intercourse as often as you would like?	N	H	O	U	A
22	Do you refuse to have sex with your partner?	N	H	O	U	A
23	Do you lose your erection during intercourse?	N	H	O	U	A
24	Do you ejaculate without wanting to almost as soon as your penis enters your partner's vagina?	N	H	O	U	A
25	Do you enjoy cuddling and caressing your partner's body?	N	H	O	U	A
26	Do you feel uninterested in sex?	N	H	O	U	A
27	Do you ejaculate by accident just before your penis is about to enter your partner's vagina?	N	H	O	U	A
28	Do you have feelings of disgust about what you and your partner do during lovemaking?	N	H	O	U	A

APPENDIX III: CONSENT FORM

VARICOCELE AND HYPOGONADISM IN AGING MALES, TAMALE, GHANA

Lead Investigator: Adams Yussif

I am a postgraduate (PhD) student from the department of Biomedical Laboratory Science, School of Allied Health, University for Development Studies (UDS) working on the above mention topic.

A brief background: Varicocele affect fertility and gonadal function. Studies have shown that, patients with varicocele are more likely to have fertility problems as well as sexual dysfunction. Microsurgical sub-inguinal varicocelectomy is a surgical procedure done to correct this abnormally. The study therefore is done to determine the effect of varicocelectomy on fertility, gonadotropins and testicular hemodynamics among patients presenting varicocele.

Data collection

You will be required to fill/answer a questionnaire which will take a maximum of 10 minutes after wards, semen specimen, and blood sample (5 millilitres) will be collected by a qualify phlebotomist. Serum total testosterone, follicle-stimulating hormone (FSH) and Luteinizing hormone (LH) will be done on the blood sample that will be collected. Semen analyses will be on the semen specimen collected.

Possible benefits

You will not be paid for participation in this study and you are also not expected to pay anything. However, if during the study, we detect any condition that needs prompt attention, you will be referred for investigation and management.

Withdrawal from study

We would like to emphasize that this is strictly **voluntary**. Should you decide not to participate in the study, it will have no consequences for you.

Confidentiality

All information gathered would be treated in strict confidentiality. We will protect any information about you taken for the research. You will not be named in any report. ***If you have any questions, please feel free to ask.***

Participant Agreement

The above document describing the benefits, risk and procedures for the research title: "**varicocele and hypogonadism in aging males, Tamale, Ghana**" has been read and explained to me. I have been given the opportunity to asked any questions about the research and have been answered to my satisfaction. I agree to participate as a volunteer.

---------- ----------

Signature or Thumbprint Date

If the volunteer cannot read the form themselves, a witness must sign here:

I was present while the benefits, risk and procedures were read to the volunteer. All questions were answered and the volunteer has agreed to take part in the research.

---------- ----------

Signature or Thumbprint Date

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

---------- ----------

Signature or Thumbprint Date

APPENDIX IV: ETHICS APPROVAL LETTER

UNIVERSITY FOR DEVELOPMENT STUDIES

Tel: 03720-93382/26634/22078
Email: registrar@uds.edu.gh
Website: www.uds.edu.gh

P. O. Box TL 1350
Tamale, Ghana

Our Ref: UDS/RB/004/22

Your Ref:.....

OFFICE OF THE REGISTRAR

25TH JANUARY, 2022.

Date:.....

ADAM YUSSIF,
DEPT. OF BIOMEDICAL LABORATORY SCIENCE
SCHOOL OF ALLIED HEALTH SCIENCES

ETHICAL APPROVAL NOTIFICATION

With reference to your request for ethical clearance on the research proposal titled '**Varicocele and Hypogonadism**' I write to inform you that the University for Development Studies Institutional Review Board (UDSIRB) found your proposal including the consent forms to be satisfactory and have duly approved same. The mandatory period for the approval is six (6) months, starting from 28th January, 2022 to 28th June, 2022.

Subject to this approval, you are please required to observe the following conditions:

1. That the anonymity of the respondents shall be guaranteed as mentioned in the consent forms.
2. That you will acknowledge the source of the data collected in any publication related to this research.
3. That you will submit a field report and a copy of the research report to the UDSIRB.
4. That you may apply to the UDSIRB for any amendments relating to recruiting methods, informed consent procedures, study design and research personnel.
5. That you will strictly abide by the code of conduct of this University.

Please do not hesitate to refer any issue (s) that you may deem necessary for the attention of the Board.

Thank you.

Prof. Herbert Kwabla Dei
Chairman, UDSIRB
Cc: file

LETTER OF AUTHORIZATION

Department of Research & Development
Tamale Teaching Hospital

TTH/R&D/SR/119
18/11/2020

TO WHOM IT MAY CONCERN

CERTIFICATE OF AUTHORIZATION TO CONDUCT RESEARCH IN TAMALE TEACHING HOSPITAL

I hereby introduce to you **Mr. Adams Yussif**, a PhD. candidate in Chemical Pathology of the Department of Biomedical Laboratory Science, School of Allied Health Sciences, University for Development Studies. The candidate has been duly authorized to conduct a study titled ***"Varicocele and hypogonadism in ageing men, Tamale - Ghana."***

Please accord the candidate the necessary assistance to enable him completes the study. If in doubt, kindly contact the Research Unit on the second floor of the administration block or on Telephone 0209281020. In addition, kindly report any misconduct of the Researcher to the Research Unit for necessary action.

The candidate is required to furnish the hospital a copy of the dissertation/Study upon completion.

Please not that this approval is given for a period of six months, beginning from 18th of November, 2020 to 17th of April, 2021.

Thank You.

ALHASSAN MOHAMMED SHAMUDEEN.
(HEAD, RESEARCH & DEVELOPMENT)

PUBLICATION CERTIFICATE

Certificate No: PUB.2022/AJRRU/82733

Asian Journal of Research and Reports in Urology

Certificate of Publication

Manuscript Title: Factors Associated with Improved Semen Characteristics Following Microsurgical Sub-inguinal Varicocelectomy among Infertile Men in Tamale, Ghana

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Authored by:

Yussif Adams, Akisibadek Alekz Afoko, Nafiu Amidu, Lawrence Quaye, Peter Paul M. Dapare, Simon Bannison Bani, and Vivian Afoko

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Effect of Varicocelectomy on Semen Parameters of Men Seeking Infertility Treatment in Tamale, Ghana

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Abstract

The study aimed to determine the effect of microsurgical sub-inguinal varicocelectomy on semen parameters among men seeking infertility treatment in Ghana. This was an intervention study conducted at Tamale Teaching Hospital in the Tamale Metropolis from September 2017 to August 2021. The study involves two groups; the surgery group (n = 75) and the observed group (n = 63). Duplicate semen samples (mean values adopted) were collected at the onset and assessed according to the criteria established by World Health Organization (WHO), 2010. Varicocelectomy was performed for the surgery group and no intervention was given to the observed group. The two groups were followed for 180 days and repeated semen samples were collected and analyzed. The data was computed using GraphPad Prism (v8.0) at an alpha of 0.05. All the men had varicocele and were aged between 46.0 and 67.0 years old. There was no difference between semen parameters among the two groups before the surgery. However, after 180 days of follow-up, all of the semen parameters significantly improved in the surgery group ($p < 0.0001$), while sperm concentration ($p = 0.0068$), progressive motility ($p = 0.0281$), and normal sperm morphology ($p = 0.0015$) decreased in the observed group. The surgery group had an overall percent increase in total sperm count (840.7%; $p = 0.0197$), sperm concentration (582.1%; $p = 0.0125$), total viable sperms (155.2%; $p < 0.0001$), and normal sperm morphology (110.9%; $p < 0.0001$) while immotile sperms (−51.71%; $p < 0.0001$) reduced. A pregnancy rate of 25.3% (19/75) was reported among the surgery group but none was reported among the observed group after 180 days. Microsurgical sub-inguinal varicocelectomy improves semen parameters and hence effective

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Factors Associated with Improved Semen Characteristics Following Microsurgical Sub-inguinal Varicocelectomy among Infertile Men in Tamale, Ghana

Yussif Adams [a*≡], Akisibadek Alekz Afoko [b,c], Nafiu Amidu [a], Lawrence Quaye [a], Peter Paul M. Dapare [a], Simon Bannison Bani [a], and Vivian Afoko [d]

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Authors' contributions

This work was carried out in collaboration among all authors. Authors YA, AAA, and NA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors YA, SBB, VA, and PPMD carried out the sample collection and immunoassays. Authors YA, LQ, SBB, and PPMD managed the analysis of the study, software and did the validation. Authors YA, AAA, PPMD, and VA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To determine factors associated with improved semen characteristics post microsurgical sub-inguinal varicocelectomy.
Study Design: An interventional study design
Place and Duration of Study: Department of Surgery (Urology Unit), Tamale Teaching Hospital (TTH), Ghana, between September 2017 to August 2021
Methodology: A total of 127 oligozoospermic patients with varicoceles requiring varicocelectomy

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Effect of Varicocelectomy on Gonadal Function among Patients Reporting with Sexual Dysfunction in Ghana

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Abstract

Background: Long-standing varicocele is often associated with testicular hypoxia and that might worsen Leydig cell function, a significant risk factor for hypogonadism. This may affect both the secretory and endocrine functions of the testis. This study aims to determine the effect of microsurgical sub-inguinal varicocelectomy on gonadal function among men reporting sexual dysfunction in Ghana. **Methods**: This was an intervention study conducted at the Tamale Teaching Hospital from September 2017 to August 2021. A total of 103 participants were randomized into two groups; the surgery group (n = 52) and the observed group (n = 51). Venous blood samples were collected at baseline, varicocelectomy was performed for the surgery group, and no intervention was given to the other. Blood samples were subsequently collected at 12-, 24-, 36-, and 48-month intervals for assay of serum total testosterone, FSH, and LH. The data were analyzed in GraphPad Prism (v8.0) at an alpha value of 0.05. **Results:** All the participants had varicocele and were aged between 55.0 to 69.0 years old. At the baseline of the study, all participants presented with sexual dysfunction but a significant improvement ($p < 0.001$) in the GRISS score, and the subscale was observed 12 months after the surgery. The mean ± SD serum total testosterone ($p = 0.6078$), FSH ($p = 0.6522$) and LH ($p = 0.2281$) between the groups at baseline did not vary but those in surgery group had improved values at 12-, 24-, 36- and 48-month post-surgery (p-trend < 0.0001). The surgery group had an overall percent increase in serum total testosterone (76.3%, 194.0%, 221.0%, and 231.9%) over 12-, 24-, 36- and 48-month and significant percent reduction in both FSH (−14.7%, −29.9%,

Review

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Changes in testicular arterial hemodynamic, gonadotropin levels, and semen parameters among varicocele patients randomized to varicocelectomy or observed in Tamale, Ghana

Yussif Adams[1], Nafiu Amidu[1] and Akisibadek Alekz Afoko[2,3]

Abstract

Objective: A randomized trial to compared testicular blood flow parameters, gonadal hormones, and semen characteristics among three groups; surgery group ($n=127$); observed group ($n=114$); and healthy controls ($n=33$).

Methods: The blood flow parameter selected was resistive index (RI) measured using color Doppler ultrasonography. Serum total testosterone, FSH, LH were measured, and semen analysis performed at baseline and repeated 12 months of follow-up. The data was computed using GraphPad Prism (v8.0) at an alpha of 0.05.

Results: In the observed group, increased +0.0060 in the right (R_RI) and in the left (L_RI) +0.0026 capsular arteries from baseline measurement to 12 months follow-up. Surgery group, reduced –0.079 in the right (R_RI) and −0.0731 in the left (L_RI) capsular arteries ($p<0.0001$). At 12 months, the changes for both left and right RIcap in the surgery group did not reach the values of the controls. In the surgery group, L_RIcap ($r=-0.63$; $p<0.0001$) and R_RIcap ($r=-0.49$; $p=0.004$) correlated with total testosterone, FSH ($r=0.57$; $p=0.001$ for left; $r=0.52$; $p=0.002$ for right), and LH ($r=0.61$; $p=0.0002$ for left; $r=0.41$; $p=0.020$ for right). Furthermore, L_RIcap correlated with changes in sperm count ($r=-0.46$; $p=0.008$) and sperm concentration ($r=-0.35$; $p=0.011$) in the surgery group.

Conclusion: Microsurgical sub-inguinal varicocelectomy improves blood supply to the testicular tissues evidenced by reduced resistive index in the surgery group. Resistive index in the left capsular artery can be used to evaluate the success of surgery because it correlates with total testosterone, FSH, LH, and semen quality.

Keywords

Varicocele, fertility, color Doppler ultrasound, resistive index, microsurgical sub-inguinal varicocelectomy, gonadotropins, semen analysis

Date received: 29 March 2022; accepted: 30 August 2022

Introduction

Varicocele is a disorder of venous returns caused by abnormal enlargement of the internal spermatic veins draining the testicles.[1,2] This treatable medical condition is found in 15% of the general population, 21%–45% in men with primary infertility, and 75%–81% of men with secondary infertility.[3–5]

Varicocele affects fertility but the pathophysiology remains debatable. Studies have shown that varicocele plays a role in decreased testicular function by altering spermatogenesis and reducing testosterone production.[2,6] The Sertoli cells act to regulate the development of the germinal cells along with the maturation and release of spermatozoa into the central lumen of the seminiferous tubule. They are joined to each other by tight junctions forming the so-called blood–testis barrier. This barrier

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