UNIVERSITY *of the*
WESTERN CAPE

A Retrospective Analysis of Semen Samples and Reproductive Hormones in Africa and the Middle East

by

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DECLARATION BY CANDIDATE

I hereby declare that this thesis submitted for the degree PhD (Medical Biosciences) in the Faculty of Natural Sciences, University of the Western Cape, is my own original work and has not previously been submitted to any other Institution of Higher Education. I further declare that all sources cited or quoted are indicated and acknowledged by means of a completed list of references.

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DEDICATION

Every challenging work needs self-effort as well as guidance of elders, especially those who are remarkably close to our heart.

I dedicate my humble effort to my sweet and loving God, and my mother (Constance Moungala) whose affection, love, encouragement, and prayers make me able to complete this journey.

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my supervisor Dr Kristian Leisegang for his incredible availability to discuss, deliberate and critique.

My co-supervisor Professor Ralf Henkel, for his invaluable advice, continuous support, and patience. Thank you for all statistical sessions.

Mr P. Loubser, General Manager at Androcryos Andrology Laboratory, for his financial support for the coverage of school fees, dedication during the collection and analysis of data and the opportunity to investigate this topic.

Dr C. van Rooyen, Head of the Immunology Laboratory at Ampath Laboratory, to assist with ethical approval from Ampath Publications Committee which gave me the permission to access and use Ampath Andrology Laboratory data.

Mrs A. Koch, Andrology Laboratory Manager at Lancet Laboratory, to assist with ethical approval from Lancet Publications Committee.

Professor M. Arafa, Urology Department, Hamad Medical Corporation, to give me the permission to access and use data from Hamad Medical Center.

Dr T. G. Cooper, Centre of Reproductive Medicine and Andrology of the University, Domagkrstrasse, Germany, who gave me access to the WHO (2010) data.

Staff at Androcryos Andrology Laboratory in Johannesburg for technical assistance, data capturing and emotional support. I would like to extend my sincere thanks to Ms Marylyn for assisting in designing a data capturing system and Mr Rajiv for data capture.

Lastly, but not least, I would like to thank my family. My mother (Constance Moungala), my father (William Moungala), and my brothers Kevin, Wilfried and Regis Immongault without their tremendous understanding and encouragement in the past few years, it would be impossible for me to complete my study.

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LIST OF ABBREVIATIONS

- DNA: Deoxyribonucleic Acid
- FSH: Follicle-Stimulating Hormone
- GnRH: Gonadotropin-Releasing Hormone
- IQR: Interquartile Range
- LH: Luteinizing Hormone
- MENA: Middle East and North Africa
- O_2: Oxygen
- ORP: Oxidation-Reduction Potential
- OS: Oxidative Stress
- ROS: Reactive Oxygen Species
- RS: Reductive Stress
- SCD: Sperm Chromatin Dispersion
- SCSA: Sperm Chromatin Structure Assay
- SD: Standard Deviation
- SDF: Sperm DNA Fragmentation
- sORP: static Oxidation-Reduction Potential
- TNSC: Total Normal Sperm Count
- TPMC: Total Progressively Motile Count
- USA: United States of America
- WHO: World Health Organization

ABSTRACT

Semen analysis is the cornerstone for the investigation of male infertility. Semen quality can be influenced by geographical location, age, ejaculatory abstinence, and season. In 2010, the WHO published criteria for human semen characteristics that were markedly lower than those previously reported. Many reports have discussed the methodology used by the WHO to set the 2010 reference values. Some of the limitations of the WHO (2010) study included an undefined ejaculatory abstinence period, the limited representation of different age groups, and a limitation in geographical representation as the study did not include any data from Africa and Middle East. Therefore, the current cohort study was designed to provide retrospective data on semen quality (Africa and Middle East) and reproductive hormones (Middle East) in patients who underwent semen analysis and endocrine investigation at Andrology Laboratories in South Africa and Qatar. The effects of geographical location, age, ejaculatory abstinence and seasonal changes were evaluated. Furthermore, data from Africa (from the current cohort study) was compared to data from America, Asia, Australia and Europe obtained from global data from Cooper et al. (2010) and Campbell et al. (2021).

Semen analysis reports (n = 70,765) for Africa and Middle East were obtained from Ampath Andrology Laboratory (n = 35,516), Lancet Andrology Laboratory (n = 24 967), Androcryos Andrology Laboratory (n = 7 450) and Hamad Medical Center (n = 2,832). Basic semen parameters such as semen volume, sperm concentration, total sperm count, progressive motility, total progressively motile count, sperm morphology, total normal sperm count, and functional sperm count such as DNA fragmentation, sperm viability and oxidation-reduction potential (ORP) were collected for the purpose of the study. Furthermore, reproductive hormones (estradiol, follicle stimulating hormone, luteinizing hormone, prolactin and testosterone), as well as seminal epithelial and red blood cells were investigated. All statistical analysis was done using the MedCalc® statistical software 19.5 with P-value of < 0.05 considered statistically significant.

Men residing in Africa and Middle East had median ejaculate volume, sperm concentration, total sperm count, progressive motility and normal morphology within the normal thresholds recommended by WHO (2010). A prevalence of 20.3% for oligozoospermia and 3.6% for

azoospermia were found in men residing in Africa and the Middle East. Men residing in the MENA region had sperm vitality below the recommended threshold and a median SDF higher than current recommended thresholds. Compared to Southern and Eastern Africa, the MENA region had generally worse semen parameters, most notably in the Middle East region. In Southern Africa, the highest semen parameters were found in men residing in Mozambique and Zimbabwe while the lowest were observed in patients residing in Zambia. In South Africa, the Free-State and Mpumalanga provinces had the lowest median sperm concentration. Age was found to negatively influence semen parameters in Africa and the Middle East. In the MENA region, an age-related decline in testosterone and prolactin, and increase in FSH was found, with no significant changes for LH and estradiol with age. The duration of abstinence had a statistically significant positive influence on semen volume, sperm concentration and progressive motility, while SDF worsened with the increased duration of abstinence. Furthermore, the results show a temporal decline in semen parameters between 2005 and 2019 among men from sub-Sahara Africa. A seasonal change in semen parameters of men residing in sub-Sahara Africa below the equator was found, with sperm concentration and total sperm count higher in winter compared to summer and autumn. The lowest sperm concentration was found in summer. Lastly, the results indicated a reduced semen quality in men residing in Africa compared to those living in America, Asia, Australia and Europe when comparing to global data from Cooper et al. (2010) and Campbell et al. (2021). The differences observed in semen quality and hormones in this study may indicate different environmental exposures and lifestyle changes in the investigated regions which requires further investigation.

KEYWORDS

- Africa
- Male Reproduction
- Middle East
- Reproductive Hormones
- Semen Analysis
- South Africa
- Sub-Saharan Africa
- World Health Organization (WHO)

Chapter 1: Introduction

1.1. Male Reproductive System

The male reproductive system consists of both internal organs and external genitalia, which include the testes, the epididymis, vas deferens, and the male accessory glands (prostate and seminal vesicles) (Figure 1.1) (Bets et al., 2013; Tsili et al., 2019). These organs are primarily involved in spermatogenesis, secretion of hormones such as testosterone and inhibin, and the production of semen during ejaculation to deliver spermatozoa into the female reproductive tract for fertilisation (Gadea et al., 2013).

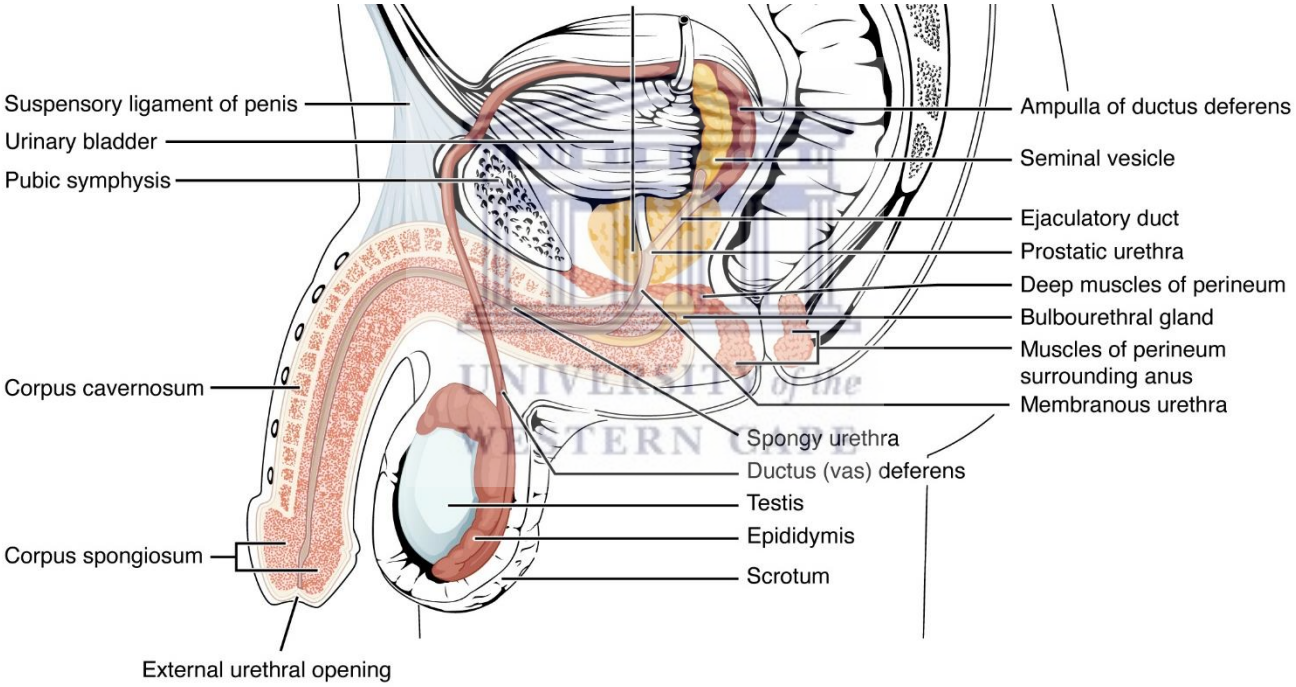

Figure 1.1: Illustration of the male reproductive system (Betts et al. (2013) Anatomy and Physiology)

1.1.1. Anatomy of the Male Reproductive System

The testes are two smooth, soft, oval-shaped glands located in the scrotum outside the body cavity, divided into lobules by the scrotal septum, and containing Sertoli cells, Leydig cells and germ cells. The size of adult testes is estimated to be 3 – 5 cm in length and 2 – 3 cm in width (Tsili et al., 2019). The superior section of the testis is held by the spermatic cord, while the inferior end is connected to the scrotum by the scrotal ligament (Tsili et al., 2017). The testis consists of the tunica albugina, the tunica vaginalis, the lobules of testes, the rete testis, and the septa of the testis (Hummitzsch et al., 2019). The tunica albugina is a thick fibrous tissue, consisting of structural collagens and extracellular proteins, without any blood vessels (Hummitzsch et al., 2019). The tunica vaginalis testis is a layer of mesothelium that covers the tunica albugina (Jones and Brachet, 1987). The lobules of testes are divided by vascularized connective tissue (Uribe and Mejía-Roa, 2015) and contain seminiferous tubules (Silber, 2018).

The epididymis is an accessory gland with a small curved shaped located on the efferent duct of the testis. With a size being appromately 20 feet (6 meters) in length when uncoiled and 6-7 cm when coiled (Butler et al., 2012), the epididymis consists of three anatomical sections: the head (caput), body (corpora), and tail (cauda) (Tiwana and Leslie, 2020). Each section is independent and organized into lobules which are divided by connective tissues (Robaire et al., 2006). These sections of the epididymis consist of Principal cells (main cell type located in the epididymal epithelium), Apical cells and Clear cells (James et al., 2020)

The prostate is a small muscular gland located anterior to the rectum and below the bladder, is the principal reproductive accessory gland in male originating from the urogenital sinus (Freire et al., 2020). This gland consists of two principal components which are interconnected, namely the stroma and the glandular epithelium (Verze and Lorenzetti, 2016). The stroma consists of vascularized fibrous and muscular tissues, while the glandular epithelium consisting of acini and ducts surrounded by the luminal cells, basal cells, and neuroendocrine cells (Ittmann, 2018). A further anatomical evaluation of the prostate gland distinguishes three different zones: the central, the transition and the peripheral zone (Verze and Lorenzetti. 2016). The central zone lies around the ejaculatory

and the bladder, while the transition zone is closely located to the urethra. The peripheral zone is the posterior section of the prostate gland (Verze and Lorenzetti. 2016).

The seminal vesicles are bilateral male accessory glands located in the pelvis at the dorsal position of the bladder and posterior side of the prostate. The ducts on the seminal vesicles connect to the vas deferens to form the ejaculatory duct (Mckay and Sharma. 2018). With an estimated size between 5 and 7 cm in length and between 3 and 5 cm in diameter (Mckay and Sharma. 2018), the seminal vesicles consist of 3 layers: the inner mucosal layer, the muscular layer, and the outer layer. The inner layer is made of epithelial cells and a lamina propria while the muscular layer consists of smooth muscle tissues. The outer layer consists of loose areolar tissues (McKay et al., 2020).

Semen is a white and opalescent fluid that is emitted from the male reproductive tract (Lawrentschuk and Perera. 2016) consisting of spermatozoa, seminal vesicle fluid and prostatic fluid. The seminal vesicles contribute approximately 50 – 65% to the volume of semen and provide high concentrations of fructose, phosphorylcholine ergothioneine, ascorbic acid, flavins prostaglandins and bicarbonate. Prostatic fluid contributes approximately 20 – 30% to the semen and provides mainly zinc and citric acid (Gómez et al., 2007). Approximately 5% of the semen consist immature germ cells and spermatozoa produced in the testis (Lawrentschuk and Perera, 2016).

A spermatozoon consists of an oval head, a midpiece and a tail region (Mortimer, 2018) with the head housing the acrosome and nucleus. The sperm tail consists of protein fibers (Lehti and Sironen, 2017). Mitochondria are found in the midpiece (Olson et al., 1990; Amaral et al., 2013, Durairajanayagam et al., 2020).

1.1.2. Physiology of the Male Reproductive System

1.1.2.1 Reproductive Hormones

Gonadotropins are polypeptide hormones synthesized by the anterior pituitary gland. These hormones stimulate the release of hormones from the gonads and the production of spermatozoa. Gonadotropins consist of Luteinizing hormone (LH) and follicle stimulating hormone (FSH) (Marques et al., 2018). The production of both hormones is stimulated by

gonadotropin-releasing hormone (GnRH) from the hypothalamus (Ma et al., 2015; Nedresky et al., 2021). LH is a glycoprotein stimulating the development of primordial germ cells and the release of testosterone by the Leydig cells in the testes (Nedresky et al., 2021). FSH is also a glycoprotein containing α- and a β-subunit (Cahoreau et al., 2015) and stimulates the Sertoli cells to produce androgen binding protein (Creasy and Chapin, 2013). FSH secretion is decreased through negative feedback by inhibin that is also produced in Sertoli cells (Luisi et al., 2005).

Testosterone is the major androgen in males, produced by the Leydig cells (Oh, 2014; Svechnikov et al., 2010) in a process of steroidogenesis that is initiated by LH (Saez, 2014). This steroid hormone promotes muscle protein synthesis (Church et al., 2019) and contributes significantly to the production of male sex characteristics (differentiation of penis, scotum and prostate), libido, spermatogenesis, and fertility (Nassar and Leslie, 2021). The synthesis of testosterone from cholesterol is done through a process called steroidogenesis (Flück and Pandey, 2017). Steroidogenesis involves the mobilization of cholesterol into the mitochondrial inner membrane to form pregnenolone. Pregnenolone is then converted into numerous steroid hormones in a process mediated by enzymes (Wang et al., 2017). Testosterone is further metabolised into dihydrotestosterone by 5α-reductase (Chang, 2011; Ogino et al., 2016) or estradiol by aromatase (Ishikawa et al., 2006).

Oestrogens are a group of steroid sex hormones which contribute to male and female sexual characteristics. This group of hormones consists primarily of estrone, estradiol, and estriol (Akarasereenont et al., 2006). Estradiol is the predominant form of oestrogen in males, produced by Leydig cells, Sertoli cells and germ cells in the seminiferous tubules (Carreau et al., 2012). It plays an important role in libido, erectile function and in spermatogenesis (Schulster et al., 2016). Oestrogens, along with androgens, have crucial function in the development and homeostasis of the prostate gland (McPherson et al., 2008). These hormones target oestrogen receptor located in various tissues and organs, with ER-α being mostly expressed in pituitary, hypothalamus, and liver, while ER-β being mostly expressed in lung and prostate (Couse et al., 1997).

Prolactin is as a polypeptide hormone produced by the anterior pituitary gland. This hormone suppresses GnRH secretion from the hypothalamus, consequently, decreases the

secretion of testosterone through inhibition of LH and FSH (Henderson et al., 2008). Prolactin also increases the LH receptor concentration in the Leydig cells (Gill-Sharma, 2009). Upregulation of prolactin is primarily by thyrotropin releasing hormone and downregulation of prolactin is primarily by dopamine (Henderson et al., 2008; Fitzgerald and Dinan, 2008; Kanasaki et al., 2015).

1.1.2.2 Spermatogenesis, Epididymal Sperm Maturation and Fertilisation

Spermatogenesis is the process during which immature germ cells develop to form mature spermatozoa (Oduwole et al., 2018) in the basal lamina of the seminiferous tubules (Cheng et al., 2010). The duration of spermatogenesis is considered to be 74 days in the human (Griswold, 2016). During spermatogenesis, cell-cell interactions between developing germ cells and Sertoli cells are essential for the maintenance of the process (Cheng and Mruk, 2002). The cellular events occurring during spermatogenesis can be divided into two phases: spermatocytogenesis and spermiogenesis (Johnson et al., 2000).

During spermatocytogenesis, diploid spermatogonial cells (type B) develop into primary spermatocytes via mitosis. Then, the primary spermatocytes undergo meiotic divisions in the basal compartment of the seminiferous tubules to form haploid secondary spermatocytes in the adluminal compartment. Then, secondary spermatocytes undergo meiosis II to form spermatids. The late spermatids will undergo the second phase of spermatogenesis (spermiogenesis) to form mature spermatozoa (Kuster and Althouse, 2007; Sharma and Agarwal, 2018).

Spermiogenesis consists of four main steps, with the first step being the Golgi phase. In this first phase the acrosome of the spermatozoa is formed by the Golgi body enzyme (Suphamungmee et al., 2008). The second step is the acrosomal phase during which the acrosome condenses around the nucleus (Muciaccia et al., 2013). The third step is the tail phase during which the centrioles present in the spermatids will elongate and form the tail. The last phase is the maturation phase, in which the spermatids lose their excess cytoplasm and form mature spermatozoa that are at this stage immotile (Kim, 2016). These spermatozoa are then released into the lumen of the seminiferous tubule by a process known as spermiation (O'Donnell et al., 2011). The male germ cells gain motility during epididymal maturation.

Epididymal maturation of spermatozoa consists of all physiological and cellular changes happening to spermatozoa in the epididymis that render the sperm motile and able to capacitate in the female reproductive tract (Gervasi and Visconti, 2017). Human spermatozoa spend between 12 – 14 days in the epididymis for maturation (Sullivan and Mieusset, 2016). In this process, spermatozoa obtain motility by being in contact with the epididymal secretions. The epididymis is made of different regions, each of which has different cell composition and proteins (Gilbert, 2000; Gervasi and Visconti, 2017). During the epididymal transit of sperm, several mechanisms such as change in sperm plasma membrane morphology, increase in chromatin condensation**,** decrease in sperm acrosome, increase in numbers of disulfide bridges in the nucleus, migration cytoplasmic droplet (which contribute to the improvement of sperm motility) and activation of functional flagella occur (James et al., 2020).

Ejaculation is comprised of three stages: pre-ejaculatory phase, first ejaculated fraction and second ejaculated fraction (Donatucci, 2006; Hebles et al., 2015). The pre-ejaculatory fluid is emitted from the Cowper and Littre´'s glands in order to minimize urethral acidity. This fluid does not contain sperm (Hebles et al., 2015). The first fraction of the ejaculate represents 15 - 45% of the whole ejaculate volume and contains epididymal and prostatic secretions. This fraction is rich in spermatozoa, magnesium, acid phosphatase, citric acid, and zinc. The first fraction of the ejaculate provides a protective effect on the sperm by promoting the formation of disulfide bridges, preventing premature chromatin decondensation and inhibiting endonuclease activity (Arver 1982; Mortimer 1994; Hebles et al., 2015). The second ejaculated fraction constitutes 55 to 85% of the whole volume. The second ejaculated fraction is characterized by a low sperm count and primarily contains secretions from the seminal vesicles (Arver 1982; Mortimer 1984).

Post-ejaculation refers to the events occurring after ejaculation. At coitus, semen is left in the anterior part of the vagina of the female. The vagina has an acidic environment and can also trigger an immune response; therefore, sperm immediately goes from the anterior vagina to enter the cervix (Carballada and Esponda, 1997). Only sperm with progressive motility reach the cervix. After the cervix, sperm will enter the uterine cavity. A portion of spermatozoa then swim through the utero-tubal junction which connect the endometrial

cavity of the uterus and the fallopian tube (Fukuda, 1994; Suarez and Pacey, 2006). Human spermatozoa are consequently stored and maintained in a reservoir at the Fallopian tubes. Following ovulation, capacitated and hyperactivated spermatozoa are selected by sperm chemotaxis. After capacitation, sperm will swim to the tubal ampulla. Sperm penetrate the cumulus oophorus and zona pellucida of the oocyte (Suarez and Pacey, 2006). This is followed by the actual fertilization, which is the process during which sperm enters the oocyte. During fertilization, the sperm nucleus undergoes morphological changes such as chromatin decondensation to form the male pronucleus, which will then fuse with the female pronucleus to form a diploid nucleus (Blodel et al., 1990; Georgadaki et al., 2016).

1.1.3. Redox Biology in Male Reproduction

Oxidation-reduction (redox) reactions are chemical processes in which one or more electrons are transferred from one element/compound (reductant, electron donator) to another one (oxidant, electron acceptor). Oxygen (O_2) molecules have a high oxidizing potential and are very important for aerobic life and during oxidative phosphorylation (OP) in the mitochondria. OP is the mechanism during which electrons are transferred from NADH or $FADH_2$ to O_2 and form ATP and water (Berg et al., 2002; Greabu et al., 2008). However, an imbalance in the electron transfer between molecules can change O_2 into reactive oxygen species (ROS) (Halliwell, 2011).

In semen, ROS are predominantly produced by leukocytes (Henkel et al., 2005), as well as spermatozoa and germ cells (Fisher and Aitken, 1997). ROS play an important role in physiological processes including reproduction as these highly reactive molecules are essential to trigger physiological events such as spermatogenesis, epididymal transport, sperm maturation and capacitation. If ROS are excessively produced, antioxidants can decrease the damaging effect of ROS by scavenging free radicals and neutralizing oxidants (He et al., 2017).

An imbalance between ROS production and antioxidants in favour of the oxidants is known as oxidative stress (OS) (Migdal and Serres, 2011), whereas a decreased ratio results in reductive stress (RS) (Castagné et al., 1999; Lipinski, 2002; Pérez-Torres et al., 2017). OS can be detrimental for sperm DNA and can also change important sperm components which can decrease sperm activity (Dutta et al., 2019). RS was found to decrease cell

growth, to lead to alterations in disulfide bonds formation in proteins, decrease mitochondrial function and negatively affect the metabolism of cells (Pérez-Torres et al., 2017). Research showed that ROS, at physiological level, can improve the male ability to fertilize (Pons-Rejraji et al., 2009; Wagner et al., 2017).

1.2. Male Infertility

1.2.1. Overview of Male infertility

Infertility is the inability to achieve a pregnancy within one year of adequate sexual exposure with no contraceptives (American Society of Reproductive Medicine, 2013). Primary infertility is the term used when a woman has never conceived, while secondary infertility is defined as the inability to conceive subsequent children after previous fertility (WHO, 2013). It is estimated that 48.5 million couples globally are facing infertility related problems (Mascarenhas et al., 2012; Agarwal et al., 2015).

Infertility can affect both the male and female partner. Male factor infertility is defined as the presence of one or more seminal parameter abnormalities or when there is a problem with sexual or ejaculatory function (Rowe, 2001). There are several factors that can cause male infertility, including chromosomal disorders, unfavourable lifestyle, gonadotoxin exposure, hormonal dysfunction and male hypogonadism, varicocele, testicular failure, ejaculatory disorders, environmental exposure to toxicants, infectious diseases, and reproductive tract obstruction (Benoff et al., 2000; Ochsendorf et al., 2008; Maartens et al., 2015; Durairajanayagam, 2018; Khourdaji et al., 2018; Leslie et al., 2021). Adding to these factors, male factor infertility is reported to be associated with geographical location (Agarwal et al., 2015), seasonal variation (Santi et al., 2018) and age (Harris et al., 2011).

1.2.2. Epidemiology of Male infertility

Male factor infertility is estimated to contribute up to 50% to couple infertility cases (Tucker, 2015), where male infertility affects approximately 7% of men globally (Krausz, 2011). In males with infertility, 35% of them are considered primary male factor infertility and 70 – 85% are considered secondary infertility (Poongothai, et al., 2009). Understanding all the factors having an influence on semen analysis results is very crucial for the development of

the reproductive medicine field (Björndahl, 2011). One of the challenges in determining the global incidence of male infertility is the fact that there are evident variations in the incidence of male infertility in different regions, which make generally the data heterogeneous and consequently not easy to analyse (Martinez et al., 2020).

The increasing incidence of infertility in Africa has made the continent to be considered as the epicentre of infertility in the world (Inhorn et al., 2015), where the burden of infertility is more pronounced compared to other regions (Gerais and Rushman, 1992). In Africa, the proportion of primary and secondary infertility is almost equal (49.91% vs 50.09%, respectively), where oligozoospermia and asthenozoospermia, as well as the presence of varicocele, were found to be the common male factors infertility abnormalities reported (Abebe et al., 2020). Furthermore, Eastern Africa region has the highest incidence of secondary infertility in comparison to Western African region and Sourthern African region (Abebe et al., 2020). However, the prevalence of primary infertility was found to be the highest in North Africa (70.56%) in comparison to other African regions (Abebe et al, 2020). The geographical distribution of male infertility in Sub-Saharan Africa indicates specific regions with decreased fertility rates observed in West Africa from Senegal, Mali, Burkina Faso to Niger, and in Central Africa, from Cameroon, Congo, and Gabon to Sudan (Maya et al., 2012).

There is a notable lack of epidemiological studies on male infertility and semen parameters in sub-Saharan Africa, and most research focusing on social and psychological consequences of male infertility. The World Health Organization (WHO) has highlighted the need to increase research efforts in the region (Khanna et al., 2012), considering that sub-Saharan Africa is indicated to have the highest incidence of infertility in the world (Bamba, 1999). However, the highest prevalence of secondary infertility in Africa occurs in sub-Saharan Africa (Gerais and Rushwan, 1992). Infectious disease and socio-cultural factors were found to be one of the majors contributing factors of male infertility in this region (Gerais and Rushwan, 1992; Program for Appropriate Technology in Health, 1997). Furthermore, male factor infertility in South Africa is still not well discussed in the literature compared to what is observed in Northern African countries, with only a few studies reported (Bornman et al., 1994; Chigumadzi et al., 1998; Dalvie et al., 2004; Aneck-Hahn et al., 2007). Considering there is limited data available, there is an evident need to provide

more epidemiological data in semen quality of men residing in African regions, including South Africa.

The literature shows that 20.2 % of Iranian couples are facing infertility related problems. This is more that the global estimation (12-15%). Furthermore, in Iran, 70% of infertility cases were found to be related to male factor (Ferlin et al., 2007). More broadly in the Middle East, male factor infertility was found in approximately 60-70% of all men presenting to IVF clinics in the contributed to the cause of infertility (Agarwal et al., 2015). However, descriptive data and male semen parameters and reproductive hormones in the Middle East region remain scanty.

In Europe, 7.5% of males were reported to face infertility (Jungwirth et al., 2012). The largest pockets of male infertility occurred in Central and Eastern Europe (8-12%) (Martinez et al., 2012). In England, male infertility contributed for 19–57% of all infertile couples (Thonneau et al., 1991). In France, male factor infertility was responsible in 20% of all infertility cases.

In North America, 15% of couples are infertile (Agarwal et al., 2015) with 4.5-6% of all males facing infertility (Martinez et al., 2012), and 50% of infertility cases involve a male factor (Agarwal et al., 2015). In the United States, about 9.4% of men are facing infertility problems (Chandra, 2014), and male infertility alone accounts for 20% (Anderson et al., 2009). In Canada research on male factors infertility are limited.

Although the world prevalence of primary infertility in couples is reported to be the lowest in South American countries (Peru, Bolivia, Ecuador, and El Salvador; 0.8%–1.0%) (Maya et al., 2012), a significant decrease in fertility rate is reported in South America (Badaracco et al., 2016). Male factor infertility reportedly involves 2.7 - 52% of infertility cases (Ikechebelu et al., 2003; Agarwal et al., 2015). In Brazil, a decline is sperm morphology was highlighted (Borges et al., 2015), where teratozoospermia was found to be the major semen abnormality in sub-fertile men (Da Silva et al., 2012).

Of 60-80 million couples that suffer from infertility every year, approximately 15-20 million reside in India (WHO, 1996). In India, male factor infertility is reportedly solely responsible in 31.6% of cases, where the most frequent factors associated with male infertility were

smoking (16%), alcohol (18%) and varicocele (24%) (Velu and Prasad, 2017). In China, it was reported that semen quality of Chinese men decreases yearly (Huang et al., 2017) and varicocele, orchitis and smoking being the majors causes of male infertility (Hong et al., 2013). In Korea, obstructive azoospermia, and varicocele were found to significantly contribute to male infertility (Lee et al., 2012).

There are very few reports discussing the epidemiology of male factors infertility in the Australasia region. One of the few studies is the one performed by Constello et al. which highlighted a trend in sperm concentration in Australia men (Constello et al., 2002). A more recent study by Martinez et al. (2012) indicated that 8% of men are facing infertility and 9% of men over 40 years are facing infertility related problems in Australia.

1.2.3. Seasonal Variations in Male Reproduction and Fertility

The literature suggests differences in semen quality following variation in seasons (Levine, 1999; Zhang et al., 2013). In Germany, the sperm count in spring is significantly higher than in summer, autumn, and winter. Furthermore, the study showed that the peak chromatin condensation was observed in summer (Henkel et al., 2001). In Chinese males, a significantly lower semen volume, sperm concentration and normal sperm morphology in midsummer (average highest temperature > 30°C) than other seasons of the year has been reported (Zhang et al., 2013). This is reflected in a significant increase in sperm morphology during spring in comparison to summer in a retrospective study from European patients, with better semen parameters for normozoospermic and oligozoospermic men in spring and winter (Ozelci et al., 2016). Furthermore, in Italy, a higher sperm motility was observed in summer, this highest percentage with normal semen pH was observed in spring, and significantly higher semen volume was observed in winter (De Giorgi et al., 2015). In America, sperm concentration was found to be significantly higher in winter than in fall, while normal sperm morphology was significantly higher in winter than summer and spring (Chen et al., 2003). Seasonal changes can also significantly influence neutral alpha-glucosidase activity in semen (Henkel et al., 2006). The impact of seasonal variation on semen characteristics is well-documented in Asia, Europe, and America. However, studies investigating the impact of seasonal shifts on human semen quality in Africa remain limited.

1.2.4. Decline in Semen Quality

Carlsen et al. (1992) first suggested a global decline in human semen quality over the past 50 years. This has led many retrospective analyses of records of semen reports in different groups and geographical regions. The results have been conflicting, with some studies indicating significant decline in semen parameters such as perm concentration (Sengupta et al., 2017; Levine et al., 2017; Mann et al., 2020), while others are not finding such trends (Costello et al., 2002; Cannarella et al., 2020). However, it is further suggested there is a more pronounced decline in male reproductive health in Western and Asian countries (Auger et al., 1995; Huang et al., 2017).

In Africa, there are reports of a temporal decrease in sperm concentration from 1965 to 2015 (Sengupta et al., 2017). Similar trends have been reported in Northern Africa (Tunisia) (Feki et al., 2009) and Western Africa (Nigeria) (Abarikwu, 2013). In Southern Africa, there is report of an increasing number of men developing varicoceles between July 1985 to June 1991 (Bornman, 1994). Similar trends are also seen in Asia (Huang et al., 2017; Sengupta et al., 2018; Li et al., 2019) (1965 – 2015) and in Europe (Splingart et al., 2012), including Scotland (Irvine et al., 1996), Denmark (Bonde et al., 1998), Italy (Bilotta et al., 1999), Austria (Lackner et al., 2008) and France (Auger et al., 1995).

While several studies worldwide seem to indicate a decline in semen quality over year, other studies have found conflicting results (Saidi et al., 1999; Ravanos et al., 2018). Saidi et al. (1999) reported that there are no decreasing trends in sperm count during the last 60 years from 9,612 fertile men residing in the USA. Ravanos et al. (2018) performed a systematic review, which highlighted a lack of consensus regarding the global decline in sperm counts and methological deficiencies between studies that have highlighted a global decrease in sperm count. The authors have called for additional large-scale prospective studies to give more relevant evidence for the worldwide trends in semen parameters.

1.2.5. Aetiology and Risk Factors of Male infertility

1.2.5.1. Genetic Causes of Male infertility

Genetic factors are responsible for approximately 60% of male infertility (Yamamoto et al., 2017). The principal genetic causes of male infertility are related to meiotic defects, mutations in the gene for cystic fibrosis, numerical and structural chromosomal aberrations, and genetically determined syndromes microdeletions in the region q11.21-23 of the Y-chromosome (Engel et al., 2004). The meiotic defects are characterized by the production of spermatozoa with autosomal and sex chromosome disomies, and can cause trisomies, monosomies and of triploids (Egozcue et al., 2000). Cystic fibrosis gene mutations lead to a degeneration of the Wolffian duct structures (Barak and Baker, 2016). Klinefelter Syndrome is a numerical chromosomal aberration characterized by the presence of an extra X chromosome (genotype XXY instead of XY) (Bonomi et al., 2017).

1.2.5.2. Ageing and Male Infertility

Several studies have found that advanced male age significantly influences sexual functions, sperm parameters, reproductive hormones, and consequently male fertility (Chen et al., 2003; Harris et al., 2011; Johnson et al., 2015; Li et al., 2019). In semen, advanced age may result in a decrease in sperm progressive motility (Chen et al., 2003; Sunanda et al., 2014; Shabani et al., 2017), morphology, and vitality (Sunanda et al., 2014). Semen volume, sperm concentration, total sperm count, total motile sperm count, and sperm morphology such as tail deffect were found to decrease as age increases (Chen et al., 2003). A review by Durairajanayagam (2018) shows a progressive decrease in semen volume and sperm motility with age and a decline in sperm concentration and morphology in men older than 40 years. Furthermore, increasing male age is associated with increased oxidative stress that may adversely affect male fertility potential (Koh et al., 2016; Leisegang et al., 2017).

Aging in men has also been associated with declines in reproductive hormones levels (Golan, 2015). Many studies have shown a significant constant decrease in circulating testosterone and free testosterone according to male age (Wu et al., 2000; Zirkin et al., 2012). Hypogonadal testosterone levels increase to 20% in males aged over 60 years, to

30% for those over 70 years old and to 50% in male aged over 80 years (Harman et al., 2001). Late-onset hypogonadism, which is a decrease in serum testosterone level in older men compared to young men (Dudek et al 2007), is associated with erectile dysfunction and decreased libido (Martits et al., 2014). Significant age-related increases in LH, FSH and sex hormone binding globulin was found (Morley et al., 1997). The estradiol concentration was found to decrease with advanced male age (Orwoll et al., 2006). However, advanced male age seems not to significantly impact prolactin concentration (Roelfsema et al., 2012).

Although aging has a significant impact on male fertility, limited data are available for men residing in African and Middle East Countries. Further research in these regions is needed to establish a global change in male fertility according to age.

1.2.5.3. Acquired Causes of Male infertility

A varicocele is an abnormal enlargement and elongation of the veins draining blood from each testicle (Leslie et al., 2020). Approximately 15% of the male population present with varicocele (Nagler and Martinis, 1997). Varicocele is present in 25% of men with abnormal sperm parameters and 12% of men having normal sperm parameters (Tulloch, 2002). Varicocele is highly prevalent in men with secondary infertility 45 to 81% in comparison to primary infertility 19 to 41% (Agarwal et al., 2007). The impact of varicocele on semen quality is strongly associated with a low sperm count, decrease sperm motility, and normal morphology (Agarwal et al., 2016c; Yetkin and Ozturk, 2018; Paick and Choi, 2019). Furthermore, varicocele can induce seminal oxidative stress (Tadros and Sabanegh, 2019). Azoospermia or severe oligozoospermia occurs in 4-13% of men with varicoceles Will et al., (2011).

Common underlying causes of male infertility are infections of the genital tract or accessory glands and are identified in 6–10% of infertile males (Schuppe et al., 2017). Epididymitis and orchitis are inflammation of the epididymis and testes (Trojian et al., 2009), while urethritis is an inflammation of the urethra (Young et al., 2020). Herpes virus infection was associated with leukocytospermia (Krause et al., 2002).

Sexually transmitted infections such as *Chlamydia trachomatis*, *Neisseria gonorrheae*, Human Immunodeficiency Virus (HIV), Human Papillomavirus (HPV), Ureaplasma, and

Hepatitis B and C viruses were found to decrease sperm concentration and motility (Gimenes et al., 2014; Kumurya and Sani, 2020; Henkel, 2021). Chlamydial infection can cause epididymitis, urethritis, orchitis and leukocytospermia (Ostaszewska et al., 2000; Gdoura et al., 2008; Mohseni et al., 2021) and decrease the integrity of sperm DNA (Moazenchi et al., 2018). *Neisseria gonorrheae* infections can cause urethritis, which is frequently reported. This inflammation can block sperm transport through the urinary tract and consequently damage the testicular tubes. Furthermore, *Neisseria gonorrheae* is also associated with vesiculitis (inflammation of seminal vesicles), penile oedema, prostatitis and orchitis (Elias et al., 2011).

Antisperm antibodies are directed against sperm antigens and can form when spermatozoa are exposed to the immune system by a breach of the blood-testes barrier (Turek, 2014). These antibodies impair fertility by decreasing sperm count, sperm motility and seminal liquefaction (Bonyadi et al., 2013; Bozhedomov, 2014, Cui et al., 2015; Bozhedomov, 2015; Cui et al., 2015). However, antisperm antibodies normally do not negatively affect the ejaculate volume, sperm viability, progressive motility, and normal morphology (Cui et al., 2015). Some of the predictive factors of antisperm antibodies include a history of vasectomy reversal (Patel and Smith, 2016; Sinha and Ramasamy, 2017), sperm agglutination on semen analysis (Jarow and Sanzone, 1992), testicular torsion (Jacobsen et al., 2020), varicocele (Majzoub et al., 2016), cryptorchidism (Domagala et al., 2006), and genital tract infection (Witkin and Toth, 1983).

Hypogonadism in males is a condition characterized by the presence of low serum testosterone levels (Bhasin et al., 2021). The condition is classified as primary hypogonadism and secondary hypogonadism. Primary hypogonadism is known as testicular failure and caused by a disturbance in the testes, while secondary hypogonadism is caused by a problem in the hypothalamus or the pituitary gland (Dohle et al., 2015). Testicular failure was reported to be the most severe form of male infertility, which, in most cases, leads azoospermia or severe oligozoospermia (Song et al., 2016). Secondary hypogonadism was found to be associated with a decrease in semen volume (Calderón et al., 2016) and sperm concentration (Naing, 2017).

The incidence of hypogonadism increases with male age (Morales et al., 2007; Gooren, 2009). Diabetic men were also found to have a decrease in serum testosterone with most of

them displaying hypogonadism-like symptoms (Kapoor et al., 2007). Furthermore, low testosterone concentration is associated with medical comorbidities such obesity (Pellitero et al., 2012; Calderón et al., 2016), metabolic syndrome (Caldas et al., 2009; Singh et al., 2011), cardiovascular diseases (Malkin et al., 2010) and chronic inflammatory diseases such as Rheumatoid arthritis (Rochira, 2017).

1.2.5.4. Risk Factors of Male infertility

Male reproduction is influenced by nutritional and lifestyle factors (Chavarro et al., 2010; Ruder et al., 2014; Ramaraju et al., 2018). Malnutrition, defined as condition of being poorly nourished, is categorized as overnutrition or undernutrition (Hickson, 2006; Leisegang, 2019a). Overnutrition and undernutrition are found to be associated with an increased in oxidative stress (Leisegang, 2019b). A systematic review of published literature found that balanced diets rich in some nutrients such as omega-3 fatty acids, antioxidants such as vitamin E, vitamin C, β-carotene, selenium, zinc, cryptoxanthin and lycopene, as well as low in saturated fatty acids and trans-fatty acids were inversely associated with low semen quality (Salas-Huetos et al., 2017).

The increasing consumption of dairy food, such as cheese was significantly associated with a decrease in sperm morphology, while whole milk decreases sperm concentration. Full-fat dairy consumption decreases progressive sperm motility (Afeiche et al., 2013). Males with decrease semen quality were found to have constant intake of some food items, containing xenobiotics and anabolic steroids that may adversely affect semen quality (Mendiola et al., 2009). A low intake of antioxidant nutrients is associated with poor semen quality (Mendiola et al., 2009).

Evidence suggests that lifestyle such as recreational drugs, caffeine consumption, psychological stress, radiation, diet, obesity, tobacco, and alcohol can impede male fertility ability (Leisegang and Dutta, 2020). Furthermore, physical exercise was found to affect male reproductive ability (Arce et al., 1993; Jóźków et al., 2017). Recreational drugs such as cannabis, opioids and anabolic steroids were found to decrease spermatogenesis and increase oxidative stress systemically (Leisegang and Dutta, 2020). The consumption of caffeine was found to be associated with an alteration of Sertoli cells glycolytic, interfering with oxidative states, decreasing semen quality, and sperm DNA integrity (Zini et al., 2014;

Dias et al., 2015). Excessive physical exercise was also found to decrease normal sperm count, motility and normal morphological changes that may compromise fertility (Arce et al., 1993; Jóźków et al., 2017).

Tobacco and alcohol are the most consumed substances in the world (Dechanet et al., 2011; Guzel et al., 2021). Tobacco smoke contains more than four thousand substances including nicotine, tar, carbon monoxide, polycyclic aromatic hydrocarbons, radioactive substances and heavy metals (Dai et al., 2015). Cotinine, a nicotine metabolite, is negatively correlated with total sperm motility (Sofikitis et al., 2000). At a high concentration, nicotine can significantly negatively affect viability parameters (Oyeyipo et al., 2014). The literature shows that alcohol consumption negatively impacts natural reproduction and fertility (Eggert et al., 2004; Gaur et al., 2010). Low sperm count, decrease in sperm motility and normal morphology were reported in men abusing alcohol (Anderson et al., 2010).

Environmental pollutants such as metals (e.g mercury, lead and chromium), endocrine disruptors (e.g. pesticites and chemical pollutants) and radiation (ionizing and nonionizing) are reported to significantly affect male fertility (Leisegang and Henkel, 2020). Increasing concentrations of lead and chromium are associated with oxidative stress (Matović et al., 2015). Chromium alters gene expression in Sertoli cells (Cheng et al., 2002). Another study reports a decrease in semen parameters of young South African men due to exposure to endocrine disruptors chemicals (Aneck-Hahn et al., 2007). Exposure to organopesticides can decrease sperm concentration and total sperm count (Recio-Vega et al., 2008). Radiation exposure through cell phones, laptops, Wi-Fi, and microwave ovens are also reported to induce oxidative stress (Kesari et al., 2018).

Systemic diseases such as diabetes (Glazer et al., 2017), obesity (Kahn et al., 2017; Leisegang, 2019a), autoimmune diseases (Brubaker et al., 2018) and malignancy (germ cell tumors) (Schrader et al., 2021) are associated with increased risk male infertility. The literature highlights an increase in sperm DNA fragmentation (SDF) in men with obesity, metabolic syndrome and diabetes (Mallidis et al., 2011; Hammoud et al., 2012). Approximately 90% of diabetic men are facing sexual dysfunction, especially erectile dysfunction, ejaculatory dysfunction, and decreased libido (Asafu-Adjei and Gittens, 2020). Like diabetes, obesity was also found to be associated with erectile dysfunction and ejaculatory dysfunction (Martini et al., 2012). Furthermore, obesity was associated with an

increase in oxidative stress (Leisegang, 2019a). Autoimmune disorders, medical conditions described as an aberrant chronic inflammatory response to self-antigens, can negatively affect male fertility (Finelli et al., 2021).

1.2.6. Oxidative Stress and Sperm DNA Fragmentation in Male Infertility

Reactive oxygen species (ROS) are highly reactive molecules derived from molecular oxygen and produced as a normal by-product of cellular aerobic respiration (Venkatesh et al., 2011). Oxidative stress is excessive production of reactive oxygen species and/or a reduction in neutralising antioxidants, thus an imbalance of oxidants and antioxidants in favour of the oxidants (Betteridge et al., 2000; Turner and Lysiak, 2008). Although a certain limited amount of ROS is essential for normal physiological reproductive functions, seminal oxidative stress has been identified as major underlying cause of male factor infertility (WHO, 2010; Arafa et al., 2019). Oxidative stress negatively affects sperm parameters, associated with a decrease in sperm motility, vitality and normal morphology (Morielli and O'Flaherty, 2015; Singh et al., 2015; Sabeti et al., 2016). Furthermore, oxidative stress in closely associated with sperm DNA damage (Henkel and Leisegang, 2020).

Spermatozoa with fragmented DNA are found in approximately 80% of male infertility cases, depending on the type of infertility (Henkel and Leisegang, 2020). Sperm DNA integrity is an important factor for fertilization and development of the embryo (Agarwal et al., 2020). The fragmentation of the sperm DNA can be caused by endogenous or exogenous causes. Endogenous causes of DNA damage are mostly associated with ROS, while exogenous factors are genital heat stress, radiation, toxins, or mutagenic exposure (Baskaran et al., 2019, Muratori et al., 2019; Henkel and Leisegang, 2020). DNA can be damaged in different forms including loss of base, mismatch of bases, base modifications, pyrimidine dimers and single strand breaks and double strand breaks DNA adducts and crosslink (Agarwal et al., 2020).

1.2.7. Clinical Assessment of Male Infertility

Clinical assessment of male infertility is critical in providing appropriate diagnostic and management options, particularly when factors can be correctable. Typically, the standard semen analysis is the first clinical assessment of male infertility (Teppa-Garrán and

Palacios-Torres, 2004). The WHO periodically provides guidelines for the evaluation of semen samples. The 5th Edition of the WHO Laboratory Manual for the Examination and Processing of Human Semen is currently used as guideline by most andrology laboratories. The reference values for semen volume, sperm concentration, progressive motility, and normal morphology recommended by this 5th Edition are summarized in Table 1.1 (WHO, 2010).

1.2.7.1. Standard Semen Analysis

The basic semen analysis protocols are routinely followed in most andrology laboratories for initial assessment of male fertility causes. During these procedures, semen is firstly macroscopically evaluated then microscopically examined. The macroscopic assessment includes the appearance, viscosity, volume, liquefaction, and the semen pH. The microscopic evaluation includes the concentration of concentration, the sperm motility and morphology. Furthermore, other seminal contents such as leucocytes and immature germ cells are also microscopically evaluated (WHO, 2010).

Sperm concentration is defined as the number of spermatozoa per unit of semen volume and is positively correlated with pregnancy (WHO, 1996; Zinaman et al., 2000; Slama et al., 2002). The WHO (2010) recommends the use of counting chamber and the improved Neubauer haemocytometer as the methods for the determination of sperm concentration. Normal sperm concentration is any value equal or more than 15 x 10^6/ml and less than or equal to 250 x 10^6/ml (WHO, 2010) (Table 1.1). A sperm concentration of 0 x 10^6/ml is referred as azoospermia. Any value more than 0 x 10^6/ml and less than 15 x 10^6/ml is referred as oligozoospermia, while a sperm concentration of more than 250 x 10^6/ml is referred as polyzoospermia (Glezerman et al., 1982). The total sperm number refers to the total number of spermatozoa in the entire ejaculate and is obtained by multiplying the sperm concentration by the semen volume (WHO, 2010).

The WHO (2010) describes different grading for sperm motility evaluation. The grading allows making distinctions between percentages of spermatozoa with progressive or non-progressive motility from those that are immotile. The motility grading consists of progressive motility, non-progressive motility and immotility. Progressive motility are spermatozoa having an active, linear or large circle movement. Non-progressive motility is

characterized by the absence of progression although we can see a movement of the flagella or the sperm head. Immotility is characterized by no movement. The recommended lower reference limit for total motility is 40% and for progressive motility is 32% (WHO, 2010). Progressive motility under the lower reference limit (32%) is referred as asthenozoospermia.

Sperm viability evaluation provides a relative proportion of live to dead spermatozoa (WHO, 2010). Live spermatozoa are critical in the ability for fertilisation, including sperm capacitation, acrosome reaction, and binding of the spermatozoon to the egg surface (Ramu et al., 2013). Sperm viability is evaluated by determining the membrane integrity of the cell by means of dye exclusion tests or by hypo-osmotic swelling (Kumar et al., 2017). Spermatozoa with an intact plasma membrane will be considered as viable (Glazar, 2014). The supravital staining procedures such as the eosin-nigrosin are the most used technique to determine sperm vitality (Jairo et al., 2021). These methods are based on the principle that sperm with damaged plasma membranes will absorb the stain while the ones with intact membrane will not. The hypo-osmotic swelling test presumes that only cells with intact membranes (live cells) will swell in hypotonic solutions (Jeyendran et al., 1992; Kumar et al., 2017). The WHO (2010) recommended 58% as lower reference limit for sperm vitality (spermatozoa with intact cell membrane).

Sperm morphology is one of the most important tests of a routine semen examination (Gatimel et al., 2017). The evaluation of sperm morphology provides a good indicator of testicular health, which is associated to physiological and environmental stresses (Menkveld et al., 2011). Several studies have highlighted an association between the percentage of abnormal forms and functional sperm abnormalities (Gatimel et al., 2017). Sperm morphology has a critical role in spermatozoa-zona binding, penetration, and spermatozoon-oocyte fusion (Ombelet et al., 1995). The classification of morphologically normal sperm has been progressively redefined. Some of the sperm abnormalities evaluated during basic semen analysis include head defect (i.e large, small, amorphous), midpiece defect and tail defect (i.e: short, multiple). The strict criteria is defined as the recommended guideline for the evaluation and classification of sperm morphology (Menkveld et al., 1990). The WHO (2010) indicates 4% as the lower reference limit for normal morphology. A proportion of normal sperm less than the lower reference limit (4%) is referred as teratozoospermia.

Semen contains cells other than spermatozoa including germ cells and leukocytes (Johanisson et al., 2000). The presence of more than 1 million leukocytes in 1 ml of ejaculate is reffered as leukocytospermia (WHO, 1999). and can indicate an inflammation of the male accessory glands (Calogero et al., 2017). The most common type of leukocytes in semen are polymorphonuclear leukocytes (PMN, neutrophils) (Tomlinson et al., 1993; Wolf, 1995; Johanisson et al., 2000). Morphologically, polymorphonuclear leukocytes are like multinucleated spermatids, but after staining, clear differenciation can be observed (Johanisson et al., 2000). The most common test used to quantify leukocyte in semen is the peroxidase positive granulocytes (neutrophils and macrophages) detection through the Endtz test (Endz, 1974).

The pH evaluation is part of the macroscopical examination of semen. Semen pH reflects the differences in pH values between the different accessory gland secretions, especially the acidic prostatic secretion and the alkaline seminal vesicular secretion (WHO, 2010). The WHO (2010) recommends that the pH should be measured once the semen has liquified, preferably after 30 minutes to 1 hour after semen collection.

Table 1.1: Categorization of semen parameters according to the WHO (2010)

Semen parameters	Categorization	Reference ranges
Volume	Normal	≥ 1.5 ml
Sperm concentration	Azoospermia	= 0 x10^6/ml
	Oligozoospermia	> 0 and < 15 x 10^6/ml
	Normal	15 - 250 x 10^6/ml
Progressive motility	Asthenozoospermia	< 3 2%
	Normal	> 32%
Morphology	Teratozoospermia	< 4%
	Normal	≥ 4%

1.2.7.2. Functional Semen Analysis

Sperm functional tests, in addition to the basic semen analysis, have been reported for many years. These functional sperm tests are mostly considered as research methods in several andrology laboratories (Oehninger et al., 2014). Some of the functional sperm tests including sperm DNA fragmentation (SDF), sperm–zona pellucida binding test, the sperm

penetration assay, the acrosome reaction and direct and indirect assessments of seminal oxidative stress. Recently, clinical evaluations such as sperm oxidative stress and DNA integrity have been of increasing clinical importance (Agarwal et al., 2008; Esteves et al., 2011).

SDF evaluates the presence of DNA strand breaks in spermatozoa and consequently evaluates the DNA integrity (Gonzalez-Marin et al., 2012; Evenson, 2016). The evaluation of SDF is an important diagnostic tool for male infertility and reproductive success (Agarwal et al., 2016a; Aitken, 2017). Furthermore, SDF can be associated with miscarriages and a high possibility for the progeny diseases (Zini et al., 2008; Robinson et al., 2012; Esteves et al., 2021). Different methods are used to assess SDF as well as chromatin integrity. Some of the methods include acridine orange test (AOT), chromomycin A3, Sperm Chromatin Structure Assay (SCSA), Comet assay, Sperm Chromatin Dispersion test (SCD) or transferase dUTP nick end labelling (Tunel) (Hamilton et al., 2020). Amongst these tests, four of them were reported to be more used namely Comet, Tunel, SCSA and AOT. The SCSA denature sperm DNA by exposing them with a low pH at the sites of DNA strand breaks (Evenson and Wixon, 2006). The determination threshold values for DNA fragmentation remains an ongoing debate.

Oxidative stress (OS) was found to be present in up to 80% of infertile men and resulted from the generation of ROS from contaminating leukocytes, abnormal sperm as well as antioxidant reduction (Agarwal et al., 2008; Esteves and Agarwal, 2011). A host of lifestyle-related factors, the majority of which are modifiable, can cause OS (Dimitriadis et al., 2017). The oxidative balance of an organism can be maintained by food habits, environmental pollution, physical activity (Chia et al., 2001) and endogenous antioxidants (Aguilar et al., 2016). However, lifestyle factors such as alcohol consumption, smoking of cigarettes as well as physical stress were reported to be associated with an increase in oxidative Stress (Khatun et al., 2018; Sikka and Hellstrom, 2016).

Presently, there is no defined patient profile to test for OS, and standard testing method to perform (Wagner et al., 2017). Different biomarkers assays used for the evaluation of OS in human semen are discussed in the literature. Some of the methods include ROS, total antioxidant capacity and malondialdehyde (MDA) (Jungwirth et al., 2012). One of the direct measurements of OS is performed by the evaluation of the oxidation-reduction potential

(ORP), which is the assessment of the balance between oxidants and antioxidants in an organism (Agarwal and Bui, 2017). The ORP provides a measure of the overall balance between oxidants and antioxidants, which gives a complete measure of the redox system and OS (Agarwal et al., 2019). ORP levels can help to detect changes in functional status of spermatozoa produced by OS in cases of idiopathic male infertility and in male partners of couples facing recurrent pregnancy loss (Agarwal et al., 2019). The clinical determination of ORP levels could be used to significantly differentiate semen quality between the control and normozoospermic group with reference to sperm concentration, motility and morphology (Agarwal et al., 2017b). A normalized ORP cut-off of 1.34 mV/10^6 sperm/ml was found when evaluating the relationship between ORP and semen quality (Agarwal et al., 2019; Arafa et al., 2020).

1.2.7.3. Abstinence Periods and Semen Analysis

Prolonged abstinence periods were found to favour the accumulation of spermatozoa in the epididymis, which can consequently increase the exposure time of spermatozoa to the detrimental effects of ROS (Agarwal and Said, 2003; Du Plessis, 2010). The WHO (2010) stipulates that semen samples should be collected after a minimum of 2 days and a maximum of 7 days of sexual abstinence (WHO, 2010). However, shorter abstinence periods can improve semen quality (Lehavi et al., 2014). Patients with a low sperm concentration have improved semen characteristics after twenty-four hours (24 hours) of sexual abstinence (Lehavi et al., 2014).

On the other hand, abstinence periods have been reported to positively influence sperm concentration, semen volume, and the number of leukocytes (De Jonge et al. 2004; Comar et al., 2017), while it has a negative influence on sperm motility and vitality (Comar et al, 2017). In addition, an increasing number of spermatozoa with DNA damage and mitochondrial membrane damage were associated with an increase in abstinence period (Comar et al., 2017).

1.2.8. Diagnostics in Male Infertility

The diagnostics in male infertility usually consist of taking the anamnesis such as the duration of the inability to achieve a pregnancy, coital frequency, previous successful

conception, recent systemic diseases, environmental exposure, medications, allergies and surgical history and physical examination such as the genitalia (penis, scrotum, testes, epididymis, vas deferens and spermatic cord) (Quallich, 2006; Practice Committee of the American Society for Reproductive Medicine, 2015). The physical examination of the patient includes the examination of the testicles, or presence of varicocele (Quallich, 2006; Singh et al., 2012). Furthermore, the diagnostics in male infertility also involve semen analysis and endocrine evaluation (free testosterone, total testosterone, FSH, LH, estradiol and prolactin levels) (Quallich, 2006; Anawalt, 2013). A high level of testosterone and LH can indicate partial androgen resistance (Tincello et al., 1997; Hellmann et al., 2012), while a decrease level of testosterone concentration might be caused by the use of anabolic steroid (Rasmussen et al., 2016) or the presence of hypogonadotropic hypogonadism (Quallich, 2006; Fraietta et al., 2013). The diagnostics in male infertility must include a minimum of one adequately performed semen evaluation (Barratt et al., 2017). The WHO provides guidelines for qualitative evaluation of semen samples; however, some fertility laboratories do not adhere to the methods describe by the WHO (Mallidis et al., 2012; Filimberti et al., 2013; Punjabi et al., 2016). The nonaherence to the WHO guidelines decreases the value of the diagnostic potential of semen analysis (Carrell and De Jonge, 2016).

1.3. Critical Review of Semen Analysis and WHO Manual for Male Infertility

1.3.1 Critical Review of Semen Analysis

Semen analysis is the primary step to investigate male factor infertility, indicated as the cornerstone in the assessment of male reproduction potential (Barratt, 2007; Butt and Akram, 2013). However, the standard semen analysis may not be a sensitive predictor of male infertility (Nagler, 2011). The literature highlights some of the reasons explaining this low predictability. One of the reasons is the inherent variability of the semen parameters within one individual and between different individuals (Leushuis et al., 2014). Although the WHO provides step-by-step methods on how to perform the semen analysis and references values for semen parameters, sperm parameters can vary within an individual and between individual (Poland et al., 1985; Jarow et al., 2013). Factors such as genetics, seasonal

variations (Levine, 1999; Santi et al., 2018) and geographical location (Agarwal et al., 2015; Elbarisi et al., 2018) can influence semen parameters.

The inability of the semen analysis to measure the fertilizing capacity of spermatozoa and to measure the physiological changes in spermatozoa occurring in the female reproductive tract prior fertilization is another argument explaining the lack of predictability of the semen evaluation (Wang and Swerdloff, 2014). There are many other factors such as the level of oxidative stress or the integrity of sperm DNA (not include in the routine semen analysis) influencing the ability of spermatozoa to fertilize the egg (Wang and Swerdloff, 2014). Finally, the technology to exactly determine different semen parameters and to associate basic semen parameters with the sperm function evaluation is still lacking (Morshedi, 2014).

1.3.2. WHO manuals for Male Infertility

The increasing need for standardization of the methods used for the analysis of human semen led the World Health Organization to periodically release guidelines for the analysis of human semen. The first manual was published in 1980 with five updated manuals subsequently published. The latest WHO manual (WHO, 2021) was published on 27 July 2021. The main purpose of the WHO guidelines is to encourage the use of standard methodologies for semen analysis to establish more accurate reference values for semen parameters.

The 1st Edition of the laboratory manual was published in 1980 and summarized the clinical experience and research from 1972. This manual was titled “The WHO Laboratory Manual for The Examination of Human Semen and Sperm–Cervical Mucus Interaction” (Scott-Wilson, 1982). The reference values in the 1st Edition are summarized in Table 1.2. There were no guidelines (reference values) for normal semen volume, total sperm count and sperm vitality.

The 2nd Edition was published 1987 and titled “The WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervicle Mucus Interaction, 2nd Edition”. This edition was more used by clinicians and researchers worldwide than the 1st edition (WHO, 1989). For the 2nd Edition, the WHO established an editorial committee to review all the procedures described in the manual. The reference values published on the 2nd edtion are

summarized in Table 1.2. Compared to the 1st Edition, there was no change for the lower reference value for sperm concentration. However, lower reference values for total motility and normal morphology were reported in the 2nd Edition. Furthermore, in the 2nd Edition, the WHO established the reference values for semen volume, total sperm count and sperm vitality, which was not in the previous edition.

In November 1992, the WHO published "The WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervicle Mucus Interaction, 3rd Edition" (WHO, 1993). The 3rd Edition was published by Cambridge University. The reference values for the 3rd Edition are highlighted in Table 1.2. Compared to the 2nd edition, there were no changes in lower reference values for semen volume, sperm concentration, total sperm count, total motility and leukocyte count. However, the lower reference value for sperm vitality was higher than the 2nd Edition. In the 3rd Edition the WHO introduced the motility grading (a and b), with "a" being rapid and rectilinear mobility and "b" slow progressive.

The 4th Edition was published in May 1999 by the WHO, entitled "Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction". The reference values in the 4th Edition are based on multicenter population studies involving normal men, however, no fertilization data was obtained from these men. Furthermore, in the 4th Edition it is advised to each laboratory to have their own reference ranges for each semen parameter. The reference values for semen parameters are represented in Table 1.2. Compared to the 3rd Edition, the lower reference values for semen volume, sperm concentration, total sperm count, progressive motility, total motility and leukocyte count remained unchanged. Only sperm concentration reference value changed.

In 2010, the WHO published the 5th Edition, namely "WHO Laboratory Manual for the Examination and Processing of Human Semen". The 5th Edition was the first to have the distribution of semen parameters being based on significant data collected from patients, more specifically from fertile men with current or previous pregnant partners and known time to pregnancy up to 12 months (Cooper et al., 2010). In comparison to the 4th Edition, the 5th Edition includes additional procedures regarding sperm preparation for clinical use or specialised assays and on cyropreservation. Furthermore, the 5th Edition is characterized by semen analysis threshold values that are markedly lower than those of previous editions.

In the 5th Edition, the 5th centiles were presented as the lower reference. The reference values for semen parameters as advised in the 5th edition are summarized in Table 1.2.

1.3.3. WHO (2010) Data

Data published by Cooper et al. (2010) is the basis of the WHO (2010) reference values for human semen characteristics. Semen parameter data was obtained from approximately 4,500 men residing in 14 countries located on four continents. However, some regions including Africa, Central and South America were under-represented. The selection criteria of the data only included men of unknown fertility status, fertile men and men selected as normozoospermic. Of the 4,500 men, those having partners with time to pregnancy less than 12 months were selected to provide the reference limits for human semen.

Since the publication of the WHO (2010), several publications have discussed the relevance of the lower reference limits as suggested by the WHO (2010) (Esteves, 2014; Cocuzza, 2015). Campbell et al. (2021) provided substantial additional published data representing an attempt on providing an update on the distribution of semen parameters values following the WHO (2010).

The data from Campbell et al. (2021) consisted of two published sources. The first source consists of data published after the WHO (2010) publication and obtained by performing a systematic review and meta-analysis of papers having semen analysis results. This was done following the Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Liberati et al., 2009). The second source consisted of some of the data used by the editorial team of the WHO (2010) to establish the reference values (Cooper et al., 2010). The data was collected from more than 3500 men, residing in twelve countries and five continents. The data is freely available using the link: https://doi.org/10.15132/10000163 .

Table 1.2: Summary of the 1st, 2nd, 3rd, 4th and 5th edition of the World Health Organization reference values guidelines for semen volume, sperm concentration, total sperm count, total motility, progressive motility, sperm vitality, normal morphology and leukocyte count

Semen parameters	WHO, 1980 (1st Edition)	WHO, 1987 (2nd Edition)	WHO, 1992 (3rd Edition)	WHO, 1999 (4th Edition)	WHO, 2010 (5th Edition)
Volume (ml)	-	≥ 2	≥ 2	≥ 2	≥ 1.5
Sperm concentration (10^6/ml)	20 - 200	≥ 20	≥ 20	≥ 20	≥ 15
Total sperm count (x10^6)	-	≥ 40	≥ 40	≥ 40	≥ 39
Total motility (%)	≥ 60	≥ 50	≥ 50	≥ 50	≥ 40
Progressive motility (%)[ab]	≥ 2[abc]	≥ 25	≥ 2 (grade a)	≥ 2 (grade a)	≥ 32 (a + b)
Vitality (%)	-	≥ 50	≥ 75	≥ 75	≥ 58
Normal Morphology (%)	80.5	≥ 50	≥ 30[abcd]	(14)[abcde]	4[abcdef]
Leukocyte count (10^6/ml)	< 4.7	< 1.0	<1.0	<1.0	<1.0

[ab]Grade a = rapid progressive motility (> 25µm/s); grade b = slow/sluggish progressive motility (5-25µm/s); Normal = 50% motility (grades a+b) or 25% progressive motility (grade a) within 60 minutes of ejaculation. [abc]Forward progression (scale 0-3); [abcd]Arbitrary value; [abcde]Value not defined but strict criteria is suggested; [abcdef]Strict (Tygerberg) criteria

1.3.4 Development of the WHO 2010 Guidelines

Semen evaluation is the most used biomarker to predict the male ability to fertilize a woman (Esteves et al., 2011). The analysis provides indications of seminal characteristics such as viscosity, volume, sperm concentration, total sperm count, sperm motility (progressive and non-progressive), sperm morphology and functional test such as DNA fragmentation test (Esteves and Agarwal, 2011). Semen parameters can considerably vary from the same or different individuals and can be affected by factors such as abstinence days and inherent biological conditions (Alvarez et al, 2003; Castilla et al., 2006; Keel, 2006). Therefore, clinical reference standards for semen quality comparison between patients are necessary.The WHO manuals provide universal guidelines and references of normal limits to help practitioners to differentiate between fertile and infertile men (Alshahrani et al., 2018).

In the first Edition, the cut-off normal values for sperm concentration were between 20 and 200 million spermatozoa per millilitre with 60% being the lower reference value for total motility and 80.5% for normal morphology. The second Edition was released in 1987 and provides more normal values compared to the first Edition. For instance, semen volume more than or equal to 2 millilitre, total sperm count more than or equal to 40 and percentage of 50% or more live spermatozoa were considered as normal. In 1992, the WHO published the 3rd Edition. Compared to the 2nd Edition, there were no changes in lower reference values for semen volume, sperm concentration, total sperm count, total motility and leukocyte count. However, the lower reference value for sperm vitality was higher than the 2nd Edition. In the 3rd Edition, the WHO introduced the motility grading (a and b). Similar semen parameters reference limits were released from the second to the fourth Edition which was released in 1999 following expert opinions (Haidl, 2011).

In 2010, following disagreements over the suitability of the reference values set in the Fourth Edition (1999), the WHO, established new references values for human semen parameters (Fifth Edition), which are considerably lower than the previous Edition. Consequently, patients who previously were diagnosed abnormal based on

their semen characteristics could now be considered normal using new reference values. Cut-off values for semen parameters such as volume (≥2 mL to 1.5 mL), sperm count (20 to 15×10^6 spermatozoa /mL) and total motility (≥50% to 40%) were lower in the latest published edition. Furthermore, reference limits for sperm vitality were lower in the latest edition (≥75% to 58%) as well as for sperm normal morphology (14% to 4%) through the introduction of the strict (Tygerberg) approach described in detail by Menkveld et al. (1990).

The validity of the WHO (2010) reference values has been questioned (Bromwich et al., 1994; Esteves et al., 2011; Christopher et al., 2011). Some limitations regarding the study performed to set the new reference values are discussed in the literature (Cocuzza et al., 2015; Murray et al., 2012). The study only involved 4,500 fertile men residing in 14 countries (with no data from Africa) and based on the 5th percentile observed in the cohort (Murray et al., 2012). Such study design does not provide the true reflection of the reality since geographical shifts in semen characteristics such as semen volume, sperm concentration and motility are reported in the literature (Bostofle et al., 1983; Auger et al., 1995; Irvin et al., 1996; Alshahra et al., 2018). There is a need to have a consensus to provide a clear definition of poor semen criteria for those men who seek to be counselled satisfactorily.

1.4. Problem Statement

Semen parameters can vary according to numerous factors including geographical location, seasonal changes, age and abstinence periods. However, data on semen parameters in men from Africa and MENA regions remains sparce compared to other regions. A decline in semen parameters is observed globally, with sparce data available from Africa and MENA regions. Therefore, an extensive retrospective analysis of semen parameter reports together with epidemiological information from males residing in Africa and MENA region is necessary in order to compare it with existing data from other regions.

1.5. Research Question

What is the distribution of semen parameters obtained from a cohort of men residing in Africa and Middle East regions, considering the age, ejaculatory abstinence, geographical regions, temporal and seasonal changes in semen quality?

1.6. Research Aim

This aim of this study is to retrospectively evaluate semen parameters and hormones obtained from a cohort of men residing in Africa and Middle East regions, considering the age, ejaculatory abstinence, geographical regions, changes over time, and seasonal changes in semen quality.

1.7. Research Objectives

The aim will be achieved through the following objectives:

i) To determine the descriptive statistics of semen parameters and hormones in the full cohort (Africa and Middle East regions);
ii) To determine the correlations of semen parameters and hormones in the full cohort (Africa and Middle East regions);
iii) To determine the impact of age on semen quality, functional sperm tests and hormones in the full cohort (Africa and Middle East regions);
iv) To investigate the influence of abstinence periods on semen quality and functional sperm tests in the full cohort (Africa and Middle East regions);
v) To investigate the geographical differences in semen quality in the full cohort (Africa and Middle East regions);
vi) To evaluate the temporal changes in semen quality over the past 15 years (2005 – 2019) in patients residing in sub-Saharan African countries;
vii) To investigate the effects of seasonal variations in semen characteristics of patients residing in sub-Saharan African countries below the equator;
viii) To perform a descriptive and comparative global analysis of Cooper (2010) data and Campbell (2021) data with the full cohort (Africa and Middle East regions).

Chapter 2: Research Design and Methodology

2.1 Study Design Overview

This study aimed to retrospectively analyse semen analysis and reproductive hormone records of males residing in Africa and Middle East regions within the context of the WHO (2010) guidelines. In order to fulfil this aim, a retrospective cohort study design was used to analyse andrology laboratory reports of semen analyses of patients from Africa and the Middle East regions obtained between 2005 - 2019. The analysis of the data is based on the study objectives. The full cohort data consisted of semen analysis reports obtained from Androcryos Andrology Laboratory, Ampath Andrology Laboratory and Lancet Andrology Laboratory located in South Africa (sub-Saharan African region data), and from Hamad Medical Center, located in Qatar (MENA region data), for retrospective analysis. In the current study the MENA region consists of Algeria, Egypt, Morocco, Qatar, Saudi Arabia, Tunisia, and United Arab Emirates.

This data was statistically analysed and presented as descriptive statistics. Comparisons and correlations were further performed to analyse impact of geographical regions, age, abstinence periods, temporal changes with time and seasonal variations on semen parameters and reproductive hormones. Additionally, data from Africa and the Middle East was statistically compared to global regional data from America, Asia, Australia and Europe obtained from Cooper et al. (2010) and Campbell et al. (2021).

2.2 Ethical Approval

This study was conducted in line with the Declaration of Helsinki for medical research. Institutional approval was granted by the Biomedical Research Ethics Committee (BMREC), University of Western Cape (UWC), South Africa (Ethics Reference Number: BM19/9/7). Permissions to use data confidentially were obtained from participating laboratories. No personal identification data such as name, ID or laboratory requisition number was extracted.

2.3 Setting

The retrospective cohort study involved semen analysis reports from patients attending Androcryos Andrology Laboratory, Ampath Andrology Laboratory, Lancet Andrology Laboratory and Hamad Medical Center. Androcryos Andrology Laboratory, located at 9 Saint David's Park, St David's Place, Parktown (Johannesburg, South Africa), was established in 1984 and was the first to offer a sperm donor program alongside an andrology laboratory in South Africa (www.androcryos.co.za). Ampath Pathology Laboratory, located at 1171 Stanza Bopape St, Hatfield (Pretoria, South Africa), is one of the Southern Africa's most prominent pathology laboratories offering pathological services such as chemical pathology, immunology, microbiology and andrology (www.ampath.co.za). Lancet Laboratories, located at Unitas Hospital, 0157 Lyttelton Manor, Clifton Ave, Centurion (Pretoria, South Africa), is amongst the main pathology laboratories operating on the African continent and provides services in sub-Saharan countries such as Botswana, Ghana, Kenya, Mozambique, Nigeria, South Africa, Swaziland, Tanzania, Uganda, Zambia and Zimbabwe (www.lancet.co.za). Hamad Medical Center, located at Al Waab St (Doha, Qatar) is the main public healthcare provider in Qatar and accredited by the Joint Commission International. The Andrology division of the Hamad Medical Center is affiliated with the Urology Department and has been recognized as a centre of excellence for male reproductive health by the United States Institute for Urological Excellence (www.hamad.qa).

2.4 Inclusion and Exclusion Criteria for Patient Reports and Data Cleaning

Inclusion and exclusion criteria are summarised in Table 2.1. Data used for the retrospective cohort study was obtained from patients who consulted for routine semen evaluations through referral by relevant medical practitioners, particularly fertility specialists and gynaecologists. Post-vasectomy evaluations and duplicated reports were excluded from the study. All pH values less than 5 and more than 10 were removed from semen reports for pH analysis only, while the remaining variables from these reports were included for data summary and analysis. Each entry was accurately conducted by the investigator (LM) and verification was performed by the supervisors (KL and RH).

Table 2.1: Summary of inclusion and exclusion criteria of semen reports

Inclusion criteria	Exclusion criteria
Semen analysis reports	Post-vasectomy
Sperm donor reports	Duplicated reports

2.5 Data Extraction and Variables

The retrospective cohort study involved semen analysis results together with patient age, geographic locations, date of semen collection, ejaculatory abstinence period and varicocele results. Data received was organized in a Microsoft Excel spreadsheet. Table 2.2 summarizes basic semen parameters (volume, pH, sperm concentration, progressive motility, sperm morphology), functional sperm tests (DNA fragmentation, sperm viability, static oxidation-reduction potential (sORP)) and hormones (estradiol, luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone and prolactin) profiles obtained from the different laboratories. Other seminal contents such as leukocytes counts, red blood cell counts and epithelial cells are also listed in Table 2.2. However, there was no data for progressive motility obtained for patients who consulted between 2013 and 2018. Additional semen quality parameters such as total progressively motile count (TPMC), total normal sperm count (TNSC) and normed sORP were calculated based on the available data. TPMC was defined as the product of total sperm count and progressive motility (sperm concentration (10^6/mL)/100 x progressive motility (%) x volume (mL)), while the TNSC was the product of total sperm count and the percentage of normal morphology (sperm concentration (10^6/mL)/100 x normal morphology (%) x volume (mL)). The normed sORP was obtained by dividing the sORP to the sperm concentration (sORP (mV) / sperm concentration (10^6/mL)).

Table 2.2: Summary of variables obtained for the retrospective cohort study

Parameters	Androcryos	Ampath	Lancet	Hamad
Collection month and year	✓	✓	✓	x
City, province, country of residence	✓	✓	✓	✓
Age	✓	✓	✓	✓
Abstinence period (days)	✓	✓	✓	✓
Semen volume (mL)	✓	✓	✓	✓
Semen pH	✓	✓	✓	✓
Sperm concentration ($x10^6$/mL)	✓	✓	✓	✓
Total sperm count ($x10^6$l)	✓	✓	✓	✓
Progressive motility (%)	✓	✓	✓	✓
Normal morphology (%)	✓	✓	✓	✓
Duplicated spermatozoa (%)	✓	✓	✓	x
Small spermatozoa (%)	✓	✓	✓	x
Leukocytes counts ($x10^6$/mL)	✓	✓	✓	x
Red blood cell counts ($x10^6$/mL)	✓	✓	✓	x
Epithelial cells	✓	✓	✓	x
Normed sORP (mV/10^6 mL)	✓	x	✓	✓
Sperm viability (%)	x	x	x	✓
DNA fragmentation (%)	x	x	x	✓
Varicocele	x	x	x	✓
Estradiol (pmol/L)	x	x	x	✓
Luteinizing hormone (IU/L)	x	x	x	✓
Follicle stimulating hormone (IU/L)	x	x	x	✓
Testosterone (nmol/L)	x	x	x	✓
Prolactin (mIU/L)	x	x	x	✓

2.6 Analysis of Semen Reports

2.6.1 Descriptive Statistics of Semen Parameters and Hormones in Africa and the Middle East

Descriptive statistics was summarised for participant age and each semen parameter, functional sperm test and hormone. Subsequently, descriptive statistics for categorical data including epithelial cells (0, 1+ and 2+), varicocele (absent, unilateral, bilateral) and semen parameters (based on semen volume, sperm concentration, progressive motility and sperm morphology) was conducted based on the reference values provided by WHO (2010). Table 2.3 summarizes the categorization for semen volume, sperm concentration, progressive motility and morphology.

Table 2.3: Categorization of semen parameters evaluated (WHO, 2010)

Semen parameters		Reference ranges
Volume	Hypospermia*	< 1.5 mL
	Normal	≥ 1.5 mL
Sperm concentration	Azoospermia	= 0 x10^6/mL
	Oligozoospermia	> 0 and < 15 x 10^6/mL
	Normal	15 - 250 x 10^6/mL
	Polyzoospermia*	> 250 x 10^6/mL
Progressive motility	Asthenozoospermia	<32%
	Normal	>32%
Morphology	Teratozoospermia	< 4%
	Normal	≥ 4%

***Classification not indicated in the WHO (2010)**

Descriptive statistics for the classified groups were reported as number of reports and percentage of reports. Comparisons between these classified groups were done using appropriate statistical analysis based on data distribution. Semen parameters (volume, progressive motility, total progressively motile count, normal morphology, total normal sperm count, DNA fragmentation, sperm viability, sORP and normed sORP) were compared according to sperm concentration categories. This was followed by a comparison of varicocele and epithelial cells results according to sperm concentration categories. Then, comparisons of semen parameters (volume, progressive motility, normal morphology, DNA fragmentation, sperm viability, and normed sORP) and hormones (estradiol, LH, FSH, testosterone and prolactin) between sperm concentration categories, progressive motility categories and normal morphology categories were performed. To finish, a classification of patient's semen volume, sperm concentration and viability tests as normal and abnormal according to the WHO (1999) and WHO (2010) criteria was done. Descriptive statistics and comparisons between the sperm concentration, progressive motility and normal morphology categories were performed using appropriate non-parametric tests which were based on data distribution.

2.6.2 Correlations between Semen Parameters and Hormones in Africa and the Middle East

The correlations between reproductive hormones (estradiol, LH, FSH, testosterone and prolactin) and seminal parameters (sperm concentration, progressive motility, normal morphology, SDF, viability and normed sORP) in the full cohort were determined. Correlations between these classified groups were done using appropriate non-parametric tests which was based on data distribution.

2.6.3 Impact of Age on Semen Parameters and Reproductive Hormones in Africa and the Middle East

Semen parameters (volume, concentration, total sperm count, progressive motility, total progressively motile count, total normal sperm count, sperm morphology), functional sperm tests (DNA fragmentation, sperm viability, sORP and normed sORP) and hormones (estradiol, LH, FSH, testosterone and prolactin) obtained in the full cohort were evaluated according to age categories. For age-related changes in semen characteristics (volume, sperm concentration, total sperm count, progressive motility, total progressively motile count, total normal sperm count and sperm morphology), functional sperm tests (DNA fragmentation, viability and normed sORP) and sex hormone (estradiol, LH, FSH, testosterone and prolactin) data was divided into four groups based on age and following previous published study by Pino et al. (2020). The following age groups were used in this study: 16 - 20 years, 21 - 30 years; 31 - 40 years; 41 - 50 years; 51 - 60 years and more than 60 years. Comparisons between these age groups were done using appropriate non-parametric statistical tests which were based on data distribution.

2.6.4 Influence of Abstinence Periods on Semen Parameters in Africa and the Middle East

The comparison of semen parameters (volume, concentration, progressive motility, normal morphology) and functional sperm tests (DNA fragmentation, sperm viability, sORP and normed sORP) obtained in the full cohort were evaluated according to abstinence periods.

To evaluate the influence of different periods of abstinence on conventional semen characteristics (volume, concentration, progressive motility, normal morphology) and functional sperm parameters (DNA fragmentation, sORP and normed sORP), semen reports were categorized according to sexual abstinence period into 3 groups: < 2 days, 2-5 days, and > 5 days as previously reported by Comar et al. (2017). Comparisons and correlations between these abstinence period groups were done using appropriate non-parametric statistical tests, which were based on data distribution.

2.6.5 Geographical Differences in Semen Parameters in Africa and the Middle East

Data collected from the full cohort study indicated patient's residential country. To assess the relationship between geographical differences and semen parameters each residential area and country were given a code. Then, countries were categorized in regions, namely Northern Africa, Southern Africa, Eastern Africa, and Middle East. The regional classification of countries was done following the World Health Organization (WHO, 2012).

Using this data, a comparison of semen parameters (volume, concentration, progressive motility, total progressively motile count, sperm morphology, total normal sperm count, sORP, normed sORP) between patients residing in Southern African countries (South Africa, Zimbabwe, Zambia, Mozambique, Lesotho and Swaziland), Eastern African countries (Ethiopia, Kenya, Rwanda, Tanzania and Uganda), Northern African countries (Algeria, Egypt, Morocco and Tunisia) and Middle East countries (Qatar and Saudi Arabia) was conducted. Subsequently, semen parameters (pH, volume, concentration, total sperm count, progressive motility, sperm morphology, red blood cell count) obtained from patients residing in Southern African countries (South Africa, Zimbabwe, Zambia, Mozambique, Lesotho and Swaziland) were compared using appropriate non-parametric statistical tests which was based on data distribution.

Data obtained from South African laboratories indicated additional information such as residential area. Consequently, a comparison evaluating the geographical differences in semen parameters (volume, sperm concentration, total sperm count, progressive motility, sperm morphology, red blood cell count) amongst patients residing in different areas within South Africa, namely Gauteng North, Gauteng Central, Gauteng East, Gauteng West, Gauteng South, Gauteng Pretoria, Eastern Cape, Free States, Mpumalanga, North - West, Western Cape and KwaZulu–Natal, Limpopo, was performed using appropriate non-parametric statistical tests based on data distribution.

2.6.6 Temporal Changes in Semen Quality (2005-2019) in the Sub-Saharan Region

Annual changes in semen characteristics between 2005 and 2019 in patients residing in sub-Saharan African countries, namely Cameroun, Congo, Ethiopia, Ghana, Kenya, Lesotho, Mozambique, Nigeria, South Africa, Swaziland, Rwanda, Senegal, Uganda, Zambia and Zimbabwe, was evaluated. For that purpose, semen characteristics such as volume, concentration, progressive motility, total progressively motile count, normal morphology, total normal sperm count, percentage of small spermatozoa and elongated spermatozoa were used as end points.

The changes over years in semen parameters such as semen volume, sperm concentration, progressive motility, total progressively motile count, sperm morphology (normal, mall and elongated) were determined using applicable non-parametric statistical tests based on data distribution.

2.6.7 Seasonal Variation in Semen Parameters in the Sub-Saharan Region Below the Equator

A comparison of semen characteristics such as pH, volume, sperm concentration, total sperm count, progressive motility, normal morphology, percentage of elongated and duplicated spermatozoa, and normed static oxidation-reduction potential (sORP), according to seasons was evaluated. Semen parameters were obtained

from patients residing in sub-Saharan African countries located below the equator (Lesotho, Mozambique, South Africa, Swaziland, Rwanda, Zambia, Zimbabwe) and visiting South African Laboratories (Androcryos Andrology Laboratory, Ampath Laboratory and Lancet Laboratory) for andrology services. For group comparison, semen reports dated between the 1st December and 28/29 February were classified as summer, while those between 1st of March and 31st of May were categorized as autumn. All semen reports from the 1st June to the 31st August were classified as winter and those between the 1st September and 30th November were classified as spring (Season of the year, www.seasonsyear.com). Comparisons between these seasonal groups were done using appropriate non-parametric statistical tests which was based on data distribution.

2.6.8 Descriptive Analysis of Cooper (2010) and Campbell (2021) Data Compared to the Africa and Middle East Data

A comparison between the Africa and Middle East cohort study data (n = 70 765), Cooper (2010) data (n = 6 299) and Campbell (2021) data (n = 3 589) was performed. Cooper (2010) data was primarily used to set the reference values for semen parameters published by the WHO (2010). This data consists of semen analysis reports obtained from fathers (with and without known time to pregnancy) and unscreened volunteers residing in America, Asia, Australia and Europe. Cooper (2010) was obtained from Dr TG Cooper, Centre of Reproductive Medicine and Andrology of the University, Domagkrstrasse, Germany. Campbell (2021) data is a combination of dataset used to determine the WHO (2010) semen references values and published data following the WHO (2010), publicly available from https://doi.org/10.15132/10000163. These data sets include semen parameters such as semen volume, sperm concentration, total sperm count, progressive motility, total progressively motile count, normal morphology, total normal sperm count and sperm viability (only for the Campbell (2021) data).

Descriptive statistics for semen parameters obtained from Cooper (2010) and Campbell (2021) data were determined. The descriptive statistics were compared to the descriptive statistics from the cohort study (Africa and the Middle East).

Comparative statistics for correlation coefficients obtained from the 3 data sets were performed.

Furthermore, differences in semen quality according to geographical locations in the cohort study data and the Cooper (2010) data were done. For this purpose, Cooper (2010) data was categorized according to geographical locations (America, Asia, Australia and Europe) of patients and compared to patients residing in Africa from the cohort study. For group comparisons, the following semen parameters were used: volume, sperm concentration, progressive motility, total progressively motile count, normal morphology and total normal sperm count.

2.7 Semen Analysis Methodologies used by Participating Laboratories in South Africa for the Processing of Semen Samples Collected After 2010

2.7.1 Sampling and Semen Collection

Semen samples collected and evaluated after 2010, were following the methods described by the World Health Organisation (WHO) criteria, 2010, while semen samples collected and evaluated before 2010 were according to the WHO (1999). Although semen samples were provided after 7 days and before 3 days of abstinence, patients requesting semen analysis evaluation were asked to provide semen sample after 3 - 7 days of abstinence. Following liquefaction at 37°C for 30 minutes, semen analysis was performed.

2.7.2 Macroscopic Evaluation

The macroscopic evaluation consisted of measuring the colour, volume, pH and viscosity of the semen sample. The volume and viscosity were measured using a 10 mL non-pyrogenic serological pipette while the pH was evaluated using a pH indicator paper. The viscosity of the raw semen sample is measured by allowing the semen to drop by gravity and observing the length of the thread with a ruler.

2.7.3 Determination of Sperm Motility

Sperm motility parameter was assessed by dropping semen sample on a warm, unfrosted slide using the non-pyrogenic serological pipette. Then, the slide was covered with a cover slip and allowed to stand for a few seconds before being evaluated using a phase contrast microscope at 20X objective, phase 2, according to the WHO 2010 Manual criteria. An estimation of the total percentage of motile spermatozoa per field was determined.

2.7.4 Sperm Concentration Evaluation and Calculation of the Total Sperm Count

Sperm concentration was evaluated using the Makler counting chamber (See Figure 2.1) method, instead of the improved neubauer hemocytometer as recommended by the WHO (2010). The evaluation of sperm concentration was performed by firstly immobilizing sperm cells. The immobilization of spermatozoa consisted of transferring around 1 mL of the well-mixed sperm specimen into a plastic screw cap tube, then to put the plastic screw cap tube with semen into water at 50°C - 60°C for at least 5 minutes. Furthermore, a drop of the immobilized well-mixed specimen was transferred in the centre of the Makler counting chamber and covered with the special cover glass. Then, the cover glass was grasped with a finger opposite the black dots allowing the drop to spread on the entire area of the disc into a thickness of 10 µm. The chamber was then lifted by its handles and placed on the stage of the microscope. A sperm count was evaluated using Primo Star microscope (Zeiss) at X20 objective and X10 eyepiece. The whole spermatozoa (with tail and head) inside the grids were counted (Figure 2.2). This number represents the sperm concentration (SC) in millions per millilitre. The total sperm number per ejaculate was determined by multiplying the sperm concentration by the volume of the whole ejaculate.

Figure 2.1: Makler counting chamber used for to determine sperm concentration

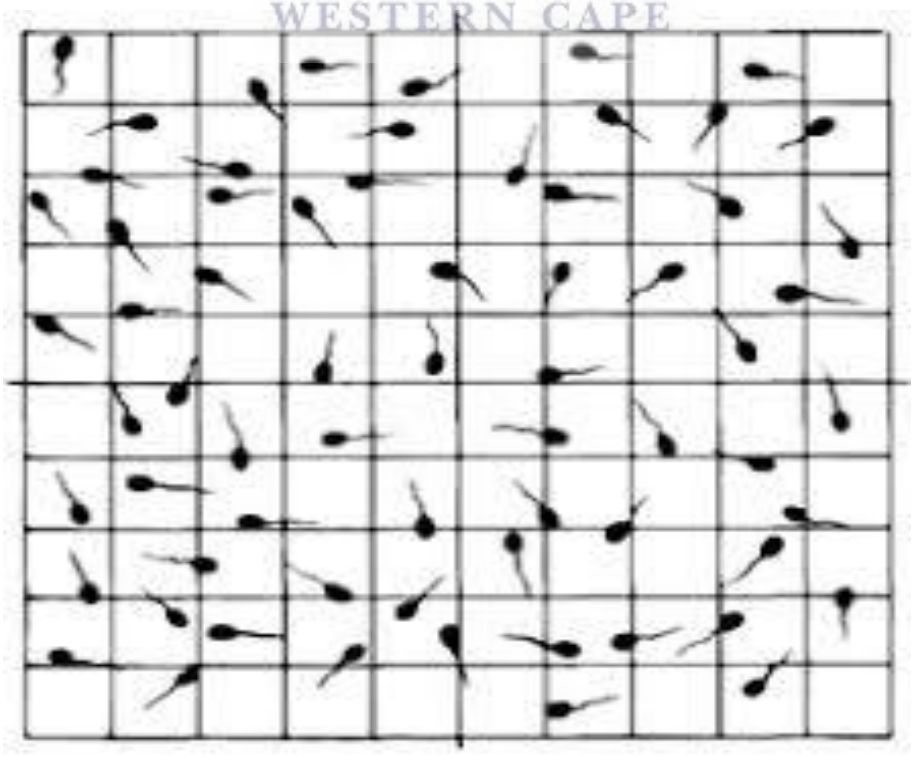

Figure 2.2: Representation of the Makler counting chamber grids

2.7.5 Evaluation of Morphological Characteristics of Spermatozoa

Sperm morphology was performed following the Papanicolaou staining procedure detailed in the WHO Laboratory manual for the examination and processing of human semen (WHO, 2010). A semen smear was prepared and air-dried. Then, the air-dried smear was fixed by immersing it into 95% (v/v) ethanol for at least 15 minutes. The fixed semen smear was subsequently immersed in ethanol 80% (v/v), ethanol 50% (v/v) and purified water for 30 seconds, respectively, before being stained in Harris's haematoxylin for 4 minutes.

The stained slide was treated in purified water for 30 seconds before being submerged 4 times in acidic ethanol. The slide was then washed in running cold tap water for 5 minutes. Following the washing, the slide was dip in ethanol 50% (v/v) and ethanol 80% (v/v) for 30 seconds then in ethanol 95% (v/v) for at least 15 minutes. The smeared slide was then treated with eosin G-6 orange stain for 1 minute before being immersed in 3 solutions of ethanol 95% (v/v) for 30 seconds per solution. After being treated with ethanol, the slide was left for 1 minute in the polychromatic eosin azure 50 green stain. Afterwards the stained slide was immersed in 2 solutions of ethanol 95% (v/v) for 30 seconds in each solution and 2 solutions of ethanol 100% for 15 seconds, respectively.

Microscopic morphology evaluation was done at a magnification of 1000-1250 X under oil immersion using a high quality 100x non-phase contrast objective. Around 200 spermatozoa were counted from each smear with a minimum of four different areas evaluated on each slide. Classification of morphological normal and abnormal spermatozoa was evaluated according to the strict (Tygerberg) criteria for evaluation of sperm morphology (Menkveld et al., 1990).

2.7.6 Leukocytes and Red Blood Cells Counts

The total white blood cell count (neutrophils, lymphocyte) was referred as leukocytes count and was calculated while evaluating the wet preparation and the complete blood count was obtained by a hematology analyzer (Coulter Gen-S Hematology

Analyzer; Beckman Coulter Corp, Hialeah, United States; www.beckmancoulter.com)

2.7.7 Epithelial Cells Determination

Debris, such as epithelial cells, is commonly found in small quantities in semen samples. The determination of epithelial cells was done on the wet preparation of semen samples. The polygonal shape epithelial cells were counted by microscopic field and classified accordingly. If no epithelial cell was observed, then a 0 value was indicated on the report. A count between 5 and 15 epithelial cells per microscopic field was indicated by 1+, while a count between 16 and 25 epithelial cells per microscopic field was indicated as 2+.

2.7.8 Sperm Viability

Sperm vitality was done to evaluate the percentage of live spermatozoa. Sperm vitality was performed using the eosin-nigrosin vital screen kit (FertiPro, FP12VI02, Beernem, Belgium, www.fertipro.com), following the manufacturer's guidelines. In this technique, dead sperm cells will be stained by the eosin dye, turning them a dark pink. The nigrosin stain produces a dark background against which dead cells and intact cells can clearly be seen (Moskovtsev and Librach, 2013). The eosin-nigrosin kit has two solutions: 0.67% eosin Y (red solution) and 10% nigrosin (black solution). Sperm vitality test was done by leaving two drops of eosin Y solution in 50 µL of semen for 30 seconds, followed by 3 drops of nigrosin for 30 seconds. One drop of the semen mixture was transferred to a microscope slide and a smear was made. A minimum of 200 spermatozoa were evaluated under a 100-times (100x) oil immersion bright field microscope**.** Unstained (live) spermatozoa will be white in colour, while stained (dead sperm) will show a pink or red coloration.

2.7.9 Sperm DNA Fragmentation

The DNA fragmentation results in this cohort study were obtained from Hamad Medical Center. The in vitro Halosperm® G2 (Halotech DNA SL, Spain) assay was used to performed the DNA fragmentation test. The test was done according to the

manufacturer's recommendations (www.halotechdna.com). The Halosperm® G2 kit contains an agarose cell support (ACS), super-coated slides (SCS), 10 units of Eppendorf tubes, a denaturant agent, a lysis solution, a float and two staining solutions (A and B). This methodology consists of immersing unfixed spermatozoa in an agarose microgel, then using an acid solution for DNA denaturation in those sperm with fragmented DNA. Then a lysis solution was used to remove nuclear proteins. Minimal or no dispersion nucleoids halos was see in sperm with fragmented, and nucleoids with large halos of spreading DNA were observed in sperm with less DNA denaturation (Figure 2.3).

The ACS was immersed in a 90-100°C water bath for 5 minutes. Subsequently, 50 µL of the ACS wee incubated at 37°C for 5 minutes using an Eppendorf tube. Then, 25 µL of semen were added to the incubated 50 µL ACS followed by the transfer of 8 µL of the cell suspension onto the Halosperm-coated slide. The slide was then left at a horizontal position at 3°C for 5 minutes for the fixation of the spermatozoa. The denaturation acid solution (solution A) was applied to the slide and then incubated for 7 minutes. After denaturation, the slide was treated with the lysis solution (solution B) and then incubated for 20 minutes. Following the lysis solution, the slide was washed for 5 minutes with distilled water and dehydrated in 70% and 100% ethanol for 2 minutes. The slide was allowed to dry. Once dried, solutions C and D were applied to the slide for 7 minutes, respectively. To finish, the slide was visualized under a bright field microscope (Axioskop 40; Carl Zeiss, Göttingen, Germany) with a minimum of 300 spermatozoa being evaluated.

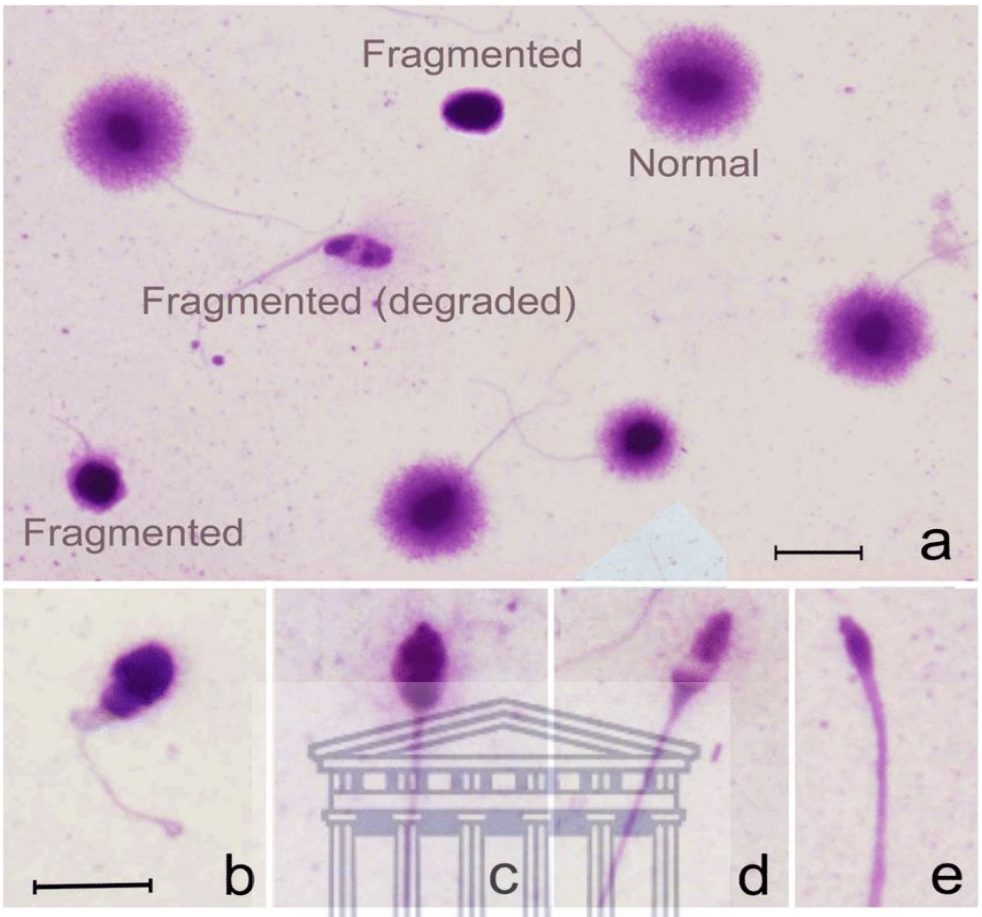

Figure 2.3: Graphical depiction of spermatozoa after processing using Halosperm® G2 stain (a: Morphology of Giemsa stained spermatozoa following the sperm chromatin dispersion test (Halosperm G2) resulting in the differentiation of three main sperm nuclear morphotypes: normal sperm free of sperm DNA fragmentation; fragmented sperm displaying small or absence of haloes; fragmented (degraded) sperm showing varying size of the faintly stained nuclear core (b–e). (Scale bar = 25 µm) (Martínez et al. 2018)

2.7.10 Oxidation-Reduction Potential (ORP)

Oxidation-reduction potential was measured using the galvanostat-based technology MiOXSYS system and performed according to the manufacturer's guidelines (AYTU BioScience, www.mioxsys.com, Englewood, USA). The calibrated MiOXSYS device was subjected to a monthly quality control testing using external control solutions. MiOXSYS External Control Solutions were supplied separately (cat# 100279) and new lots were identified for use. These solutions contained known sORP values and

were used to confirm that the MiOXSYS sensor and MiOXSYS analyser were functioning properly together. Two control solutions were provided by the manufacturer, the low control, and the high control. To run the controls, a volume of 30 µL of each control were transferred into a MiOXSYS sensor using aerosol resistant micropipette tips and processed by the MiOXSYS analyser.

After performing the controls, each semen sample was evaluated. The evaluation of the semen sample consisted of fully inserting an unsealed individual MiOXSYS sensor into the MiOXSYS analyser, an indication as *"Waiting for sample*" appeared on the display screen and a 2-minute sample detection countdown timer begun. A volume of 30 µl of the sample was immediately transferred into the sensor using an aerosol resistant micropipette tip. The semen sample was then processed by the machine bbefore the sORP results in mV were provided by the MiOXSYS device. The normed sORP was calculated by dividing the machine reading by the sperm concentration, the following formula was used: sORP (mV)/sperm count ($x10^6$/mL)

2.8 Statistical Analyses

Statistical analysis was performed using the MedCalc® statistical software version 19.5 (MedCalc Software Ltd, Ostend, Belgium; https:www.medcalc.org; 2020). Descriptive statistics for variables were presented as sample size, percentage of sample size, mean ± SD, median (IQR) and range for each variable. The Chi-Square test was used to determine the distribution of all the data sets. Based on the distribution of data, non-parametric statistical analyses were applied. The Mann-Whitney Test was used to evaluate the statistical differences between groups. Correlations were determined using the non-parametric Spearman Rank correlation expressed as r^2. The non-parametric Kruskal-Wallis test combined with the Jonckheere-Terpstra trend test were used to evaluate the changes in semen parameters over years. For all statistical tests, a P-value of < 0.05 was considered statistically significant.

Chapter 3: Results

3.1 Semen Analysis Reports

A total of 74,811 semen analysis reports were retrospectively collected from andrology laboratories in South Africa and Qatar. These included 37,561 reports from Ampath, 24,967 reports from Lancet, 8,283 reports from Androcryos and 4,000 from Hamad Medical Center, Doha, Qatar. Some laboratories did not provide full complete data for all variables. This has led to missing data for the final analysis. To comply with the inclusion and exclusion criteria, 2,045 post-vasectomy reports from Ampath, 833 patients report with a requisition number but no data from Androcryos and 1,168 duplicated patients reports from Hamad Medical Center were removed from the data analysis. Therefore, a total of 70,765 semen analysis reports were verified and included for statistical analysis, including 35,516 patient reports from Ampath, 24,967 patient reports from Lancet, 7,450 patient reports from Androcryos and 2,832 patient reports from Hamad Medical Center. This is summarised in Figure 3.1.

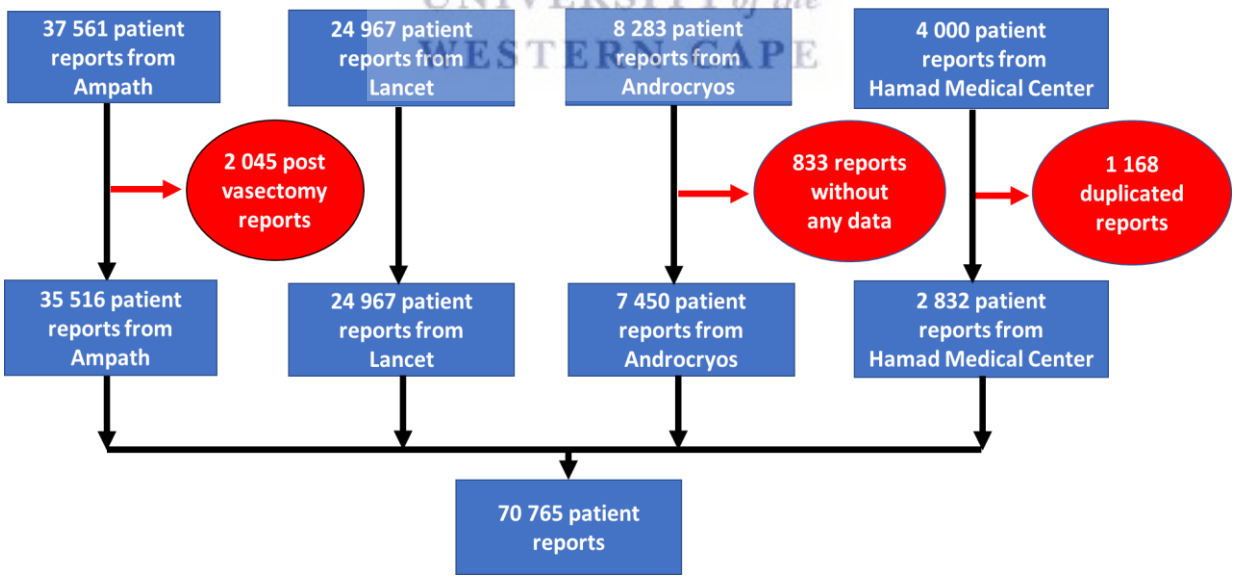

Figure 3.1: Flow diagram of the inclusion and exclusion of patient reports.

Confidential information, such as the patient's name, identity number and requisition number, were removed from the data set. The Chi-Square test for normality shows that all parameters are not normally distributed and are therefore reported and analysed using non-parametric statistical tests based on the median and inter-quartile range (IQR).

Figure 3.2 (A) illustrates the frequency of semen analyses per year at Androcryos, Ampath and Lancet Andrology laboratories. Data collected from Hamad Medical Centre did not indicate semen collection date. The figure shows a constant increase in the number of semen analyses from 2005 (n = 549) to 2014 (n = 7,065), followed by a constant decrease in the number of semen reports from 2014 to 2019 (n = 4,920). Figure 3.2 (B) illustrates a monthly decline in the number of semen analyses from steep decline from November to December based on annual averages of semen analyses per month.

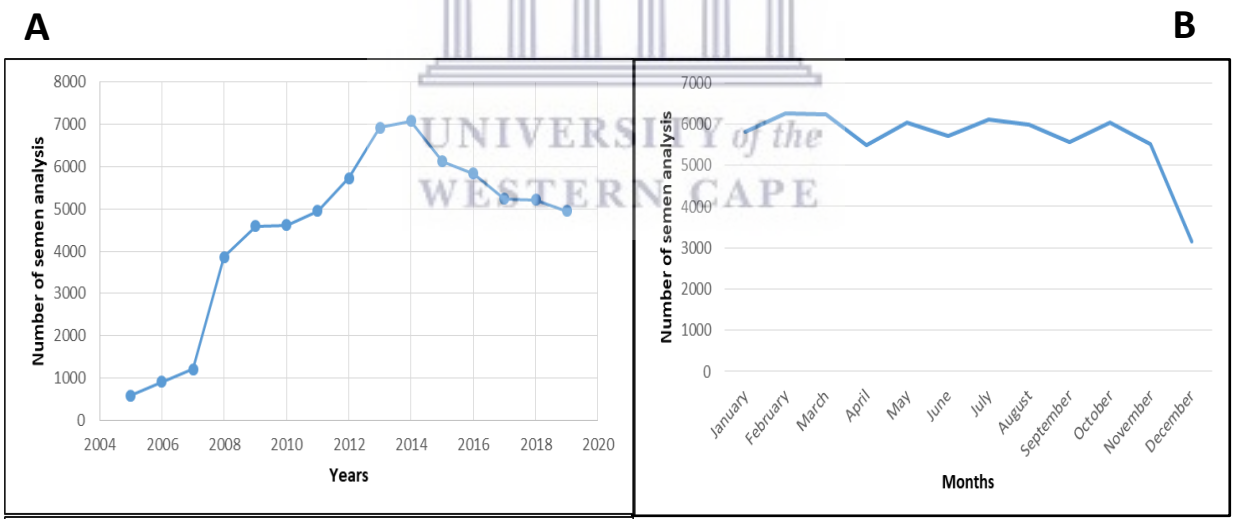

Figure 3.2: Illustration of the number of semen analyses (A) per year and (B) per month in Ampath, Androcryos and Lancet Andrology Laboratories in South Africa.

3.2 Descriptive Statistics of Semen Parameters and Hormones Africa and the Middle East

The descriptive statistics for the cohort study are presented in Table 3.1. The median (IQR) age of the full cohort is 38 (34 - 43) years. The highest number of semen analysis reports is obtained from patients aged between 35 and 40 years (29.7%) while the lowest is from patients aged less than 20 years (0.1%) (Figure 3.3).

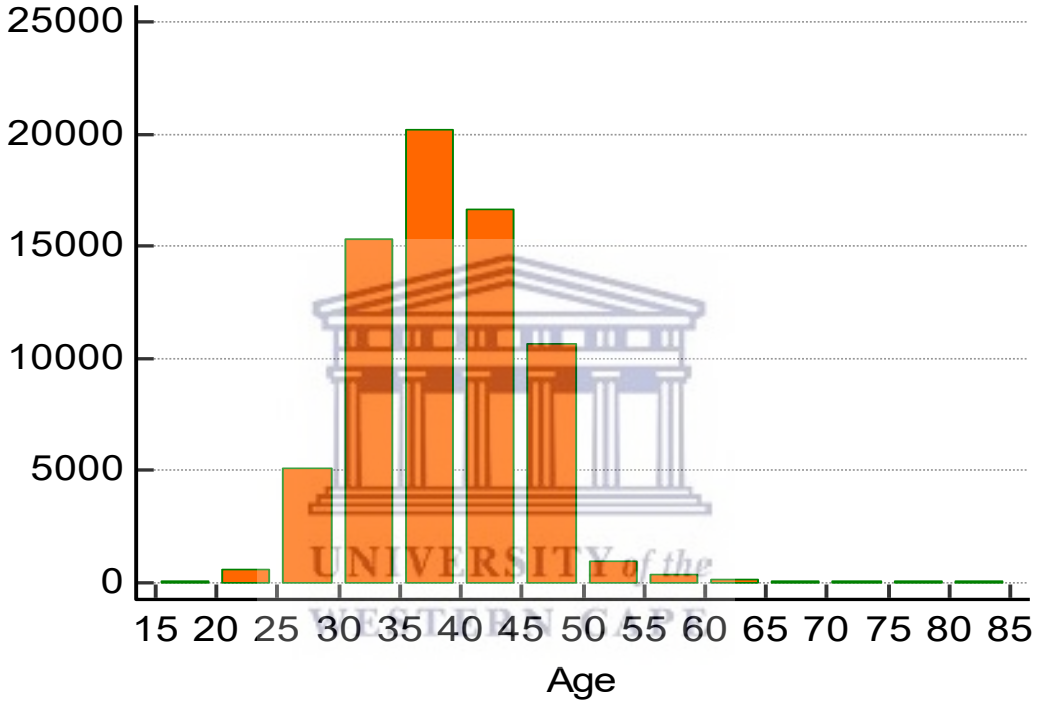

Figure 3.3: Frequency of semen analysis reports collected per age group.

3.2.1 Descriptive Statistics of Semen Parameters

For semen pH parameters only, data indicating pH < 5 and >10 were excluded (n = 22). The median (IQR) for semen pH is 7.80 (7.5 - 8.0). The median (IQR) for semen volume is 2.80 (2 - 4) mL. The median (IQR) for sperm concentration and total sperm count is 41 (15 - 81) x 10^6/mL and 104 (35 - 226) x 10^6, respectively. The median (IQR) for progressive motility and total progressively motile count is 32 (15 - 45)% and 29.8 (4.8 - 81.9) x 10^6, respectively. The median (IQR) for the percentage of sperm with normal morphology is 5 (3 - 9)%, while the total normal sperm count is 6.07 (1.5 - 18)%. Other sperm abnormalities such as small, elongated and duplicated

spermatozoa are 2 (1 - 5)%, 2 (1 - 6)% and 1 (0 - 2)%, respectively. The median (IQR) of the percentage of viable spermatozoa is 49 (33 - 60)% and the median (IQR) percentage of spermatozoa with DNA damage is 25.00 (20 - 70)%. The median (IQR) for leukocytes cells count and red blood cell count is 0 (15 - 40) x 10^6/mL and 0.60 (0.1 - 1.3) x 10^6/mL, respectively. The median (IQR) results for ORP and normed ORP are 50.9 (37.2 - 68.7) mV and 1.82 (1 - 4.2) mV/10^6 sperm/mL, respectively.

3.2.2 Reproductive Hormones

The median (IQR) for luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone is 3.7 (2.7 - 5.2) IU/L, 3.3 (2.1 - 5.8) IU/L and 16.4 (12.5 - 21.7) nmol/L, respectively, while the median (IQR) level of estradiol and prolactin is 97 (72 - 126) pmol/L and 195.4 (144.3 - 268.6) mIU/L, respectively.

Table 3.1: Descriptive statistics of age, semen parameters and reproductive hormones evaluated Africa and the Middle East.

Variable		Unit	Total Sample (n)	Total Sample (%)	Mean ± SD	Median	IQR	Range	5th	95th	Distribution
Age		years	70,142	99.1	38.2 ± 6.4	38	34 - 43	15 – 81	28	48	Not normal
Semen pH		pH	62,930	88.9	7.8 ± 0.4	7.8	7.5 - 8	5 – 10	7.2	8.5	Not normal
Semen Volume		mL	58,976	83.3	2.9 ± 1.6	2.8	2 - 4	0.1 – 20	1	6	Not normal
Sperm Concentration		x 10^6/ml	65,761	92.9	58.5 ± 62.5	41	15 - 81	0 – 896	0.68	180	Not normal
Total sperm count		x 10^6	58,613	82.8	165.9 ± 196.3	104	35 - 226.8	0 – 3900	0.9	539.5	Not normal
Total normal sperm count		%	24,067	34.0	15.2 ± 24.8	6.1	1.5 - 18	0 – 398	0.09	61.7	Not normal
Progressive motility		%	10,808	15.2	30.0 ± 18.6	32	15 - 45	0 – 100	0	57	Not normal
Total progressively motile count		x 10^6	10,808	15.2	60.4 ± 85.0	29.8	4.8 - 81.9	0 – 1323	0	226.4	Not normal
Sperm Morphology	**Normal**	%	24,133	34.1	6.8 ± 5.1	5	3 - 9	0 – 100	1	16	Not normal
	Small	%	41,682	58.9	4.5 ± 6.5	2	1 - 5	0 – 90	0	18	Not normal
	Elongated	%	41,678	58.8	4.4 ± 6.5	2	1 - 6	0 – 96	0	16	Not normal
	Duplication	%	37,751	53.3	7.9 ± 21.4	1	0 - 2	0 – 90	0	74	Not normal
Viability *		%	667	0.9	46.3 ± 19.1	49	33 - 60	0 – 96	10	75	Not normal
SDF *		%	838	1.2	30.7 ± 20.0	25	20 - 70	0 – 100	10	75	Not normal
Leukocyte concentration		x 10^6/mL	20,420	28.8	0.24 ± 1.38	0.0	15 - 40	0 – 93	0	1	Not normal
Red blood cell concentration		x 10^6/mL	22,939	32.4	1.16 ± 2.48	0.6	0.1 - 1.3	0 – 115	0	4	Not normal
sORP		mV	2,986	4.2	56.8 ± 37.4	50.9	37.2 - 68.7	-65.3 – 666.2	18.5	108	Not normal
Normed sORP		mV/10^6 sperm/mL	2,908	4.1	5.0 ± 10.3	1.82	1.0 - 4.2	-6.3 – 222.1	0.44	20.77	Not normal
Estradiol *		pmol/L	1,031	1.4	105.1 ± 50.7	97	72 - 126	1.7 – 408	1	2	Not normal
LH *		IU/L	1,130	1.6	4.6 ± 9.2	3.7	2.7 - 5.2	0.5 – 294	1.6	9	Not normal
FSH *		IU/L	1,130	1.6	4.9 ± 8.7	3.3	2.1 - 5.8	0.08 – 186.7	1.2	12.4	Not normal
Testosterone *		nmol/L	1,130	1.6	18.3 ± 9.2	16.4	12.5 - 21.7	2.4 – 121.3	8.2	33.6	Not normal
Prolactin *		mIU/L	1,115	1.6	236.1 ± 234.5	195.4	144.3 - 268	1.08 – 5832	94.9	485.6	Not normal

*** Results were only available from patients residing in the MENA region. FSH = Follicular Stimulating Hormone; LH = Luteinizing Hormone; SDF = Sperm DNA Fragmentation; sORP = Static Oxidation-Reduction Potential.**

3.2.3 Categorical Classification of Semen Analysis Parameters

Descriptive statistics for categorical parameters such as epithelial cells, varicocele and viscosity and leukocytes count are reported in Table 3.2. Data reporting the presence of epithelial cells in semen (n = 2,909) shows that 86.4% (n = 2,514) of the patient reports indicate that no epithelial cells were found, 11.5% (n = 334) indicate a 1+ (5 - 15 epithelial cells per microscopic field) epithelial cells and 2.1% (n = 61) indicate a 2+ (16 - 25 epithelial cells per microscopic field) classification. A total of 1,858 patients reports indicate varicocele results, of which 79.9% (n = 1,486) indicate no varicocele in patients, 15.0% (n = 280) reports highlight unilateral varicocele and 4.9% (n = 92) show bilateral varicocele in patients. A total of 63,273 reports indicated semen viscosity, of which 87.7% (n = 55,445) had normal viscosity while 12.3% (n = 7,828) had abnormal viscosity. The leukocyte count was reported in 20,420 reports, with 89.9% (n= 18,371) of reports indicated a leukocyte count of less than 1 x 10^6/mL and 10.0% (n = 2,049) reporting leukocytospermia (leukocyte count of more than 1 x10^6/mL).

Descriptive statistics for semen volume, sperm concentration, sperm motility and morphology classifications are also reported in Table 3.2. The majority of patient reports have normal viscosity (87.7%) and normal semen volume (87.1%). Categorizations of sperm concentration show that 74.5% (n = 49,025) patients reports have a normal sperm concentration (≥15 x 10^6/mL and ≤250 x 10^6/mL), 20.3% (n = 13,371) of patients reports indicate oligozoospermia (sperm concentration < 15 x 10^6/mL and > 0 x 10^6/mL), 3.6% (n = 2,387) patients reports show azoospermia (sperm concentration = 0 x 10^6/mL) and 1.5% (n = 978) of patients reports show a polyzoospermic condition (sperm concentration > 250 x 10^6/mL). Furthermore, 49.2% (n = 5,320) highlight asthenozoospermia (motility < 32%), 74.3% (n = 17,931) of patients have normal sperm morphology (≥ 4% normal morphological forms), while 25.7% (n = 6,202) have teratozoospermia (sperm morphology < 4% normal morphological forms).

Table 3.2: Descriptive statistics for semen analysis parameters.

Variable (Total Number of Reports)	Parameter Reported	Number of Reports	Percentage of Reports
Epithelial cells (n = 2,909)	0	2,514	86.4%
	1+	334	11.5%
	2+	61	2.1%
Varicocele * (n = 1,858)	Absent	1,486	79.9%
	Unilateral	280	15.0%
	Bilateral	92	4.9%
Viscosity (n = 63,273)	Abnormal	7,828	12.3%
	Normal	55,445	87.7%
Leukocytes count (n = 20,420)	< 1 x 10^6/mL	18,371	89.9%
	> 1 x 10^6/mL	2,049	10.0%
Volume (n = 58,976)	Hypospermia	7,586	12.8%
	Normal	51,390	87.1%
Sperm Concentration (n = 65,761)	Azoospermia	2,387	3.6%
	Oligozoospermia	13,371	20.3%
	Normal	49,025	74.5%
	Polyzoospermia	978	1.5%
Progressive Motility (n = 10,808)	Asthenozoospermia	5,320	49.2%
	Normal	5,488	50.8%
Morphology (n = 24,133)	Teratozoospermia	6,202	25.7%
	Normal	17,931	74.3%

*** Results were only available from patients residing in the MENA region.**

3.2.4 Semen Parameters in Africa and the Middle East Based on WHO (2010) Criteria

Tables 3.3 and 3.4 report variations in semen characteristics according to sperm concentration classifications. Patients with normal sperm concentration are found to have significantly ($P < 0.001$) higher semen volume (median (IQR) = 2.8 (2-4) mL) than polyzoospermic patients (median (IQR) = 2 (1.2-2) mL). Patients displaying oligozoospermia have a significantly ($P < 0.001$) higher semen volume (median (IQR) = 2.9 (2-4) mL) than polyzoospermic patients (median (IQR) = 2 (1.2-2.9) mL). No significant difference in semen volume is observed between patients having normal sperm concentration and oligozoospermic patients. Polyzoospermic patients are found to have statistically higher ($P < 0.001$) progressive motility (median (IQR) = 44 (37-52)%), total progressively motile count (median (IQR) = 267.34 (155-390) x 10^6), normal sperm morphology (median (IQR) = 11 (6-16)%) and total normal sperm count (median (IQR) = 60.46 (30-105)%) than oligozoospermic patients and patients with normal sperm concentration.

Patients with oligozoospermia displayed the lowest progressive motility (median (IQR) = 15 (0-36)%), total progressively motile count (median (IQR) = 1.33 (0-6.3) x 10^6), normal morphology (median (IQR) = 3 (2-5)%) and total normal sperm count (median (IQR) = 0.44 (0.1-1)%). Furthermore, patients with oligozoospermia have a significantly ($P < 0.001$) greater number of spermatozoa with DNA damage (median (IQR) = 28 (20-48.2)%) and a significantly ($P < 0.001$) lower number of live spermatozoa (median (IQR) = 47 (33-59.7)%) in comparison to patients having a normal sperm concentration (median (IQR) = 22 (15-35)% and 50 (33-62)% for sperm viability, respectively). The oligozoospermic group exhibits also higher sORP (median (IQR) = 62.12 (36.3-67.4) mV) and normed sORP values (median (IQR) = 8.35 (4-16.5) mV/10^6 sperm/mL) than those patients with normal sperm concentration (median (IQR) = 50.90 (37.5-69) mV for the sORP evaluation and median (IQR) = 1.32 (0.8-2) mV/10^6 sperm/mL for the normed sORP result). No results for DNA fragmentation, sperm viability, sORP test and normed sORP evaluations are obtained for polyzoospermic patients.

Table 3.4 shows results for the frequency of varicocele and epithelial cells for azoospermic, oligozoospermic, polyzoospermic patients and patients with normal sperm concentration. No data for varicocele is reported for azoospermic and polyzoospermic groups. Even though unilateral varicocele is less prevalent in oligozoospermic group (14.73%) than in the normal sperm concentration group (15.62%), bilateral varicocele is more prevalent in oligozoospermic patients (5.24%) in comparison to patients with normal sperm concentration (5.15%). Furthermore, Table 3.4 shows that patients with azoospermia have the highest prevalence of 1+ (20%) and 2+ (10%) epithelial cells, while polyzoospermic have the lowest prevalence (6.6% for 1+ epithelial cells and 0% for 2+ epithelial cells).

Table 3.3: Comparison of normal and abnormal semen parameters profiles, with statistically significant values obtained using the Mann–Whitney test for independent values. Bolded P-values indicate statistical significance.

		Volume (mL)	Progressive motility (%)	Total progressively motile count (x 10^6)	Normal morphology (%)	Total normal sperm count (%)	DNA fragmentation (%)	Viability (%)	sORP (mV)	Normed sORP (mV/10^6sperm/mL)
Normal concentration (n = 49,025) A	**Mean ± SD**	3.02 ± 1.57	32.91 ± 17.24	73.75 ± 79.10	7.53 ± 4.97	17.83 ± 23.32	27.57 ± 18.32	47.71 ± 19.73	56.77 ± 37.37	1.67 ± 1.43
	Median (IQR)	2.80 (2-4)	35 (20-47)	48.38 (20.4-100)	6.00 (4-10)	9.40 (3.9-22)	22.00 (15-35)	50.00 (33-62)	50.90 (37.5-69.)	1.32 (0.8-2)
	n	42,575	7,882	7,882	18,288	18,245	566	224	1,986	1,994
Oligozoo-spermia (n = 13,371) B	**Mean ± SD**	3.05 ± 1.64	20.20 ± 19.02	4.70 ± 7.62	3.53 ± 2.82	0.86 ± 1.18	35.19 ± 20.90	44.98 ± 19.04	73.54 ± 27.62	13.46 ± 16.2
	Median (IQR)	2.90 (2-4)	15.00 (0-36)	1.33 (0-6.3)	3.00 (2-5)	0.44 (0.1-1.0)	28.00 (20-48.2)	47.00 (33-59.7)	62.12 (36.3-67)	8.35 (4-16.5)
	n	12,112	2,425	2,425	5,114	5,103	173	367	829	832
Polyzoo-spermia (n = 978) C	**Mean ± SD**	2.18 ± 1.28	43.43 ± 12.59	292.48 ± 199	11.34 ± 5.69	76.61 ± 63.02	-	-	-	-
	Median (IQR)	2.00 (1.2-2.9)	44.00 (37-52)	267.34 (155-390)	11.00 (6-16)	60.46 (30-105)	-	-	-	-
	n	890	200	200	464	464	-	-	-	-
P-value (A vs B)		0.135	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.05**	**< 0.001**	**< 0.001**
P-value (A vs C)		**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	-	-	-	-
P-value (B vs C)		**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	-	-	-	-

Table 3.4: Frequency of varicocele and epithelial cells according to sperm concentration categories.

	Varicocele			Epithelial cells		
	Absent	**Unilateral**	**Bilateral**	**0**	**1+**	**2+**
Normal concentration (n = 49 025)	79.21%	15.62%	5.15%	87.88%	10.52%	1.59%
Azoospermia (n = 2 387)	-	-	-	70%	20%	10%
Oligozoospermia (n = 13 371)	80.02%	14.73%	5.24%	83.15%	13.97%	2.87%
Polyzoospermia (n = 978)	-	-	0%	93.33%	6.66%	0%

Table 3.5 shows basic semen characteristics (volume, sperm concentration, progressive motility, normal morphology) and functional tests (normed sORP, sperm viability and DNA fragmentation) in normozoospermic, teratozoospermic, asthenozoospermic, oligozoospermic patients and patients with normal sperm concentration. Normozoospermic patients have a significantly ($P < 0.05$) higher sperm concentration (median (IQR) = 70 (41-122) x 10^6/mL), progressive motility (median (IQR) = 47.00 (42-53)%) and normal morphology (median (IQR) = 10 (7-15)%) compared to the other groups. Normozoospermic patients are also found to have the lowest percentage of spermatozoa with fragmented DNA (median (IQR) = 15.00 (10-17)%). Patients displaying normal sperm concentration (15 - 250 x 10^6/mL) have a significantly ($P < 0.001$) higher sperm concentration (median (IQR) = 57 (33-94) x 10^6/mL), progressive motility (median (IQR) = 35 (20-47)%), percentage of spermatozoa with normal morphology (Median (IQR) = 6 (4-10)%) and percentage of live spermatozoa (median (IQR) = 50.00 (33-62)%) compared to oligozoospermic, teratozoospermic and asthenozoospermic groups. In addition, patients having a normal sperm concentration have a significantly ($P < 0.001$) greater number of viable spermatozoa (median (IQR) = 50 (33-62)%) and significantly ($P < 0.001$) lower percentage of spermatozoa with DNA damage (median (IQR) = 22 (15-35)%) and normed sORP results (median (IQR) = 1.28 (0.8-2) mV/10^6sperm/mL) than the other sperm classification groups.

The lowest progressive motility (median (IQR) = 10 (0-34)%) and normal morphology (median (IQR) = 2 (1-3)%) are obtained in teratozoospermic patients which also have the highest percentage of spermatozoa with DNA fragmentation (median (IQR) = 30 (20-52)%). Asthenozoospermic patients have a significantly ($P < 0.05$) higher semen volume (median (IQR) = 3 (2-4) mL) than patients with normal sperm concentration (median (IQR) = 2.8 (2-4) mL) and patients with teratozoospermia (median (IQR) = 2.8 (2-4) mL). No significant differences in semen volume are observed between patients with normal sperm concentration and oligozoospermic patients, between patients with normal sperm concentration and teratozoospermic patients and between oligozoospermic and asthenozoospermic patients. Oligozoospermic patients reported significantly ($P <0.005$) higher normed sORP results (median (IQR) = 8.35 (4.4-16.5) mV/10^6sperm/mL) compared to patients having normal sperm concentration (median (IQR) = 1.32 (0.8-2) mV/10^6sperm/mL).

Table 3.5: Comparison of normal and abnormal semen parameters profiles, with statistically significant values obtained using the Mann–Whitney test for independent values. Bolded P-values indicate statistical significance.

		Volume (mL)	Concentration (x 10^6/mL)	Progressive motility (%)	Normal morphology (%)	Viability (%)	DNA fragmentation (%)	Normed sORP (mV/10^6sperm/mL)
Normal concentration (n = 49,025) A	**Mean ± SD Median (IQR) n**	3.02 ± 1.57 2.80 (2-4) 42,575	70.58 ± 48.63 57.00 (33-94) 49,025	33.08 ± 17.17 35.00 (20-47) 7,882	7.57 ± 4.98 6.00 (4-10) 18,288	48.33 ± 19.68 50.00 (33-62) 224	27.65 ± 18.38 22.00 (15-35) 566	1.59 ± 1.31 1.28 (0.8-2) 1,994
Oligozoospermia (n = 13,371) B	**Mean ± SD Median (IQR) n**	3.05 ± 1.64 2.90 (2-4) 12,112	6.50 ± 4.23 6.00 (2.8-10) 13,371	20.38 ± 19.07 16.00 (0-36) 2,425	3.55 ± 2.82 3.00 (4-10) 5,114	44.98 ± 19.04 47.00 (33.5-59.7) 367	35.19 ± 20.90 28.00 (20-48.2) 242	13.46 ± 16.26 8.35 (4.4-16.5) 832
Teratozoospermia (n = 6,202) C	**Mean ± SD Median (IQR) n**	2.98 ± 1.58 2.80 (2-4) 6,171	26.87 ± 34.71 16.00 (5.8-35) 6,199	18.06 ± 18.81 10.00 (0-34) 2,815	1.95 ± 1.25 2.00 (1-3) 6,202	45.60 ± 19.10 48.00 (32-60) 620	37.88 ± 22.47 30.00 (20-52) 435	7.77 ± 13.07 3.11 (1.4-8.4) 1,514
Asthenozoospermia (n = 5,320) D	**Mean ± SD Median (IQR) n**	3.09 ± 1.63 3.00 (2-4) 5,306	41.84 ± 48.11 28.00 (8-58) 5,270	13.88 ± 10.32 15.00 (4-23) 5,320	6.11 ± 5.77 4.00 (2-9) 5,434	46.27 ± 19.12 49.00 (33-60) 660	31.45 ± 20.51 25.00 (16-41) 734	5.51 ± 10.85 2.02 (1.09-4.79) 2,531
Normozoospermia (n=10,394) E	**Mean ± SD Median (IQR) n**	3.05 ± 1.55 2.90 (2-4) 3,584	93.19 ± 77.58 70.00 (41-122) 3,599	47.9 ± 47.72 47.00 (42-53) 3,599	11 ± 4.48 10.00 (7-15) 3,599	-	16.18 ± 11.00 15.00 (10-17) 11	1.79 ± 1.04 1.65 (1.12-2.09) 51
P-value (A vs B)		0.083	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
P-value (A vs C)		0.063	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
P-value (A vs D)		**0.048**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
P-value (A vs E)		**0.230**	**< 0.001**	**< 0.001**	**< 0.001**	**-**	**0.0001**	**< 0.001**
P-value (B vs C)		**0.036**	**< 0.001**	0.054	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
P-value (B vs D)		0.074	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
P-value (B vs E)		0.355	**< 0.001**	**< 0.001**	**< 0.001**	**-**	**0.0001**	**< 0.001**
P-value (C vs D)		**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
P-value (C vs E)		0.648	**< 0.001**	**< 0.001**	**< 0.001**	**-**	**0.0001**	**< 0.001**
P-value (D vs E)		0.732	**< 0.001**	**< 0.001**	**< 0.001**	**-**	**0.002**	0.0600

Descriptive statistics for serum hormone profiles amongst sperm categories classifications of azoospermia, oligozoospermia, normal sperm concentration, teratozoospermia and asthenozoospermia are reported in Table 3.6. Significantly lower ($P < 0.001$) estradiol concentrations are found in normozoospermic patients (median (IQR) = 72.00 (58.2-97.5) pmol/L) in comparison to patients with normal sperm concentration (median (IQR) = 97 (75-125) pmol/L), oligozoospermic (median (IQR) = 102.5 (73-133) pmol/L), teratozoospermic (median (IQR) = 97 (73-129) pmol/L) and asthenozoospermic patients (median (IQR) = 98.50 (73-128) pmol/L). No significant differences in the estradiol concentrations are observed between normal sperm concentration, oligozoospermia, teratozoospermia and asthenozoospermia groups. Significantly ($P < 0.05$) higher LH concentrations are found in patients displaying oligozoospermia (median (IQR) = 4.40 (3-5.9) IU/L) compared to azoospermic (median (IQR) = 4 (2.7-5.1) IU/L), teratozoospermic (median (IQR) = 3.70 (2.9-5.4) IU/L) and asthenozoospermic (median (IQR) = 3.80 (2.7-5.3) IU/L) patients. Between the oligozoospermic group and patients having normal sperm concentration, no differences in the LH concentrations are noted ($P = 0.125$).

The FSH results show that the oligozoospermic group has a significantly ($P < 0.001$) greater FSH concentration (median (IQR) = 4.70 (2.6-7.6) IU/L) than patients with normal sperm concentration (median (IQR) = 2.90 (1.9-4.6) IU/L), teratozoospermic (median (IQR) = 3.3 (2.1-5.9) IU/L) and asthenozoospermic patients (median (IQR) = 3.3 (2.1-5.9) IU/L). There are also no differences in the FSH concentration between the azoospermic and teratozoospermic groups ($P = 0.455$) and between the azoospermic and asthenozoospermic groups ($P = 0.909$). The testosterone levels are significantly ($P < 0.05$) higher in patients with oligozoospermia (median (IQR) = 17.16 (12.8-23.1) nmol/L) than in the group of patients with normal sperm concentration (median (IQR) = 16.20 (12.2-21) nmol/L) and asthenozoospermia (median (IQR) = 16.6 (12.5-22.1) nmol/L). Significant differences ($P < 0.05$) are also observed between patients having normal sperm concentration and teratozoospermic patients (median (IQR) = 16.42 (12.5-22.8) pmol/L) as well as asthenozoospermic patients. No significant results are obtained for the comparison of prolactin levels amongst the evaluated sperm classification groups.

Table 3.6: Comparison of normal and abnormal semen parameters profiles with reproductive hormones in the MENA region, with statistically significant values obtained using the Mann–Whitney test for independent values. Bolded P-values indicate statistical significance.

		Estradiol (pmol/L)	Luteinizing hormone (IU/L)	Follicle stimulating hormone (IU/L)	Testosterone (nmol/L)	Prolactin (mIU/L)
Normal sperm concentration (n = 49,025) A	**Mean ± SD Median (IQR) n**	103.89 ± 45.74 97.00 (73-125) 561	4.54 ± 11.66 3.50 (2.6-4.8) 631	4.50 ± 10.74 2.90 (1.9-4.6) 631	17.79 ± 8.96 16.20 (12.2-21.0) 635	236.80 ± 261.9 190.80 (142-266) 625
Azoospermia (n = 2,387) B	**Mean ± SD Median (IQR) n**	86.10 ± 32.62 82.00 (62-105) 94	4.09 ± 1.94 4.00 (2.7-5.1) 94	4.34 ± 3.06 3.30 (2.2-5.4)94	17.36 ± 6.27 15.77 (12.2-20.9) 94	224.70 ± 117.3 194.85 (160-258) 94
Oligozoospermia (n = 13,371) C	**Mean ± SD Median (IQR) n**	112.70 ± 60.97 102.50 (73-133) 336	4.95 ± 3.07 4.40 (3.0-5.9) 361	5.93 ± 4.50 4.70 (2.6-7.6) 361	19.47 ± 10.14 17.16 (12.8-23.1) 358	238.16 ± 201.6 206.40 (147-276) 353
Teratozoospermia (n = 6,202) D	**Mean ± SD Median (IQR) n**	105.09 ± 50.74 97.00 (73-129) 609	4.64 ± 9.18 3.70 (2.9-5.4) 652	4.94 ± 8.74 3.30 (2.3-6.4) 652	18.28 ± 9.19 16.42 (12.5-22.8) 646	236.08 ± 234.45 195.40 (148-275) 637
Asthenozoospermia (n = 5,320) E	**Mean ± SD Median (IQR) n**	107.49 ± 52.15 98.50 (73-128) 910	4.74 ± 9.73 3.80 (2.7-5.3) 1 001	5.09 ± 9.21 3.30 (2.1-5.9) 1 001	18.42 ± 9.49 16.60 (12.5-22.1) 999	239.32 ± 246.08 196.10 (144-271) 987
Normozoospermia (n=10,394) F	**Mean ± SD Median (IQR) n**	80.06 ± 52.15 72.00 (58.2-97.5) 15	3.92 ± 52.15 3.00 (2.60-5.55) 15	3.48 ± 52.15 2.70 (2.25-4.43) 15	14.10 ± 52.15 12.19 (10.26-14.48) 16	238.2 ± 97.16 210.8 (177.9-273.8) 15
P-value (A vs B)		**< 0.001**	**< 0.001**	**0.001**	0.886	0.345
P-value (A vs C)		0.062	0.125	**< 0.001**	**< 0.001**	0.641
P-value (A vs D)		0.129	0.623	**< 0.001**	**0.034**	0.960
P-value (A vs E)		0.094	0.325	**< 0.001**	**0.019**	0.852
P-value (A vs F)		**0.0144**	0.942	0.763	**0.020**	0.278
P-value (B vs C)		**< 0.001**	**0.010**	**0.001**	0.110	0.423
P-value (B vs D)		**< 0.001**	0.237	0.455	0.313	0.991
P-value (B vs E)		**< 0.001**	0.715	0.909	0.599	0.670
P-value (B vs F)		**0.324**	0.774	0.405	0.0091	0.473
P-value (C vs D)		0.139	**< 0.001**	**< 0.001**	0.208	0.722
P-value (C vs E)		0.470	**< 0.001**	**< 0.001**	**0.025**	0.316
P-value (C vs F)		**0.027**	0.263	0.052	**0.0051**	0.464
P-value (D vs E)		0.137	**< 0.001**	**< 0.001**	0.061	0.117
P-value (D vs F)		**0.012**	0.481	0.288	**0.008**	0.481
P-value (E vs F)		**0.015**	0.661	0.525	**0.010**	0.350

Table 3.7 shows that 6,524 (11.06%) patients would have been considered abnormal according to the WHO 1999 classification (WHO, 1999) recommendations, yet normal following the new 2010 guidelines (WHO, 2010) for semen cut-off values. A total of 2,858 (4.35%) patients who are classified as oligozoospermic according to the 1999 guidelines are now considered as having normal sperm concentration according to the WHO (2010). Approximately 63.30% of teratozoospermic patients (following the WHO, 1999) are now classified as having normal sperm morphology (following the WHO, 2010), and 171 (5.60%) patients changed their classification for sperm viability.

Table 3.7: Classification of normal and abnormal semen parameters according to WHO (1999) and WHO (2010) criteria.

Parameter	≥ WHO 1999	≥ WHO 2010	≥ WHO 2010 but < WHO 1999
Semen volume (mL) (n = 58,976)	44,866 (76.07%)	51,390 (87.13%)	6,524 (11.06%)
Sperm concentration (x 10^6/mL) (n = 65,761)	46,167 (70.20%)	49,025 (74.55%)	2,858 (4.35%)
Normal morphology (%) (n = 24,133)	2,658 (11%)	17,932 (74.30%)	15,277 (63.30%)
Viability (%) (n = 667)	46 (6.88%)	217 (32.48%)	171 (5.60%)

3.3 Correlations of semen parameters and hormones Africa and the Middle East

The correlations and statistical significance between reproductive hormones (LH, FSH, testosterone, estradiol and prolactin) and seminal parameters (sperm concentration, progressive motility, normal morphology, sperm DNA fragmentation, viability and normed sORP) are summarized in Table 3.8. Positively significant correlations are observed between LH and normed sORP (r^2 = 0.186, P < 0.0001), FSH and normed sORP (r^2 = 0.267, P < 0.0001), sperm concentration and progressive motility (r^2 = 0.357, P < 0.0001), sperm concentration and normal morphology (r^2 = 0.504, P < 0.0001), normed sORP and DNA fragmentation (r^2 = 0.224, P < 0.0001), sperm concentration and DNA fragmentation (r^2 = 0.192, P<0.0001), progressive motility and normal morphology (r^2 = 0.426, P < 0.0001), progressive motility and sperm viability (r^2 = 0.162, P < 0.0001) and between normal

morphology and sperm viability (r^2 = 0.402, P < 0.0001). Weaker positively significant correlations are reported between estradiol and normed sORP (r^2 = 0.079, P = 0.0151), testosterone concentration and normed sORP (r^2 = 0.095, P = 0.0021), prolactin and normed sORP (r^2= 0.079, P = 0.0112) and between sperm concentration and sperm viability (r^2 = 0.094, P = 0.014).

Significant negative correlations are observed between LH and normal morphology (r^2 = -0.120, P < 0.0001), estradiol and progressive motility (r^2= -0.108, P = 0.005), estradiol and normal morphology (r^2= -0.081, P = 0.0096), FSH and progressive motility (r^2= -0.113, P = 0.0002), FSH and normal morphology (r^2 = -0.128, P < 0.0001), testosterone and normal morphology (r^2 = -0.074, P = 0.0134), progressive motility and DNA fragmentation (r^2 = -0.311, P < 0.0001), normal morphology and DNA fragmentation (r^2 = -0.344, P < 0.0001), normed sORP and progressive motility (r^2 = -0.441, P < 0.0001), normed sORP and normal morphology (r^2= -0.504, P < 0.0001), normed sORP and sperm viability (r^2 = -0.162, P = 0.0001) and between sperm concentration and normed sORP (r^2 = -0.815, P < 0.0001)

There is no association between LH and progressive motility (r^2= -0.012, P = 0.670), LH and DNA fragmentation (r^2 = 0.023, P = 0.609), LH and sperm viability (r^2 = 0.061, P = 0.298), estradiol and DNA fragmentation (r^2 =-0.024, P = 0.6043), estradiol and sperm viability (r^2 = -0.089, P = 0.1394), FSH and DNA fragmentation (r^2 = 0.087, P = 0.0564), FSH and sperm viability (r^2 = -0.088, P = 0.1374), T concentration and progressive motility (r^2= -0.027, P = 0.3698), testosterone and DNA fragmentation (r^2= -0.032, P=0.4888), testosterone and sperm viability (r^2 = -0.017, P = 0.7710), prolactin and progressive motility (r^2 = -0.023, P = 0.4345), prolactin and normal morphology (r^2= -0.034, P = 0.2556), prolactin and DNA fragmentation (r^2 = 0.049, P = 0.2864) and between prolactin and sperm viability (r^2 = 0.036, P = 0.5458).

Table 3.8: Correlation between semen characteristics and reproductive hormones. Bolded P-values indicate statistical significance.

		Progressive motility (%)	Normal morphology (%)	DNA fragmentation (%)	Viability (%)	Normed sORP (mV/10^{6}sperm/mL)
Luteinizing hormone (IU/L)	r^2 P-value n	-0.012 0.670 1,127	**-0.120** **< 0.0001** **1,130**	0.023 0.609 480	-0.061 0.298 290	0.186 < 0.0001 1,034
Estradiol (pmol/L)	r^2 P-value n	**-0.108** **0.005** **1,028**	**-0.081** **0.0096** **1,031**	-0.024 0.6043 463	-0.089 0.1394 275	**0.079** **0.0151** **935**
Follicle stimulating hormone (IU/L)	r^2 P-value n	**-0.113** **0.0002** **1,127**	**-0.128** **< 0.0001** **1,130**	0.087 0.0564 482	-0.088 0.1374 289	**0.267** **< 0.0001** **1,034**
Testosterone (nmol/L)	r^2 P-value n	-0.027 0.3698 1,127	**-0.074** **0.0134** **1,130**	-0.032 0.4888 475	-0.017 0.7710 290	**0.095** **0.0021** **1,034**
Prolactin (mIU/L)	r^2 P-value n	-0.023 0.4345 112	-0.034 0.2556 1,115	0.049 0.2864 477	0.036 0.5458 285	**0.079** **0.0112** **1,019**
Concentration (x 10^6/mL)	r^2 P-value n	**0.357** **< 0.0001** **1,075**	**0.504** **< 0.0001** **24,121**	**0.192** **< 0.0001** **838**	**0.094** **0.014** **661**	**-0.815** **< 0.0001** **2,908**
Progressive motility (%)	r^2 P-value n		**0.426** **< 0.0001** **10,396**	**-0.311** **< 0.0001** **837**	**0.162** **<0.0001** **666**	**-0.441** **< 0.0001** **2,896**
Normal morphology (%)	r^2 P-value n			**-0.344** **<0.0001** **836**	**0.402** **<0.0001** **667**	**-0.504** **< 0.0001** **2,901**
DNA fragmentation (%)	r^2 P-value n				**-0.697** **<0.0001** **185**	**0.224** **< 0.0001** **766**
Viability (%)	r^2 P-value n					**-0.162** **0.0001** **609**

Table 3.9 shows the correlations between LH, FSH, testosterone, estradiol and prolactin and sperm concentration. Significant positive correlations are demonstrated between LH and FSH (r^2 = 0.570, P < 0.001), estradiol and testosterone (r^2 = 0.347 P < 0.001) and between LH and testosterone (r= 0.254, P < 0.001). Weaker significant positive correlations are observed between FSH and testosterone (r^2 = 0.113, P < 0.001), estradiol and LH (r^2 = 0.110, P < 0.001) and between prolactin and LH (r^2 = 0.100, P<0.001). Negative significant correlations are indicated between FSH and sperm concentration (r^2 = -0.088, P < 0.0029), sperm concentration and testosterone (r^2 = -0.100, P < 0.0007) and between prolactin and estradiol (r^2 = 0.064

P < 0.043). No significant correlations are reported between FSH and estradiol (r^2 = 0.033, P = 0.210), FSH and prolactin (r = -0.003, P=0.913), testosterone and prolactin (r^2 = 0.037, P = 0.224) and between sperm concentration and estradiol (r^2 = -0.056 P = 0.068).

Table 3.9: Correlation between reproductive hormones and sperm concentration. Bolded P-values indicate statistical significance.

		Estradiol (pmol/L)	Follicle stimulating hormone (IU/L)	Testosterone (nmol/L)	Prolactin (mIU/L)	Sperm concentration (x 10^6/mL)
Luteinizing hormone (IU/L)	r^2 P-value n	**0.115** **0.0002** **1,025**	**0.570** **< 0.0010** **1,125**	**0.254** **< 0.0010** **1,112**	**0.107** **0.0003** **1,107**	**-0.171** **< 0.0001** **1,128**
Estradiol (pmol/L)	r^2 P-value n		0.033 0.2100 1,025	**0.347** **< 0.0010** **1,019**	**0.064** **0.0431** **1,014**	0.000 0.9960 1,029
Follicle stimulating hormone (IU/L)	r^2 P-value n			**0.113** **< 0.001** **1,112**	-0.003 0.9130 1,106	**-0.249** **< 0.0001** **1,128**
Testosterone (nmol/L)	r^2 P-value n				0.037 0.2240 1,102	**-0.085** **0.0044** **1,128**
Prolactin (mIU/L)	r^2 P-value n					**-0.075** **0.0119** **1,113**

3.4 The Impact of Age on Semen Quality and Reproductive Hormones in Africa and the Middle East

The results from the association between age and semen quality, functional sperm tests and reproductive hormones such as LH, FSH, testosterone, estradiol and prolactin, are summarized in Table 3.10 and Table 3.11. Patients are grouped according to the following age intervals: 16-20 years (n = 96); 21-30 years (n = 8,470); 31-40 years (n = 36,900); 41–50 years (n = 22,154); 51-60 years (n = 2,359); and more than 60 years (n = 163). The oldest age group (more than 60 years) has a significantly (P < 0.01) lower semen volume (median (IQR) = 1.50 (1-2.8) mL) than the other age groups. The same age group (more than 60 years) is also indicated to have the lowest sperm concentration (median (IQR) = 26 (4.9-74) x 10^6/mL), total sperm count (median (IQR) = 37.50 (27-210) x 10^6), progressive motility (median (IQR) = 15.5 (0-30)%), total progressively motile count (median (IQR) = 4.41 (3.1-21) x 10^6), total normal sperm count (median (IQR) = 2.45 (0.31-9.7)%), and percentage of spermatozoa with normal morphology (median (IQR) = 4 (2-7)%). Sperm concentration starts declining after the age of 50 years. Significantly greater (P <

0.05) total progressively motile count is obtained in patients aged between 21 and 30 years (median (IQR) = 35.28 (7.9-87.9) x 10^6). The same age group has a significantly ($P < 0.05$) higher semen volume (median (IQR) = 3 (2-4) mL), percentage of morphological normal spermatozoa (median (IQR) = 6 (4-10)%), total normal sperm count (median (IQR) = 7.5 (1.9-21.4)%), and percentage of small spermatozoa (median (IQR) = 6 (2-13)%) compared to older age groups. Results summarized in Table 3.11 show a constant increase in the number of spermatozoa with damaged DNA and a constant decrease in the number of viable spermatozoa with age from 21 years old. Patients under 30 years old have a significantly ($P < 0.05$) lower number of spermatozoa with DNA fragmented (median (IQR) = 22 (15-34)%) than patients older than 41 years old, and significantly higher number of viable spermatozoa (median (IQR) = 50 (38.5-64.5)%) compared to patients aged between 41-60 years old. No significant difference in normed sORP is noted.

Results obtained from the comparison of reproductive hormones amongst age groups are summarized in Table 3.12. The lowest concentrations of estradiol and LH are found in the oldest age group (median (IQR) = 82 (49.7-102.7) pmol/L and 3.45 (3-5.4) IU/L). There is a decrease in FSH according to age groups. Significant ($P < 0.01$) increases in FSH concentrations are observed in patients older than 31 and younger than 60. Indeed, patients aged less than 31 years old have significantly ($P < 0.01$) lower FSH concentrations (median (IQR) = 2.20 (1.2-2.3) IU/L for patients aged between 16 to 20 years and 2.70 (1.7-4.6) IU/L for patients aged between 21 and 30 years old) compared to the 31-40 years (median (IQR) = 3.20 (2.1-5.8) IU/L), 41-50 years (median (IQR) = 3.90 (2.6-7.2) IU/L) and 51-60 years (median (IQR) = 4.43 (2.7-6.7) IU/L) age groups. An age-group related decreases in median (IQR) serum testosterone and prolactin are observed from patients aged between 16 years old and 60 years old. Furthermore, the youngest age group (16-20 years) has a significantly higher ($P < 0.05$) testosterone concentration (median (IQR) = 21.50 (19.4-24.5) nmol/L) in comparison to the oldest age group (> 60 years old) which has a median (IQR) testosterone level of 21.21 (12.6-25.4) nmol/L). Patients aged between 41-50 years have a significantly ($P < 0.05$) lower prolactin concentration (median (IQR) = 180.20 (137.7-247.7) mIU/L) than the younger age groups.

Table 3.10: Basic semen parameters by age decade Africa and the Middle East, with statistically significant values obtained using the Mann–Whitney test for independent values. Bolded P-values indicate statistical significance.

Age groups		Volume (mL)	Concentration (x 10^6/mL)	Total sperm count (x 10^6)	Progressive motility (%)	Total progressively motile count (x 10^6)	Total normal sperm count (%)	Sperm morphology (%)		
								Normal	Small	Elongated
16-20 years (n=96) A	**Mean ± SD Median (IQR) n**	2.74 ± 1.70 2.60 (1.5-3.3) 65	40.89 ± 45.00 28.00 (8-57.5) 65	111.06 ± 122.77 72.50 15.7-157.3) 65	25.32 ± 16.96 25.00 (14.2-35.7) 31	35.06 ± 56.90 18.00 (3.5-38.4) 31	10.82 ± 15.3 4.95 (1.2-15.8) 50	7.08 ± 5.47 5.00 (3-10) 50	7.17 ± 7.86 4.00 (0-13.2) 17	10.64 ± 21.72 2.00 (0.7-9) 17
21-30 years (n=8,470) B	**Mean ± SD Median (IQR) n**	3.17 ± 1.60 3.00 (2-4) 7,445	57.07 ± 61.40 40 (15-78) 7,423	173.16 ± 200.09 112 (37.5-240) 7,401	30.76 ± 18.23 32.00 (16-46) 2,525	64.17 ± 87.77 35.28 (7.9-87.9) 2,525	17.22 ± 26.3 7.50 (1.9-21.4) 5,187	7.28 ± 5.61 6.00 (4-10) 5,194	8.86 ± 9.33 6.00 (2-13) 3,284	4.37 ± 6.95 2.00 (0-6) 3,284
31-40 years (n=36,900) C	**Mean ± SD Median (IQR) n**	3.06 ± 1.60 2.90 (2-4) 32,587	58.59 ± 62.48 41.00 (15.4-80) 34,526	169.68 ± 197.60 108 (37.5-232) 32,402	30.39 ± 18.24 32.00 (15-46) 6,066	62.83 ± 84.99 31.58 (5.9-85) 6,066	15.54 ± 24.8 6.30 (1.6-18.7) 13,490	6.83 ± 5.04 6.83 (3-10) 13,497	4.76 ± 6.73 2.00 (1-6) 21,564	4.59 ± 6.89 2.00 (1-6) 21,559
41-50 years (n=22,154) D	**Mean ± SD Median (IQR) n**	2.84 ± 1.55 2.50 (1.8-3.6) 17,806	59.27 ± 62.55) 41.00 (15-82) 22,642	159.55 ± 193.54 97.50 (31.5-216) 17,695	28.34 ± 19.22 30.00 (10-45) 1,780	51.52 ± 81.19 21.74 (2.27-66) 1,780	12.79 ± 23.2 4.93 (1.1-14) 4,449	6.20 ± 4.57 5.00 (3-8) 4,450	3.25 ± 4.78 2.00 (1-3) 16,603	4.31 ± 5.87 2.00 (1-3) 16,604
51-60 years (n=2 359) E	**Mean ± SD Median (IQR) n**	2.37 ± 1.55 2.00 (1.2-3.1) 843	54.69 ± 74.26 30.00 (6.5-74.5) 828	115.83 ± 166.47 55.00 (10.8-153.3) 827	24.29 ± 20.67 22.00 (1-43) 287	48.29 ± 88.12 13.06 (0.00-56) 287	10.67 ± 22 3.46 (0.7-11.4) 712	5.76 ± 4.78 5.00 (3-7) 713	12.30 ± 9.2 11.00 (5-16) 169	4.01 ± 5.79 2.00 (0-5) 169
> 60 years (n=163) F	**Mean ± SD Median (IQR) n**	2.02 ± 1.44 1.50 (1-2.8) 128	51.29 ± 69.23 26.00 (4.9-74) 122	98.86 ± 168.56 37.50 (27-210) 122	17.97 ± 18.18 15.50 (0-30) 48	34.48 ± 74.42 4.41 (3.1-21) 48	8.74 ± 16.21 2.45 (0.31-9.7) 98	5.26 ± 3.97 4.00 (2-7) 98	15.95 ± 12 11.50 (4-16) 22	3.50 ± 5.44 2.00 (0-4) 22
P-value (A vs B)		**0.011**	**0.019**	**0.006**	**0.079**	**0.019**	0.094	0.480	0.337	0.550
P-value (A vs C)		0.055	**0.009**	**0.007**	0.098	**0.046**	0.287	0.864	0.430	0.870
P-value (A vs D)		0.459	0.072	**0.037**	0.341	0.440	0.985	0.556	0.185	0.880
P-value (A vs E)		0.052	0.579	0.425	0.721	0.725	0.208	0.182	0.013	0.513
P-value (A vs F)		**0.009**	0.998	0.051	0.057	0.109	0.066	0.104	0.010	0.516
P-value (B vs C)		**< 0.0001**	0.296	0.296	0.394	**0.047**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0001**
P-value (B vs D)		**< 0.0001**	**0.0025**	**< 0.0001**	**0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
P-value (B vs E)		**0.0014**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.7820
P-value (B vs F)		**< 0.0001**	**0.006**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.001**	0.7266
P-value (C vs D)		**< 0.0001**	0.1786	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
P-value (C vs E)		**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.1006
P-value (C vs F)		**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.3431
P-value (D vs E)		**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.0009**	**0.0051**	**< 0.0001**	**0.0018**	**< 0.0001**	**0.0113**
P-value (D vs F)		**< 0.0001**	**0.0016**	**< 0.0001**	**0.0002**	**0.0025**	**0.0024**	0.0268	**< 0.0001**	0.1683
P-value (E vs F)		**0.0051**	0.4910	**0.0482**	**0.0441**	0.0968	0.2477	0.3749	0.2299	0.8512

Table 3.11: Functional semen parameters by age decade Africa and the Middle East , with statistically significant values obtained using the Mann–Whitney test for independent values. Bolded P-values indicate statistical significance.

Age groups		DNA Fragmentation (%)	Viability (%)	sORP (mV)	Normed sORP (mV/10^6 sperm/mL)
16-20 years (n = 96) A	**Mean ± SD Median (IQR) n**	13.50 ± 2.12 13.50 (12-15) 2	51.50 ± 19.09 51.50 (38-65) 2	45.20 ± 16.32 42.60 (34.7-56) 19	2.66 ± 2.90 1.39 (0.9-4.1) 18
21-30 years (n = 8,470) B	**Mean ± SD Median (IQR) n**	27.21 ± 17.41 22.00 (15-34) 194	49.71 ± 18.57 50.00 (38.5-64.5) 131	54.41 ± 32.41 49.30 (36.4-66.9) 709	4.98 ± 9.16 1.77 (0.9-4.1) 696
31-40 years (n = 36,900) C	**Mean ± SD Median (IQR) n**	28.92 ± 19.08 23.00 (15-38) 443	47.41 ± 18.51 50.00 (35-60) 324	57.70 ± 37.35 52.35 (37.7-70) 1,526	5.16 ± 11.26 1.89 (1-4.3) 1,482
41-50 years (n = 22,154) D	**Mean ± SD Median (IQR) n**	36.29 ± 22.15 30.00 (20-50) 161	43.63 ± 19.90 47.00 (26.5-57.5) 151	57.29 ± 39.55 50.70 (37.1-68.8) 528	5.69 ± 10.01 1.92 (1-4.9) 504
51-60 years (n = 2,359) E	**Mean ± SD Median (IQR) n**	44.46 ± 22.99 42.00 (25-59) 30	38.14 ± 19.80 45.00 (19.5-53.5) 48	65.19 ± 58.21 53.75 (38.7-73) 112	3.88 ± 8.10 1.831-3.4) 113
> 60 years (n = 163) F	**Mean ± SD Median (IQR) n**	50.62 ± 25.70 55.00 (28-60) 8	44.18 ± 18.02 37.00 (15-54.5) 11	49.40 ± 51.42 37.00 (21-56) 22	3.85 ± 6.36 1.31 (1-2.5) 23
P-value (A vs B)		0.152	0.970	0.167	0.490
P-value (A vs C)		0.150	0.734	**0.074**	0.345
P-value (A vs D)		0.057	0.536	0.113	0.248
P-value (A vs E)		**0.035**	0.413	**0.039**	0.495
P-value (A vs F)		0.066	0.429	0.314	0.979
P-value (B vs C)		0.501	0.249	**0.030**	0.237
P-value (B vs D)		**< 0.0001**	**0.0106**	0.295	**0.077**
P-value (B vs E)		**< 0.0001**	**0.0013**	**0.048**	0.861
P-value (B vs F)		**0.0076**	0.3284	0.9824	0.412
P-value (C vs D)		**0.0001**	**0.0367**	0.4492	0.3330
P-value (C vs E)		**0.0001**	**0.0044**	0.2783	0.4285
P-value (C vs F)		**0.0123**	0.5253	0.6866	0.2541
P-value (D vs E)		**0.0477**	0.1277	0.1747	0.2208
P-value (D vs F)		0.1063	0.2843	0.8426	0.2048
P-value (E vs F)		0.4732	0.4078	0.4123	0.4734

Table 3.12: Reproductive hormones by age decade in MENA region, with statistically significant values obtained using the Mann–Whitney test for independent values. Bolded P-values indicate statistical significance.

Age groups		Estradiol (pmol/L)	Luteinizing hormone (IU/L)	Follicle stimulating hormone (IU/L)	Testosterone (nmol/L)	Prolactin (mIU/L)
16-20 years (n = 96) A	**Mean ± SD**	88.66 ± 29.73	4.57 ± 3.18	2.35 ± 1.71	20.87 ± 4.88	262.42 ± 73.37
	Median (IQR)	94.00 (62-115)	3.50 (2.62-5.7)	2.20 (1.2-2.3)	21.50 (19.4-24.5)	248.90 (218.5-281)
	n	32	35	35	34	25
21-30 years (n = 8,470) B	**Mean ± SD**	106.87 ± 53.44	4.62 ± 4.40	4.22 ± 8.68	19.27 ± 9.72	250.28 ± 231.64
	Median (IQR)	98.00 (71.75-126)	4.00 (2.9-5.3)	2.70 (1.7-4.6)	17.10 (12.8-22.8)	204.65 (149.7-282)
	n	642	649	649	637	609
31-40 years (n = 36,900) C	**Mean ± SD**	104.15 ± 50.19	4.72 ± 12.29	5.05 ± 10.14	18.06 ± 8.75	241.13 ± 274.60
	Median (IQR)	96.00 (72-125)	3.60 (2.6-5.1)	3.20 (2.1-5.8)	16.32 (12.5-21.6)	196.30 (146-267)
	n	812	873	873	864	731
41-50 years (n = 22,154) D	**Mean ± SD**	103.94 ± 46.14	4.41 ± 2.91	5.54 ± 4.69	17.88 ± 9.99	208.65 ± 115.26
	Median (IQR)	97.00 (73.7-126)	3.70 (2.7-5.1)	3.90 (2.6-7.2)	16.10 (12-20.9)	180.20 (137.7-247.7)
	n	617	709	709	683	565
51–60 years (n = 2,359) E	**Mean ± SD**	122.43 ± 69.20	4.83 ± 3.07	5.32 ± 4.50	15.86 ± 7.06	214.60 ± 119.35
	Median (IQR)	109.5 (79-132)	4.00 (2.5-6.4)	4.43 (2.7-6.7)	14.80 (11.7-17.2)	175.30 (132.5-292.5)
	n	308	329	329	312	209
> 60 years (n = 163) F	**Mean ± SD**	84.80 ± 44.74	4.37 ± 3.25	5.36 ± 3.61	20.94 ± 7.74	248.48 ± 116.04
	Median (IQR)	82.00 (49.7-102.7)	3.45 (3-5.4)	5.50 (2.7-6.1)	21.21 (12.6-25.4)	208.2 (176-269)
	n	12	65	65	62	41
P-value (A vs B)		0.4767	0.6906	0.1405	0.1952	0.1678
P-value (A vs C)		0.5643	1.000	**0.0305**	0.0906	0.0934
P-value (A vs D)		0.5693	0.8213	**0.0073**	0.0766	**0.0286**
P-value (A vs E)		0.2697	0.7727	**0.0112**	**0.0193**	0.0840
P-value (A vs F)		0.7150	1.000	0.0737	0.7751	0.3914
P-value (B vs C)		0.7139	0.0616	**0.0002**	0.0956	0.3914
P-value (B vs D)		0.8669	0.4915	**< 0.0001**	0.0740	**0.0131**
P-value (B vs E)		0.2309	0.6457	**0.0014**	**0.0164**	0.1945
P-value (B vs F)		0.2583	0.8225	0.1602	0.4225	0.6617
P-value (C vs D)		0.9232	0.3301	**0.0010**	0.6252	**0.0323**
P-value (C vs E)		0.1604	0.2626	0.0885	0.0728	0.3070
P-value (C vs F)		0.2770	0.9354	0.4118	0.2773	0.4868
P-value (D vs E)		0.1939	0.4861	0.9403	0.1309	0.9790
P-value (D vs F)		0.2761	0.9285	0.8624	0.2198	0.2429
P-value (E vs F)		0.1254	0.6486	0.7452	0.1329	0.2563

3.5 The influence of Abstinence Periods on Semen Quality in Africa and the Middle East

These results on the influence of different periods of abstinence on semen volume, sperm concentration, progressive motility, normal morphology, sperm DNA fragmentation and normed sORP, are summarized in Table 3.13.

For group comparisons, sexual abstinence period is divided into 3 groups: < 2 days (n = 29,743); 2-5 days (n = 19,729) and > 5 days (n = 428). Significantly (P < 0.01) higher semen volumes (median (IQR) = 3.00 (2-4) mL) and sperm concentrations (median (IQR) = 48.33 (15-95) x 10^6/mL) are found in patients having > 5 days of abstinence than in those having < 2 days abstinence (median (IQR) = 1.80 (1-2.6) mL and 32 (15-60) x 10^6/mL, respectively) and the group of patients having 2 to 5 days of abstinence (median (IQR) = 2.8 (2-3.8) mL and 40 (15-80) x 10^6/mL, respectively). Additionally, patients who have the longest abstinence days (> 5) have a significantly (P < 0.01) greater progressive motility (median (IQR) = 35 (18-46)%), normal morphology (median (IQR) = 6 (64-10)%) and lowest normed sORP (median (IQR) = 1.75 (0.9-3.9) mV/10^6 sperm/mL).

The Spearman's rank correlation results are summarised in Table 3.14. Positive significant (P < 0.0001) correlations are indicated for semen volume (r^2 = 0.084, n = 50 078), sperm concentration (r^2 = 0.07, n = 54 337), progressive motility (r^2 = 0.07, n = 10 702), normal morphology (r^2 = 0.05, n = 23 934) and DNA fragmentation test (r^2 = 0.14, n = 838). No significant correlation between duration of abstinence and normed sORP results is found.

Table 3.13: Comparison between semen parameters and sexual abstinence periods Africa and the Middle East, with statistically significant values obtained using the Mann–Whitney test for independent values. Bolded P-values indicate statistical significance.

		Volume (mL)	Concentration (x 10^6/mL)	Progressive motility (%)	Normal morphology (%)	DNA Fragmentation (%)	sORP (mV)	Normed sORP (mV/10^6 sperm/mL)
< 2 days (n = 29,743) A	**Mean ± SD**	2.02 ± 1.48	43.60 ± 41.99	13.14 ± 13.84	5.55 ± 8.67	23.42 ± 13.71	49.24 ± 26.07	4.82 ± 9.28
	Median (IQR)	1.80 (1-2.6)	32.00 (15-60)	10.00 (0-21)	4.00 (2-8)	22.00 (12.7-28.7)	44.55 (33.2-65.6)	2.00 (0.9-4.2)
	n	175	245	85	98	19	74	74
2 – 5 days (n = 19,729) B	**Mean ± SD**	2.96 ± 1.55	57.08 ± 60.80	29.35 ± 18.81	6.61 ± 4.87	29.54 ± 19.51	55.29 ± 37.57	5.11 ± 10.35
	Median (IQR)	2.80 (2-3.8)	40.00 (15-80)	30.00 (13-45)	5.00 (3-9)	25.00 (15-37)	50.00 (37-67.1)	1.88 (1-4.5)
	n	15,571	19,300	6,067	13,822	558	1,913	1,870
> 5 days (n = 428) C	**Mean ± SD**	3.28 ± 1.75	68.98 ± 75.92	31.85 ± 17.62	7.17 ± 5.06	36.30 ± 21.86	65.54 ± 37.65	1.75 ± 10.54
	Median (IQR)	3.00 (2-4)	48.33 (15-95)	35.00 (18-46)	6.00 (4-10)	30.00 (20-50)	59.10 (42.6-78.8)	1.75 (0.9-3.9)
	n	8,406	8,627	2,866	6,096	157	542	521
P-value (A vs B)		**< 0.0001**	**0.009**	**< 0.0001**	**< 0.0001**	0.227	**< 0.0001**	0.761
P-value (A vs C)		**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.001**	**0.013**	**< 0.0001**	0.802
P-value (B vs C)		**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.0001**	**< 0.0001**	0.951

Table 3.14 Correlation between semen parameters and sexual abstinence period. Bolded P-values indicate statistical significance.

	Volume (mL)	Sperm concentration (x 10^6/mL)	Progressive motility (%)	Normal morphology (%)	DNA Fragmentation (%)	sORP (mV)	Normed sORP (mV/10^6 sperm/mL)
n	50,078	54,337	10,702	23,934	838	2,931	2,853
Spearman's rank correlation (r^2)	0.084	0.070	0.070	0.050	0.140	0.097	0.003
Significant level (P)	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.870

3.6 Geographical Differences in Semen Quality in Africa and the Middle East

The results obtained from the investigation pertaining the relationship between geographical differences and semen characteristics are summarized in Table 3.15, Table 3.16 and Table 3.17. Table 3.15 summarizes semen parameters of patients residing in Southern African countries (n = 64,439), Eastern African countries (n = 2,069), Northern African countries (n = 660) and Middle East region (1,198). Patients residing in North Africa are found to have a significantly ($P < 0.05$) higher semen volume (median (IQR) = 3 (2-4.5) mL) than those obtained from the other groups investigated. Residents from Middle East countries have a significantly ($P < 0.05$) lower semen volume (median (IQR) = 2.5 (2-4) mL) than patients residing in Northern African countries (median (IQR) = 3.00 (2-4.5) mL), Eastern African (median (IQR) = 3 (2-4.5) mL) and Southern African countries (median (IQR) = 2.8 (2-4) mL).

Residents from countries located in Southern Africa have significantly greater sperm concentration (median (IQR) = 42 (68.00) x 10^6/mL), progressive motility (median (IQR) = 39 (35-49)%), total progressively motile count (median (IQR) = 47.79 (7.4-82) x 10^6) and total normal sperm count (median (IQR) = 7.14 (1.6-20.08)%) in comparison to the other regions. Furthermore, the sORP and normed sORP results are significantly ($P < 0.005$) lower in patients residing in Southern African countries (median (IQR) = 42.50 (38-57) mV and 1.14 (0.6-2.73) mV/10^6 sperm/mL, respectively) than in patients residing in North African countries (median (IQR) = 50.70 (28.7-60) mV and 1.84 (0.8-3.97) mV/10^6 sperm/mL, respectively) and patients residing in Middle East countries (49.50 (32-61) mV and 2.03 (0.3-3.85) 10^6 sperm/mL, respectively).

The results for the percentage of normal morphology indicate that patients residing in countries located in Eastern Africa have significantly ($P < 0.001$) higher percentage of spermatozoa with normal morphology (median (IQR) = 6 (4-13)%) than those residing in Southern African countries (median (IQR) = 6 (4-10)%), Northern African countries (median (IQR) = 3 (1-5)%) and Middle East countries (median (IQR) = 3 (1-5)%). Significantly ($P = 0.0092$) higher percentage of morphological elongated spermatozoa is seen in Southern Africa group of patients (median (IQR) = 2 (0-5)%) than in the Eastern Africa group (median (IQR) = 1 (0-3)%).

Table 3.15: Geographical differences in semen characteristics Africa and the Middle East, with statistically significant values obtained using the Mann–Whitney test for independent values. Bolded P-values indicate statistical significance.

		Volume (mL)	Concentration (x 10^6/mL)	Progressive motility (%)	Total progressively motile count (x 10^6)	Sperm morphology (%)		Total normal sperm count (%)	sORP (mV)	Normed sORP (mV/10^6 sperm/mL)
						Normal	Elongated			
Southern Africa (n = 64,439) A	**Mean ± SD** **Median (IQR)** **n**	2.99 ± 1.59 2.80 (2-4) 60,614	58.72 ± 63.78 42 (15-81) 63,891	36.6 ± 15.95 39 (35-49) 7,880	77.58 ± 92.80 47.79 (7.4-82) 7,880	7.04 ± 4.72 6.00 (4-10) 19,673	4.47 ± 6.53 2.00 (0-5) 41,359	16.61 ± 25.89 7.14 (1.6-20.08) 19,673	45.98 ± 23.89 42.50 (38-57) 2,857	2.13 ± 3.23 1.14 (0.6-2.73) 2,847
Eastern Africa (n = 2,069) B	**Mean ± SD** **Median (IQR)** **n**	3.01 ± 1.52 3.00 (2-4.5) 2,004	42.86 ± 51.65 23.00 (15-59) 1,951	19.70 ± 12.29 18.50 (0-34) 34	45.80 ± 66.70 17.00 (0-59.60) 34	7.40 ± 4.80 6.00 (4-13) 1,560	2.86 ± 5.21 1.00 (0-3) 31	15.49 ± 25.28 6.00 (1.2-19.31) 1,560	-	-
Northern Africa (n = 660) C	**Mean ± SD** **Median (IQR)** **n**	3.25 ± 1.71 3.00 (2-4.5) 956	33.66 ± 25.47 30.00 (15-63) 956	10.70 ± 11.18 10.00 (0-17) 956	15.41 ± 24.85 5.25 (0-21.00) 956	4.70 ± 7.61 3.00 (1-5) 956	-	6.64 ± 12.92 2.40 (1.1-6.1) 956	57.45 ± 42.40 50.70 (28.7-60) 658	5.52 ± 13.77 1.84 (0.8-3.97) 654
Middle East (n = 1,198) D	**Mean ± SD** **Median (IQR)** **n**	2.94 ± 1.66 2.50 (2-4) 1,664	29.18 ± 23.38 24.00 (14-47) 1,664	10.61 ± 10.79 10.00 (0-18) 1,662	12.40 ± 21.04 3.50 (0-15.75) 1,662	4.23 ± 5.45 3.00 (1-5) 1,664	-	4.83 ± 9.30 1.72 (1.0-3.58) 1,664	54.04 ± 33.07 49.50 (32-61) 1,198	5.51 ± 9.75 2.03 (0.3-3.85) 1,197
P-value (A vs B)		0.111	**< 0.0001**	**< 0.0001**	**0.0046**	**0.0006**	**0.0092**	**0.0006**	-	-
P-value (A vs C)		**0.0004**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	-	**< 0.0001**	**0.0008**	**0.0041**
P-value (A vs D)		**0.039**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	-	**< 0.0001**	**0.0049**	**0.0001**
P-value (B vs C)		**0.018**	**0.034**	**< 0.0001**	**0.0003**	**< 0.0001**	-	**< 0.0001**	-	-
P-value (B vs D)		**0.0086**	0.767	**< 0.0001**	**< 0.0001**	**< 0.0001**	-	**< 0.0001**	-	-
P-value (C vs D)		**< 0.0001**	**0.0003**	0.8990	0.1000	0.533	-	**0.0003**	0.1540	0.0560

In the current cohort study, the median (IQR) age for patients residing in Southern African countries was as followed: South Africa: 37 (31 – 49) years, Zimbabwe: 36 (29 – 42) years, Zambia: 46 (39 – 52) years, Mozambique: 41 (37 – 48), Lesotho: 39 (33 – 46) years and Swaziland: 39 (30 – 42) years. The results from the regional analysis of semen quality amongst patients residing in Southern African countries (South Africa (n = 60,732), Zimbabwe (n = 1,315), Zambia (n = 1,043), Mozambique (n = 703), Lesotho (n = 330) and Swaziland (n = 316) are summarized in Table 3.16.

Patients residing in Mozambique and Swaziland are found to have significantly ($P < 0.001$) higher semen pH (median (IQR) = 8.1 (7.9-8.3) and 8.1 (8-8.3)) than the other group evaluated, while patients residing in Lesotho have a significantly ($P < 0.001$) lower pH (median (IQR) = 7.5 (7.2-7.7) in the cohort. Significantly ($P < 0.001$) greater sperm concentration (median (IQR) = 49.5 (12-115) x 10^6/mL) and total sperm count (median (IQR) = 129.6 (27-323) x 10^6) are observed in patients residing in Mozambican compared to those residing in Zimbabwe, Zambia, Lesotho, and Swaziland patients. The lowest sperm concentration (median (IQR) = 19 (3.5-44) x 10^6/mL) and total sperm count (median (IQR) = 40.1 (7.3-106) x 10^6) are found in patients residing in Zambia, although some results are non-significant.

There is no data for progressive motility, morphological small, elongated and duplicated spermatozoa, for patients residing in Zambia, additionally, no data for progressive motility, normal morphology and red blood cell count is available for patients residing in Lesotho. Patients residing in South Africa and Mozambique have a significantly higher ($P < 0.01$) progressive motility (median (IQR) = 40 (26-49)% and 40 (27-49)% respectively), than those residing in Zambia (median (IQR) = 30.5 (20-45)%) and Swaziland (median (IQR) = 25 (20-35)%). Patients residing in South Africa are also found to have a significantly ($P < 0.01$) greater percentage of morphological normal spermatozoa (median (IQR) = 6 (4-10) %) than patients residing in Zimbabwe (median (IQR) = 4 (2-7)%), Zambia (median (IQR) = 5 (3-9)%) and Mozambique (median (IQR) = 4 (3-7)%). In addition, South African patients have a significantly higher number of elongated spermatozoa (median (IQR) = 2 (1-6)%) than those residing in Zambia (median (IQR) = 1 (0-5)%) and Lesotho (median (IQR) = 2 (0-5)%).

Semen profiles of patients residing in Zambia and Mozambique show a significantly ($P < 0.001$) higher number of morphological small spermatozoa (median (IQR) = 10 (5-20)% and 11.1 (5-12)% respectively) and duplicated spermatozoa (median (IQR) = 76 (68-80)% and 78 (70-81)% respectively) compared to those residing in South Africa (median (IQR) = 2 (1-5)% and 1 (0-2)%, respectively) and Lesotho (median (IQR) = 2 (0-2)% and 0 (0-2)%, respectively). Red blood cell count results indicate that patients residing in Zambia have a significantly ($P < 0.001$) higher concentration (median (IQR) = 0.8 (0-2) x 10^6/mL) compared to patients residing in South Africa (median (IQR) = 0.6 (0.2-1.4) x 10^6/mL), Mozambique (median (IQR) = 0.6 (0.4-1) x 10^6/mL) and Swaziland (median (IQR) = 0 (0-0.4) x 10^6/mL).

Table 3.16: Geographical differences in semen quality in Southern African countries, with statistically significant values obtained using the Mann–Whitney test for independent values. Bolded P-values indicate statistical significance.

Southern African nationalities	pH	Volume (mL)	Concentration (x 10^6/mL)	Total sperm count (x 10^6)	Progressive motility (%)	Sperm morphology (%)				Red blood cell count (x 10^6/mL)
						Normal	Small	Elongated	Duplicated	
South Africa (n = 60,732) A	7.7 ± 0.43 7.7 (7.5-8) 57,523	3.0 ± 1.6 2.8 (2-4) 50,629	61.0 ± 76.50 43 (16.9-84) 57,525	174 ± 200 112 (39-239) 50,381	36.8 ± 16 40.0 (26-49) 5,787	7.2 ± 4.8 6.0 (4-10) 17,072	4.5 ± 6.5 2.0 (1-5) 41,082	4.5 ± 1.4 2.0 (1-6) 41,078	7.4 ± 20.6 1.0 (0-2) 37,172	1.1 ± 2.28 0.6 (0.2-1.4) 18,454
Zimbabwe (n = 1,315) B	7.9 ± 0.27 7.9 (7.9-8.1) 1,250	2.7 ± 1.5 2.5 (1.7-3.6) 1,255	46.0 ± 5-65 28 (27-78.5) 1,221	113.2 ± 150.4 64.0 (10.8-156) 1,221	-	5.4 ± 3.6 4.0 (2-7) 1,022	-	-	-	0.6 ± 1.56 0.2 (0-0.6) 1,221
Zambia (n = 1,043) C	7.9 ± 0.4 8.0 (7.9-8.1) 982	2.5 ± 1.3 2.5 (1.5-3.1) 985	32.3 ± 44.88 19 (3.5-44) 968	82.7 ±131 40.1 (7.3-106) 968	32.0 ± 41 30.5 (20-45) 166	6.3 ± 4.3 5.0 (3-9) 806	13.9 ± 11 10.0 (5-20) 157	3.9 ± 6.8 1 (0-5) 157	73.3 ± 9.6 76.0 (68-80) 157	1.7 ± 4.25 0.8 (0-2) 885
Mozambique (n = 703) D	8.1 ± 0.31 8.1 (7.9-8.3) 569	3.0 ± 1.56 2.7 (2-4) 574	78.0 ± 90.27 49.5 (12-115) 567	226.9 ± 278 129.6 (27-323) 567	39.5 ± 15 40.0 (27-49) 23	5.1 ± 3.6 4.0 (3-7) 497	11.1 ± 8.0 11.1 (5-12) 23	4.2 ± 7.0 2.0 (0-5.7) 23	74.7 ± 8.9 78.0 (70-81) 23	1.1 ± 1.65 0.6 (0.4-1) 563
Lesotho (n = 330) E	7.5 ± 0.36 7.5 (7.2-7.7) 324	2.8 ± 1.64 2.5 (1.6-3.5) 324	43.4 ± 41.61 35 (11.2-65) 324	131.0 ± 161 81.8 (26.5-175) 324	-	-	2.0 ± 3.5 2.0 (0-2) 288	3.4 ± 5.4 2 (0-5) 288	0.8 ± 1.1 0 (0-2) 0.0 288	-
Swaziland (n = 316) F	8.1 ± 0.3 8.1 (8-8.3) 310	2.7 ± 1.4 2.6 (2-3.2) 314	53.5 ± 53.11 28.4 (13.3-94) 310	145.5 ± 167 79.0 (26-220) 310	28.1 ± 16 25.0 (20-35) 104	7.0 ± 4.5 6.0 (4-10) 277	9.6 ± 7.29 8.0 (4-13) 101	3.8 ± 5.3 2 (0-6) 101	76.7 ± 8.1 78.0 (72-83) 81	0.4 ± 1.05 0.0 (0-0.4) 303
P-value (A vs B)	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**-**	**< 0.0001**	**-**	**-**	**-**	**< 0.0001**
P-value (A vs C)	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.0014**	**< 0.0001**	**0.009**	**< 0.0001**	**< 0.0001**
P-value (A vs D)	**< 0.0001**	0.7260	0.160	0.1290	0.614	**< 0.0001**	**< 0.0001**	0.394	**< 0.0001**	0.107
P-value (A vs E)	**< 0.0001**	**0.0390**	**< 0.0001**	**< 0.0001**	**-**	**-**	**< 0.0001**	**0.0007**	**< 0.0001**	-
P-value (A vs F)	**< 0.0001**	**0.0270**	**0.0152**	**0.0028**	**< 0.0001**	0.5575	**< 0.0001**	0.497	< 0.0001	**< 0.0001**
P-value (B vs C)	0.988	**< 0.0001**	0.5290	**0.0008**	**-**	**< 0.0001**	-	-	-	0.2730
P-value (B vs D)	**< 0.0001**	**0.0003**	**< 0.0001**	**< 0.0001**	**-**	**0.031**	-	-	-	**< 0.0001**
P-value (B vs E)	**< 0.0001**	0.440	0.089	**0.014**	**-**	**-**	-	-	-	-
P-value (B vs F)	**< 0.0001**	0.377	**0.0018**	**0.003**	**-**	**< 0.0001**	-	-	-	**< 0.0001**
P-value (C vs D)	**< 0.0001**	**0.0046**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.680	0.302	0.494	**< 0.0001**
P-value (C vs E)	**< 0.0001**	**< 0.0001**	0.328	0.930	**-**	-	**< 0.0001**	0.124	**< 0.0001**	**-**
P-value (C vs F)	**< 0.0001**	**< 0.0001**	**0.0026**	0.2116	**0.0171**	**0.0446**	0.7225	0.0524	0.912	**< 0.0001**
P-value (D vs E)	**< 0.0001**	0.064	**< 0.0001**	**< 0.0001**	**-**	**-**	**< 0.0001**	0.964	**< 0.0001**	**-**
P-value (D vs F)	0.0878	**0.0475**	**0.0130**	**0.0031**	**0.0016**	**< 0.0001**	0.347	0.651	0.412	**< 0.0001**
P-value (E vs F)	**< 0.0001**	0.968	0.175	0.330	-	-	**< 0.0001**	0.271	**< 0.0001**	-

An evaluation of semen parameters obtained from South African patients residing in different locations is summarized in Table 3.17, with the higher average age of patients found in those residing in the Free States (48 (44 – 61) years old and the lowest in patients residing in Gauteng Central (32 (27 – 38) years old). Patients are categorized according to their residing areas. A total of 13 South African regions are included, namely Gauteng North (n = 15,090), Gauteng Central (n = 4,711), Gauteng East (n = 2,068), Gauteng West (n = 3,029), Gauteng South (n = 9,671), Gauteng Pretoria (n = 5,828), Eastern Cape (n = 4,357), Free State (n = 442), Mpumalanga (n = 6,265), North–West (n = 2,625), Western Cape (n = 3,690), KwaZulu-Natal (n = 485) and Limpopo (n = 2,471) are identified. The data collected from the laboratories did not provide data for progressive motility, normal morphology and red blood cell concentration for patients from Gauteng South, Eastern Cape, Free State, North-West, Western Cape and Limpopo.

Patients residing in Gauteng Central have the highest sperm concentration (66.5 (30-106) x 10^6 /mL) and total sperm count (median (IQR) = 216 (102-431) x 10^6) while those staying in the Free State have the lowest sperm concentration (median (IQR) = 51 (18-67) x 10^6/mL) and total sperm count (median (IQR) = 151 (92-230) x 10^6). The highest percentage of morphological small spermatozoa (median (IQR) = 11 (5-20)%) are obtained in patients residing in Gauteng Pretoria. Although patients living in Mpumalanga have the highest progressive motility (median (IQR) = 58 (32-69)%), they are also found to have the lowest percentage of spermatozoa with normal morphology (median (IQR) = 6 (4-10)%) with a high number of elongated spermatozoa (median (IQR0 = 3 (2-3)%) and the highest red blood cell concentration (median (IQR) = 1.3 (1-3) x 10^6/mL). Furthermore, the highest percentage of morphological normal spermatozoa (median (IQR) = 13 (8-16)%) and lower percentage of progressive motile spermatozoa (median (IQR) = 37 (19-45)%) are observed in patients residing in KwaZulu-Natal.

Table 3.17: Semen characteristics of patients residing areas within South Africa.

Locations within South Africa		Volume (mL)	Concentration (x 10^6/mL)	Total sperm count (x 10^6)	Progressive motility (%)	Sperm morphology (%)			Red blood cell count (x 10^6/mL)
						Normal	Small	Elongated	
Gauteng North (n = 15,090)	**Mean ±SD** **Median (IQR)** **n**	3.08 ± 2.46 2.80 (1.6-4) 14,990	79.39 ± 61.59 63.00 (15-134) 9,652	269 ± 375 205(101-378) 9,584	48 ± 7.0 49 (22-49) 229	7.8 ± 4.0 6 (4-10) 959	2.7 ± 3.7 2 (0-2) 8,748	2.5 ± 10.5 2 (1-6) 8,723	0.90 ± 1.70 0.50 (0.2-1) 1,925
Gauteng Central (n = 4,711)	**Mean ± SD** **Median (IQR)** **n**	2.94 ± 1.58 2.8 (1.7-4) 4,773	84.69 ± 63.37 66.50 (30-106) 2,037	277 ± 232 216 (102-431) 2,052	46 ± 7.0 47 (26-65) 835	9.0 ± 4.6 8 (5-12) 2,037	11 ± 7.4 10 (2-20) 834	2.9 ± 4.5 1 (0-1) 834	1.19 ± 2.4 0.70 (0.5-1) 4,635
Gauteng East (n = 2,068)	**Mean ± SD** **Median (IQR)** **n**	2.97 ± 1.67 2.80 (1.7-4) 2,091	75.91 ± 58.58 57.06 (20-118) 920	252 ± 199 193.5(98-398) 920	47 ± 7.0 47 (24-53) 279	8.1 ± 4.1 7 (5-12) 920	12 ± 8.6 10 (2-18) 279	3 ± 4.9 1 (0-2) 279	1.14 ± 3.30 0.60 (0-1.2) 2,010
Gauteng West (n = 3,029)	**Mean ± SD** **Median (IQR)** **n**	2.98 ± 1.53 2.90 (1.7-4) 3,060	79.97 ± 62.89 62.51 (22-89) 1,366	257 ± 202 204 (106-309) 1,366	50 ± 11 49 (28-62) 438	8.2 ± 4.2 7 (6-10) 1,366	11.5 ± 8.0 10 (2-20) 437	2.5 ± 4.0 1 (0-2) 437	1.09 ± 1.80 0.60 (0.1-1) 2,932
Gauteng South (n = 9,671)	**Mean ± SD** **Median (IQR)** **n**	3.05 ± 1.68 2.90 (1.7-4) 9,630	66.73 ± 48.54 54.00 (26-77) 6 218	222 ± 178 173.8 (96-262) 6,212	-	-	2 ± 2.4 2 (0-2) 6,168	3.5 ± 4.8 2 (3) 6,167	4.0 ± 6.08 0.00 (0-0.1)
Gauteng Pretoria (n = 5,828)	**Mean ± SD** **Median (IQR)** **n**	3.17 ± 1.61 3.1 (1.7-4) 5,872	83.34 ± 66.34 64.00 (32-98) 2,539	273 ± 217 213.9 (97-373) 2,536	46 ± 7.0 46 (27-52) 1,197	9.0 ± 4.5 8 (60-10) 2 537	12 ± 7.0 11 (5-20) 1,253	3.6 ± 6.1 2 (1-4) 1,253	1.38 ± 1.9 0.80 (1-2) 2,487
Eastern Cape (n = 4,357)	**Mean ± SD** **Median (IQR)** **n**	2.81 ± 1.82 2.50 (1.5-4) 4,342	79.36 ± 86.58 57.00 (26-89) 2,767	260 ± 295 174 (87-204) 2,766	-	-	2.4 ± 2.9 2 (0-5) 1,253	4.7 ± 6.7 3 (1-6) 1,253	-
Free State (n = 442)	**Mean ± SD** **Median (IQR)** **n**	2.98 (± 1.43) 3.00 (1.5-4) 439	66.39 ± 71.83 51.00 (18-67) 307	215 ± 214 151 (92-230) 304	-	-	2.5 ± 2.8 2 (0-2) 299	4.4 ± 6.2 2 (0-2) 299	-
Mpumalanga (n = 6,265)	**Mean ± SD** **Median (IQR)** **n**	3.11 ± 1.82 3.0 (1.7-4.5) 6,245	73.77 ± 66.47 54.40 (20-82) 3,575	262 ± 326 178.5 (99-206) 3,566	56 ± 11 58 (32-69) 63	7.4 ± 4.0 6 (4-10) 373	2.6 ± 3.1 2 (0-4) 2,998	4.4 ± 6.1 3 (2-4) 2,999	1.5 ± 1.5 1.3 (1-3) 376
North-West (n = 2,625)	**Mean ± SD** **Median (IQR)** **n**	3.08 ± 1.60 3.0 (1.8-4) 2,616	74.26 ± 67.06 52.00 (28-92) 1,606	252 ± 255 177 (105-195) 1,606	-	-	2.4 ± 3.5 2 (0-2) 1 380	4.2 ± 6.4 2 (0-2) 1,380	-
Western Cape (n = 3,690)	**Mean ± SD** **Median (IQR)** **n**	3.05 ± 1.49 3.0 (1.6-4) 297	78.16 ± 61.04 59.00 (25-79) 180	279 ± 300 198 (102-209) 180	-	-	2.1 ± 2.5 2 (1-5) 172	3.5 ± 5.1 2 (1-2) 172	-
KwaZulu-Natal (n = 485)	**Mean ± SD** **Median (IQR)** **n**	3.02 ± 1.9 3.0 (1.7-4) 486	65.52 ± 44.37 50.00 (22-75) 257	244 ± 224 162.5 (88-197) 257	38 ± 2.38 37 (19-45) 5	13.6 ± 6 13 (8-16) 5	2.2 ± 3.8 2 (0-2) 246	2.8 ± 5.2 2 (0-2) 244	0.8 ± 0.7 0.8 (0.5-1) 5
Limpopo (n = 2,471)	**Mean ± SD** **Median (IQR)** **n**	2.9 ± 1.6 2.5 (1.7-4) 2,468	77.01 ± 57.44 60.00 (23-96) 1,587	244 ± 209 180 (123-246) 1,587	-	-	2.4 ± 2.9 2 (0-2) 1,562	4.9 ± 6.5 3 (2-4) 1,562	-

3.7 Temporal Changes in Semen Quality (2005-2019) in the sub-Saharan Region

The investigation of changes in semen characteristics between January 2005 and January 2020 in patients residing in sub-Saharan African countries, specifically for semen volume, sperm concentration, progressive motility, total progressively motile count, normal morphology, total normal sperm count, percentage of small and elongated spermatozoa, was performed. A total of 95.9% (n = 67,866) patient reports indicate the collection year of the reports, in which only 11.66% (n = 7,915) reported progressive motility and total progressively motile count data. Data collected from the andrology laboratories did not indicate progressive motility and total progressively motile count from 2013 to 2018. Temporal significant ($P < 0.0001$) decreases in semen volume, sperm concentration, percentage of small spermatozoa and more pronounced decreases in the percentage of spermatozoa with normal morphology and total normal sperm count are observed. Results are summarized in Table 3.18.

The median (IQR) for semen volume decreased from 2.9 (2-4) mL in 2005 (n = 549) to 1.9 (1.3-3.8) mL in 2019 (n = 4,895). A decrease in the median (IQR) sperm concentration from being 46 (16-94) x 10^6/mL in 2005 (n = 549) to 40 (15-83) x 10^6/mL in 2019 (n = 4,919) is also observed. The total median (IQR) normal sperm count declined from 12.95 (3.6-35)% in 2005 (n = 498) to 3.84 (1.14-9)% in 2019 (n = 1,746), while percentage of spermatozoa with normal morphology decreased from 9 (5-14)% in 2005 (n = 498) to 4 (3-5)% in 2019 (n = 1,797). A similar pattern is observed for the median (IQR) number of small spermatozoa which dropped from 14 (9-20)% in 2005 (n = 497) to 2 (4.00)% in 2019 (n = 2,505). Although no data for progressive motility and total progressively motility count are collected between 2013 and 2018 from the participating laboratories, significant ($P < 0.05$) increasing trends in progressive motility, total progressively motile count and number of elongated spermatozoa are seen. In 2005, the median (IQR) progressive motility is 35 (24-45)% (n = 429) which increased to 50 (40-51)% in 2019 (n = 97). The median (IQR) total progressively motile count increased from 46.84 (15-104) in 2005 (n = 439) to 61.48 (12-101) in 2019 (n = 97). Similarly, the median (IQR) percentage of elongated spermatozoa increased from 3 (1-7)% in 2005 (n = 497) to 4 (1-10)% in 2019 (n = 2,505).

Table 3.18: Descriptive statistics of semen characteristics per year in patients residing in sub-Saharan Africa. Bolded P-values indicate statistical significance. performed using the Kruskal-Wallis with Jonckheere-Terpstra trend test.

		2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	P-value
n		549	808	1034	3787	4377	4596	4671	5466	6197	7 065	5334	5210	4874	4931	4920	
Volume (mL)	**Median (IQR range) n**	2.90 (2-4) 549	3.00 (2.1-4) 815	3.00 (2-4) 1,041	3.00 (2-4.1) 1,097	2.90 (2-4) 1,338	2.90 (2-4) 3,280	3.00 (2-4) 4,679	3.00 (2-4) 5,485	2.90 (2-4) 6,243	2.80 (2-3.9) 6,189	2.70 (1.8-3.8) 5,378	2.70 (1.9-3.8) 5,233	2.70 (2-3.8) 4,905	2.60 (1.9-3.8) 4,958	1.90 (1.9-3.8) 4,895	**< 0.0001**
Concentration ($x 10^6$/mL)	**Median (IQR range) n**	46.00 (16-94) 549	42.00 (12-83) 808	50.25 (16-97) 1,034	48.00 (20-91) 3,786	47.00 (18-87) 4,377	43.2 (16-85) 4,594	44.51 (16-86) 4,667	42.00 (15-85) 5,466	41.80 (15-83) 6,194	41.00 (14-81) 6,126	40.00 (15-80) 5,334	39.00 (15-80) 5,209	36.00 (14-73) 4,872	41.00 (16-80) 4,931	40.00 (15-83) 4,919	**< 0.0001**
Progressive motility (%)	**Median (IQR range) n**	35.00 (24-45) 439	34.00 (23-44) 762	37.00 (24-46) 994	38.00 (26-47) 1,035	38.00 (25-47) 1,246	38.00 (25-48) 1,305	45.00 (30-53) 1,277	44.00 (28-52) 759	-	-	-	-	-	-	50.00 (40-51) 97	**< 0.0001**
Total progressively motile count x 10^6	**Median (IQR range) n**	46.84 (15-104) 439	41.47 (14-89) 762	50.43 (16-120) 994	53.19 (20-115) 1,035	47.47 (17-99) 1,246	41.99 (12-99) 1,305	52.78 (17-110) 1,216	57.17 (18-115) 759	-	-	-	-	-	-	61.48 (12-101) 97	**< 0.0001**
Normal morphology (%)	**Median (IQR range) n**	9.00 (5-14) 498	8.00 (4-12) 706	8.00 (4-13) 937	8.00 (4-13) 983	8.00 (4-12) 1,197	9.00 (6-13) 1,237	10.00 (6-13) 1,216	9.00 (5-12) 1,649	8.00 (5-12) 2,239	7.00 (4-11) 2,176	4.00 (3-6) 1,666	4.00 (3-6) 1,596	4.00 (3-5) 1,608	4.00 (3-5) 1,729	4.00 (3-5) 1,797	**< 0.0001**
Total normal sperm count (%)	**Median (IQR range) n**	12.95 (3.6-35) 498	11.48 (2.8-31) 705	13.40 (2.9-36) 937	12.31 (2.9-35) 983	12.51 (2.8-29) 1,197	12.37 (2.9-31) 1,236	13.31 (3.5-30) 4,148	11.26 (3-29) 1,649	10.03 (2.4-26) 2,238	8.20 (2-22) 2,176	4.80 (1.4-12) 1,666	4.65 (1.4-11) 1,595	4.03 (1.2-10.5) 1,607	4.133 (1.3-9) 1,727	3.84 (1.14-9) 1,746	**< 0.0001**
Small spermatozoa (%)	**Median (IQR range) n**	14.00 (9-20) 497	13.00 (8-19) 706	12.00 (7-18) 938	3.00 (2-6) 3,392	3.00 (2-6) 3,903	3.00 (2-6) 4,100	2.00 (2-6) 4,148	2.00 (1-3) 3,897	2.00 (1-2) 3,188	1.00 (0-3) 3,095	1.00 (0-3) 3,054	2.00 (0-4) 2,980	2.00 (0-4) 2,694	3.00 (0-6) 2,585	2.00 (0-4) 2,505	**< 0.0001**
Elongated spermatozoa (%)	**Median (IQR range) n**	3.00 (1-7) 497	2.00 (1-7) 706	2.00 (0-5) 938	2.00 (1-4) 3,392	2.00 (1-5) 3,903	2.00 (1-5) 4,100	2.00 (1-4) 4,148	2.00 (1-4) 3,897	3.00 (2-5) 3,186	1.00 (0-4) 3,095	2.00 (0-5) 3,054	2.00 (1-7) 2,980	3.50 (1-8) 2,694	4.00 (2-10) 2,585	4.00 (1-10) 2,505	**< 0.0001**

3.8 Effects of Seasonal Variations in Semen Characteristics of Patients Residing in Sub-Saharan African Below the Equator

The results from the comparison of semen characteristics (pH, volume, sperm concentration, total sperm count, progressive motility, normal morphology, percentage of elongated and duplicated spermatozoa, and normed sORP) obtained from different seasons (winter (n = 17,812), spring (n = 17,011), summer (n = 15,042) and autumn (n = 17,750) are reported in Table 3.19.

The results obtained for semen pH, progressive motility and normal morphology are not significant. However, the sperm concentration is significantly ($P < 0.05$) higher in winter (median (IQR) = 43.54 (16.5-85) x 10^6/mL) than summer (median (IQR) = 40.10 (15-81) x 10^6/mL) and autumn (median (IQR) = 40.40 (15-81) x 10^6/mL). Total sperm count in winter (median (IQR) = 110.40 (38.9-236) x 10^6) is significantly ($P < 0.05$) higher than in summer (median (IQR) = 105.04 (34-228) x 10^6) and autumn (median (IQR) = 102.30 (33-224) x 10^6). Semen volume is significantly ($P < 0.05$) lower in winter (median (IQR) = 2.8 (2-3.9) mL) and autumn (median (IQR) = 2.70 (2-3.9) mL) compared to spring (median (IQR) = 2.8 (2-4) mL) and summer (median (IQR) = 2.8 (2-4) mL). Significantly ($P < 0.05$) higher numbers of elongated spermatozoa are observed in spring (median (IQR) = 2 (1-6)%) and summer (median (IQR) = 2 (1-6)%) in comparison to winter (median (IQR) = 2 (1-5)%) and autumn (median (IQR) = 2 (1-6)%), while the lowest percentage of duplicated spermatozoa is found in winter (median (IQR) = 1 (0-2)%). Significantly ($P < 0.05$) higher normed sORP is indicated in winter (median (IQR) = 3.60 (1.1-18.7) mV/10^6 sperm/mL) than in spring (median (IQR) = 1.14 (1.13-1.14) mV/10^6 sperm/mL), summer (median (IQR) = 1.14 (1.14-1.15) mV/10^6 sperm/mL) and autumn (median (IQR) = 1.63 (1.1-1.8) mV/10^6 sperm/mL).

Table 3.19: Analysis of semen quality associated with seasonal variations. Bolded P-values indicate statistical significance.

Seasons		pH	Volume (mL)	Concentration (x 10^6/mL)	Total sperm count (x 10^6)	Progressive motility (%)	Sperm morphology (%)			Normed sORP (mV/10^6sperm/mL)
							Normal	Elongated	Duplicated	
Winter (n = 17,812) A	**Mean ± SD** **Median (IQR)** **n**	7.71 ± 0.83 7.68 (7.4-8.0) 16,703	3.02 ± 2.27 2.80 (2-3.9) 15,222	61.59 ± 66.88 43.54 (16.5-85) 16,641	177.58 ± 302.2 110.40 (38.9-236) 15,136	36.62 ± 15.34 39.00 (25-48) 1,976	7.07 ± 4.68 6.00 (4-10) 5,706	4.19 ± 6.24 2.00 (1-5) 10,970	6.46 ± 19.18 1.00 (0-2) 9,796	8.68 ± 9.25 3.60 (1.1-18.7) 8
Spring (n = 17,011) B	**Mean ± SD** **Median (IQR)** **n**	7.79 ± 0.89 7.80 (7.5-8.1) 15,603	3.09 ± 1.90 2.80 (2-4) 14,241	61.05 ± 68.64 43.00 (16-84.8) 15,576	178.86 ± 240.28 112.0 (37.5-240) 14,107	36.40 ± 16.29 40.00 (25-49) 1,892	6.94 ± 4.65 5.00 (4-10) 5,455	4.67 ± 6.38 2.00 (1-6) 10,007	8.11 ± 21.64 1.00 (0-2) 9,089	1.14 ± 0.001 1.14 (1.13-1.14) 6
Summer (n = 15,042) C	**Mean ± SD** **Median (IQR)** **n**	7.77 ± 0.44 7.70 (7.5-8) 13,888	3.04 ± 1.67 2.80 (2-4) 12,128	59.69 ± 102.00 40.10 (15-81) 13,944	172.15 ± 236.73 105.04 (34-228) 12,051	36.30 ± 16.40 39.00 (25-49) 1,705	7.12 ± 4.75 6.00 (4-10) 4,433	4.55 ± 6.70 2.00 (1-6) 9,436	8.52 ± 22.12 1.00 (0-2) 8,621	1.14 ± 0.001 1.14 (1.14-1.15) 3
Autumn (n = 17,750) D	**Mean ± SD** **Median (IQR)** **n**	7.78 ± 0.88 7.70 (7.5-8) 16,565	2.97 ± 1.64 2.70 (2-3.9) 14,446	58.30 ± 62.55 40.40 (15-81) 16,576	166.76 ± 200.02 102.30 (33-224) 1, 376	36.92 ± 15.90 40.00 (26-49) 2,217	7.14 ± 4.82 6.00 (4-10) 5,519	4.49 ± 6.78 2.00 (1-6) 11,167	8.51 ± 22.18 1.00 (0-2) 10,147	1.47 ± 0.36 1.63 (1.1-1.8) 74
P-value (A vs B)		0.121	**0.028**	0.191	0.710	0.817	0.084	**0.000**	**0.000**	**< 0.001**
P-value (A vs C)		0.691	**0.020**	**< 0.001**	**0.001**	0.943	0.742	**0.000**	**0.000**	**< 0.001**
P-value (A vs D)		0.817	0.274	**0.000**	**0.000**	0.385	0.909	**0.000**	**0.000**	**0.033**
P-value (B vs C)		0.060	0.820	**0.000**	**0.000**	0.788	0.057	1.000	0.261	1.000
P-value (B vs D)		0.187	**0.001**	**0.000**	**0.000**	0.574	0.082	0.320	**0.000**	0.730
P-value (C vs D)		0.535	**0.000**	0.802	0.116	0.402	0.839	1.000	**0.028**	0.812

3.9 Global Comparison of Semen Parameters from Africa and the Middle East

The results from the comparison between data from the full cohort (Africa and the Middle East, n = 70,765), and data available from global geographical regions obtained from Cooper (2010) (n = 6,299) and Campbell (2021) data (n = 3,589) are summarized from Table 3.20 to Table 3.25. Furthermore, the results from the geographical differences in semen quality obtained in the cohort data and the Cooper (2010) data are summarized in Table 3.26. The Cooper (2010) data is a published data by Cooper et al. (2010) which was used to formulate the WHO (2010) reference intervals for semen parameters. The Campbell (2021) data consisted of published data by Campbell et al. (2021).

3.9.1 Descriptive Statistics and Comparisons of Cooper (2010) and Campbell (2021) Data with Africa and the Middle East

The descriptive statistics for the Cooper (2010) data are presented in Table 3.20, while the descriptive statistics of the Campbell (2021) data are summarized in Table 3.21. The Chi-Square test for normality for both data sets indicate that all parameters are not normally distributed and reported using non-parametric (median and IQR) data.

In Table 3.20, the median (IQR) age is 31 (28-36) years. The median (IQR) for semen volume is 3 (2.1-4.2) mL while the median (IQR) for sperm concentration and total sperm count are 63 (36-101) x 10^6/mL and 189.2 (97-337) x 10^6, respectively. The median (IQR) for the percentage of sperm with normal morphology is 18 (11.5-36)% while the total normal sperm count is 36.17 (13.57-81.40)%. The median (IQR) results for progressive motility and total progressively motile count are 55.00 (48-63)% and 117 (56.4-208) x10^6, respectively. In Table 3.21, the median (IQR) for semen volume is 3 (2.3-4.2) mL while the median (IQR) for sperm concentration and total sperm count are 66 (36 - 110) x 10^6/mL and 209.5 (107-362) x 10^6, respectively. The median (IQR) for the percentage of sperm with normal morphology is 14.00 (8 - 23)%, while the total normal sperm count is 23.45 (11.5-78.9)%. The median (IQR) results for progressive motility and total progressively motile count are 55 (45-63)% and 112 (49-204) x10^6, respectively. The median (IQR) for sperm viability evaluation is 78 (69 -88)%.

Table 3.20: Descriptive statistics of semen parameters obtained from the Cooper (2010) data (n = 6 299).

Variable	Evaluated sample size (n)	Percentage of total sample (n = 6 299)	Mean ± SD	Median (IQR)	Range	5th	95th	Distribution (Normal/ not normal)
Age	5,844	93.41	32.00 ± 6.06	31.00 (28 - 36)	17.00 - 67.00	25.00	36.00	Not normal
Volume (mL)	6,269	99.52	3.35 ± 1.68	3.00 (2.1 - 4.2)	0.1 - 20.00	1.07	6.50	Not normal
Concentration (x 10^6/mL)	6,273	99.58	78.01 ± 62.31	63.00 (36 - 101)	0.00 - 677	12.50	194	Not normal
Total sperm count (x 10^6)	6,293	99.90	255.5 ± 242.3	189.2 (97 - 337)	0.00 - 3 115	25.00	707	Not normal
Normal morphology (%)	5,968	94.74	27.90 ± 24.22	18.00 (11.5 - 36.0)	1.00 - 100	5.00	82.00	Not normal
Total normal sperm count (%)	6,160	97.79	67.92 ± 99.20	36.17 (13.57 - 81.40)	0.00 - 1689	0.12	298	Not normal
Progressive motility (%)	3,836	60.89	55.01 ± 12.81	55.00 (48 - 63)	0.00 - 102.00	0.00	63.16	Not normal
Total progressively motile count (x 10^6)	3,841	60.97	155.86 ± 141.51	117 (56.4 - 208)	0.00 - 1 298.88	0.00	72.30	Not normal

Table 3.21: Descriptive statistics of semen parameters obtained from the Campbell (2021) data (n = 3 589).

Variable	Evaluated sample size (n)	Percentage of total sample (n = 6 299)	Mean ± SD	Median (IQR)	Range	5th	95th	Distribution (Normal/not normal)
Volume (mL)	3,586	99.91	3.42 ± 1.53	3.00 (2.3 - 4.2)	0.1 - 12.50	1.40	6.20	Not normal
Concentration (x 10^6/mL)	3,587	99.94	82.57± 64.50	66.00 (36 - 110)	0.9 - 532	16	208	Not normal
Total sperm count (x 10^6)	3,584	99.86	270.31 ± 232.78	209.5 (107 - 362)	2.5 - 3 115	39	701	Not normal
Normal morphology (%)	3,335	92.92	16.72 ± 11.32	14.00 (8.0 - 23.0)	0.00 - 76.0	4.00	39.00	Not normal
Total normal sperm count (%)	3,334	97.79	52.87 ± 85.42	23.45 (11.5 - 78.9)	0.00 - 1 432	0.15	246	Not normal
Progressive motility (%)	3,389	94.42	53.64 ± 14.09	55.00 (45.0 - 63.0)	0.00 - 94.00	30.00	77.00	Not normal
Total progressively motile count (x 10^6)	3,389	94.42	150.12 ± 138	112 (49 - 204)	0.00 - 1 265	0.00	61.76	Not normal
Viability (%)	1,232	34.32	77.1 ± 13.32	78.0 (69 - 88)	29.0 - 100	53	97	Not normal

Table 3.22 summarizes the comparison of semen volume, sperm concentration, progressive motility, total progressively motile count, normal morphology, total normal sperm count (TNSC) and sperm viability between patients reports obtained from the cohort study (n =70,765), the Cooper (2010) (n = 6,299) and the Campbell (2021) data (n = 3,589). Semen volume and sperm concentration obtained in the current cohort study are significantly ($P < 0.05$) lower than the Cooper (2010) data and the Campbell (2021) data (median (IQR) = 2.80 (2.0-4.0) mL / 41.00 (15-81) x 10^6/mL vs 3 (2.1-4.2) mL / 63 (36-101) x 10^6/mL and 3 (2.3-4.2) mL / 66 (36-110) x 10^6/mL, respectively). No significant difference in semen volume is found between the Cooper (2010) data and the Campbell (2021) data. Progressive motility and total progressively motile count from the cohort study data are significantly ($P < 0.05$) lower than those from the Cooper (2010) data and the Campbell (2021) data (median (IQR) = 32 (15-45)% / 29.76 (4.8-81.9) x 10^6 vs 55 (48-63)% / 45.86 (8.6-97) x 10^6 and 55 (45-63)% / 42 (49-204) x 10^6, respectively). The progressive motility and total progressively motile count obtained from the Cooper (2010) data are significantly ($P < 0.05$) higher than those obtained from the Campbell (2021) data. Normal morphology and the total normal sperm count are significantly ($P < 0.05$) lower in the cohort study data than the Cooper (2010) data and the Campbell (2021) data (median (IQR) = 8 (4-13)% / 12.54 (2.9-33.12)% vs 18.00 (11.5-36.0)% / 36.17 (13.57-81.40)% and 14.00 (8-23)% / 23.45 (11.5-78.9)%). Normal morphology and total normal sperm count from the Cooper (2010) data are significantly ($P < 0.05$) higher than those obtained in the Campbell (2021) data. Sperm viability is significantly ($P < 0.05$) lower in the cohort study data compared to the Campbell (2021) data (median (IQR) = 49 (33-60)% vs 78.00 (69-88)%). No data for sperm viability is available for the original WHO (2010) data.

Table 3.22: Comparison of semen parameters between the current cohort data, Cooper (2010) data and Campbell (2021) data, with statistically significant values obtained using the Mann–Whitney test for independent values. Bolded P-values indicate statistical significance.

		Volume (mL)	Concentration (x 10^6/mL)	Progressive motility (%)	Total progressively motile count (x 10^6)	Normal morphology (%)	Total normal sperm count (%)	Sperm Viability (%)
Cohort data (n = 70,765) A	**Mean ± SD** **Median (IQR)** **n**	2.99 ± 1.59 2.80 (2.0-4.0) 58,976	58.52 ± 62.52 41.00 (15-81) 65,761	30.00 ± 18.56 32.00 (15-45) 10,808	60.38 ± 85.01 29.76 (4.8-81.9) 10,808	8.86 ± 5.56 8.00 (4.00-13.00) 4,321	25.15 ± 34.52 12.54 (2.9-33.12) 4,320	46.30 ± 19.11 49 (33-60) 667
Cooper (2010) data (n = 6,299) B	**Mean ± SD** **Median (IQR)** **n**	3.35 ± 1.68 3.00 (2.1-4.2) 6,269	78.01 ± 62.31 63.00 (36-101) 6,273	55.01 ± 12.81 55.00 (48-63) 3,836	105.86 ± 131.51 45.86 (8.6-97) 3,841	27.90 ± 24.22 18.00 (11.5-36.0) 5,968	67.92 ± 99.20 36.17 (13.57-81.40) 6,160	-
Campbell (2021) data (n = 3,589) C	**Mean ± SD** **Median (IQR)** **n**	3.42 ± 1.53 3.00 (2.3 – 4.2) 1,335	82.57 ± 64.50 66.00 (36 - 110) 3,587	53.64 ± 14.09 55.00 (45-63) 3,389	96.2 ± 108 42 (49-204) 3,389	16.72 ± 11.32 14.00 (8-23) 3,335	52.87 ± 85.42 23.45 (11.5-78.9) 3,334	77.12 ± 13.32 78.00 (69 - 88) 1,335
P-value (A vs B)		**0.0001**	**0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**-**
P-value (A vs C)		**< 0.0001**	**< 0.0001**	**0.0001**	**< 0.0001**	**0.0001**	**0.0001**	**0.0001**
P-value (B vs C)		0.1605	**0.0001**	**0.001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**-**

3.9.2 Correlations between Cooper (2010) and Campbell (2021) Data with Africa and the Middle East

The correlations and statistical significance (P-value) of semen parameters obtained from the cohort study, the Cooper (2010) and the Campbell (2021) data are summarized in Tables 3.23, 3.24 and 3.25, respectively. Positive significant correlations are observed in the cohort study data and the Campbell (2021) data between sperm concentration and normal morphology (r^2 = 0.504, P < 0.0001 and r^2 = 0.204, P < 0.0001. respectively), progressive motility and normal morphology (r^2 = 0.426, P < 0.0001 and r^2 = 0.230, P < 0.0001, respectively) and between sperm viability and normal morphology (r^2 = 0402, P < 0.0001 and r^2 = 0.218, P<0.0001, respectively). Weaker significant positive correlations are reported in the cohort study data, the Cooper (2010) and the Campbell (2021) data between progressive motility and sperm concentration (r^2 = 0.357, P < 0.0001; r^2 = 0.147, P < 0.0001, and r^2 = 0.097, P < 0.0001, respectively) and between progressive motility and total sperm count (r^2 = 0.345, P < 0.0001; r^2 = 0.159, P < 0.0001, and r^2 = 0.130, P < 0.0001, respectively). Significant negative correlations are observed in the 3 sets of data (cohort study, Cooper (2010) and the Campbell (2021) data) between sperm concentration and semen volume (r^2 = -0.037, P < 0.0001; r^2= -0.052, P < 0.0001 and r^2= -0.124, P < 0.0001, respectively).

Table 3.23: Correlations between semen parameters from the cohort study. Bolded P-values indicate statistical significance.

		Volume (mL)	Normal morphology (%)	Progressive motility (%)	Total sperm count (x 10^6)	Viability (%)
Concentration (x 10^6/mL)	r^2 P-value n	**-0.037** **< 0.0001** **58,613**	**0.504** **< 0.0001** **24,121**	**0.357** **< 0.0001** **1,075**	**0895** **< 0.0001** **58,613**	**0.094** **0.014** **661**
Volume (mL)	r^2 P-value n		**0.034** **< 0.0001** **24,073**	**0.031** **0.0012** **10,756**	**0.356** **< 0.0001** **58,613**	0.017 0.6694 666
Normal morphology (%)	r^2 P-value n			**0.426** **< 0.0001** **10,396**	**0.482** **< 0.0001** **24,067**	**0.402** **< 0.0001** **667**
Progressive motility (%)	r^2 P-value n				**0.345** **< 0.0001** **10,708**	**0.162** **< 0.0001** **666**
Total sperm count (x 10^6)	r^2 P-value n					**0.100** **0.0099** **666**

Table 3.24: Correlations between semen parameters from the Cooper (2010) data. Bolded P-values indicate statistical significance.

		Volume (mL)	Normal morphology (%)	Progressive motility (%)	Total sperm count (x 10^6)
Concentration (x 10^6/mL)	r^2 P-value n	**-0.052** **< 0.0001** **6 266**	**0.047** **< 0.0003** **5,944**	**0.147** **< 0.0001** **3,871**	**0.742** **<0.0001** **6,268**
Volume (mL)	r^2 P-value n		**-0.111** **< 0.0001** **5,942**	**0.093** **< 0.0001** **3,868**	**0.476** **< 0.0001** **6,267**
Normal morphology (%)	r^2 P-value n			**-0.114** **< 0.0001** **3,563**	-0.016 0.2187 5,965
Progressive motility (%)	r^2 P-value n				**0.159** **< 0.0001** **3,868**

Table 3.25: Correlations between semen parameters from the Campbell (2021) data. Bolded P-values indicate statistical significance.

		Volume (mL)	Normal morphology (%)	Progressive motility (%)	Total sperm count (x 10^6)	Viability (%)
Concentration (x 10^6/mL)	r^2 P-value n	**-0.124** **< 0.0001** **3,584**	**0.204** **< 0.0001** **3,334**	**0.097** **< 0.0001** **3,388**	**0.767** **< 0.0001** **3,584**	0.033 0.2217 1,335
Volume (mL)	r^2 P-value n		-0.025 0.1523 3,332	**0.077** **< 00001** **3,386**	**0.398** **< 0.0001** **3,584**	**-0.057** **0.0357** **1,335**
Normal morphology (%)	r^2 P-value n			**0.230** **< 0.0001** 3,135	**0.174** **< 0.0001** **3,331**	**0.218** **< 0.0001** **1,335**
Progressive motility (%)	r^2 P-value n				**0.130** **< 0.0001** **3,385**	**0.650** **< 0.0001** **1,335**

3.9.3 Regional differences in semen parameters between the cohort data and Cooper (2010) data

The Cooper (2010) data is from fathers (with and without known time to pregnancy) and unscreened volunteers residing in America (Chile and United States of America), Asia (China), Australia and Europe (Scotland, Norway, Denmark, Germany, Italy, United Kingdom). A regional sub-analysis of the Cooper (2010) data and data from patients residing in Africa (Northern Africa, Southern Africa and Eastern Africa) from the full cohort is performed. Table 3.26 summarizes semen parameters of patients residing in Africa (n = 67,168), obtained from the cohort study, and patients residing in America (n = 2,505), Asia (n = 616), Australia (n = 957) and Europe (n = 2,221), obtained from the Cooper (2010) data. Patients residing in Europe are found to have a significantly (P < 0.05) higher semen volume

(median (IQR) = 3.50 (2.5-4.6) mL) than those obtained from the other groups investigated. Semen parameter reports obtained from patients residing in Australia show a significant ($P < 0.05$) higher sperm concentration of total sperm count and total progressively motile count (median (IQR) = 95.66 (45.6-126) x 10^6/mL; 229.6 (123.7-403) x 10^6 and 156.49 (83-277) x 10^6, respectively) than those obtained from the other groups investigated. Patients residing in Asia have a significantly ($P < 0.05$) higher percentage of spermatozoa with normal morphology and total normal sperm count (median (IQR) = 75 (65-85)% and 96.97 (55-173)%, respectively) than those obtained from the other groups investigated. Patients residing in Africa have a significantly ($P < 0.05$) lower sperm concentration (median (IQR) = 36 (15-73) x 10^6/mL), total sperm count (median (IQR) = 79.13 (54-89) x 10^6), progressive motility (median (IQR) = 34 (31-46) %), total progressively motile count (median (IQR) = 44 (5.4-78) x10^6), normal morphology (median (IQR) = 5.98 (4.1-11)% and total normal sperm count (median (IQR) = 7.02 (1.5-19.9)%) than those obtained from the other groups investigated.

Table 3.26: Regional differences in semen quality from patients residing in Africa and patients residing in America, Asia, Australia, Europe, with statistically significant values obtained using the Mann–Whitney test for independent values. Bolded P-values indicate statistical significance.

Geographical locations		Volume (mL)	Concentration (x 10^6/mL)	Total sperm count (x10^6)	Progressive motility (%)	Total progressively motile count (x 10^6)	Normal morphology (%)	Total normal sperm count (%)
America (n = 2,505) A	**Mean ± SD Median (IQR) n**	3.20 ± 1.66 3.00 (2-4.1) 2,500	72.90 ± 56.96 60.40 (35-94) 2,501	227.11 ± 209.13 174.5 (86.52-299) 2,501	54.84 ± 12.59 56.00 (47.8-62.6) 1,145	148.38 ± 125.39 119.08 (57.98-204) 1,147	12.85 ± 5.46 13.00 (9-16.5) 2,413	29.36 ± 32.91 19.84 (8-38) 2,505
Asia (n = 616) B	**Mean ± SD Median (IQR) n**	2.80 ± 1.45 2.50 (2-3.1) 616	71.84 ± 73.23 51.00 (20.6-98) 616	178.0 ± 148.65 130.0 (81.5-220) 616	58.95 ± 10.90 55 (51-65) 616	109.0 ± 102.0 74.88 (44-133) 616	73.89 ± 14.34 75.00 (65-85) 616	130.2 ± 111.4 96.97 (55-173) 616
Australia (n = 957) C	**Mean ± SD Median (IQR) n**	3.30 ± 1.69 3.00 (2-4.3) 936	95.66 ± 74.46 95.66 (45.6-126) 937	303.47 ± 290.88 229.6 (123.7-403) 936	53.32 ± 11.27 55.00 (47-60) 274	191.46 ± 149.65 156.49 (83-277) 274	42.53 ± 30.18 43.00 (16-71) 948	122.48 ± 166.9 68.55 (25.4-156) 957
Europe (n = 2,221) D	**Mean ± SD Median (IQR) n**	3.60 ± 1.69 3.50 (2.5-4.6) 2,218	80.66 ± 65.93 64.00 (35-106) 2,220	288.35 ± 264.8 217.3 (106-382) 2,218	55.00 ± 13.49 54.00 (46-63) 1,801	171.3 ± 156.52 129.8 (62.8-229) 1,803	24.90 ± 13.63 24.00 (15-34) 1,991	70.85 ± 83.91 44.94 (16.36-93) 2,081
Africa (n = 67,168) E	**Mean ± SD Median (IQR) n**	3.00 ± 1.57 2.89 (2-4.5) 63,574	50.10 ± 55.40 36.00 (15-73) 66,798	158.05 ± 123.7 79.13 (54-89) 63,574	32.45 ± 14.7 34.00 (31-46) 8,870	76.03 ± 89.90 44 (5.4-78) 8,870	7.06 ± 4.89 5.98 (4.1-11) 22,189	15.76 ± 25.30 7.02 (1.5-19.9) 22,189
P-value (A vs B)		**< 0.0001**	**0.0045**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
P-value (A vs C)		**0.0053**	**< 0.0001**	**< 0.0001**	**0.0219**	**< 0.0001**	**< 0.0001**	**< 0.0001**
P-value (A vs D)		**< 0.0001**	0.455	**< 0.0001**	**0.042**	**0.0074**	**< 0.0001**	**< 0.0001**
P-value (A vs E)		**0.0012**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
P-value (B vs C)		**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
P-value (B vs D)		**< 0.0001**	0.555	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
P-value (B vs E)		**0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
P-value (C vs D)		**< 0.0001**	**< 0.0001**	0.0672	0.369	**0.0008**	**< 0.0001**	**0.0001**
P-value (D vs E)		**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**

Chapter 4: Discussion

Semen quality can be affected by several factors, including age (Gu et al., 2018; Li et al., 2019; Wu et al., 2021), the duration of sexual abstinence (Agarwal et al., 2016; Comar et al., 2017; Hanson et al., 2018), geographical location (Jørgensen et al., 2001; Swan et al., 2002; Iwamoto et al., 2007; Osadchuk et al., 2021), ethnicity (Khandwala et al., 2017; Punjani et al., 2020), seasonal variations (Zhang et al., 2013; Aslam et al., 2021) and varicocele (Zhang et al., 2017; Pallotti et al., 2018). However, only few publications have reported the impact of these factors in semen parameters of men residing in Africa and the Middle East (Henkel et al., 2001; Owolabi et al., 2013; Bahri et al., 2021).

The World Health Organization (WHO) periodically releases the Laboratory Manual indicating reference values or, more recently decision limits, for semen analysis (WHO, 1999; WHO, 2010). In late 2010, the WHO published the 5th Edition of the manuals for the laboratory examination of human semen (WHO, 2010). Although this has been recently replaced by decision limits in WHO (2021), this study was conducted within the WHO (2010) framework and will be discussed in this context.

The WHO (2010) guidelines are considered as the standard methods for laboratories performing semen analyses worldwide. However, some geographical areas such as Africa were under-represented in the studies that generated the reference values (WHO, 2010; Cooper et al., 2010; Esteves et al., 2012). This retrospective study was performed to provide additional epidemiological semen parameter data of fertile and infertile men residing in Africa and the Middle East.

The results of this study included a total of 74,811 semen analysis reports retrospectively collected centrally from andrology laboratories in South Africa and Qatar that service Africa and Middle East countries and residents (Figure 3.1). This includes data of semen parameters and hormones profiles, alongside age, duration of abstinence period, geographical location, and seasonal variations of patients residing in Africa and Middle East regions from 2005 - 2019. However, although the

results of this study include men residing in Africa and Middle East, 85.8% of the reported are from men residing in South Africa.

4.1. Semen Parameters in Africa and the Middle East Based on WHO (2010) Criteria

The standard semen analysis is typically the first and central clinical assessment of male infertility for evaluation and management options (Teppa-Garrán and Palacios-Torres, 2004; Barratt, 2007). Seminal parameters, such as ejaculate volume, sperm count, viability, motility morphology and leukocyte concentration are important clinical indicators of male fertility (Barratt, 2007; Baskaran et al., 2020; Agarwal et al., 2021). In the full study cohort of males in Africa and the Middle East, the median found for ejaculate volume, sperm concentration, total sperm count, progressive motility and normal morphology were above the recommended thresholds, and leukocyte concentration was below the recommended thresholds, as determined by WHO (2010) (Table 3.1).

However, the median for sperm viability was below the recommended threshold values (WHO, 2010) (Table 3.1). Sperm viability results in the current study are from men residing in the Middle East and North Africa (MENA) region. As discussed below, males in the MENA region have reduced semen quality compared to other regions, which may be reflected in the viability results. A low percentage of viable spermatozoa is associated with high SDF and can impair male fertility (Samplaski et al., 2015).

The evaluation of SDF has been proposed to differentiate fertile from infertile men (Shamsi et al., 2011; Santi et al., 2018). Numerous different reports for SDF of infertile males have shown markedly different outcomes, ranging from 16% to 46% in relatively small studies using different techniques. Using the TUNEL assay, Sharma et al. (2010) reported a cut off value of 19.3% for SDF in 194 infertile men, while Sharma et al. (2016) indicated a cut-off point of 16.8% in 261 infertile men. An SDF cut-off value of 20.3% was found in a study involving 354 infertile and 40 fertile Egyptian men using the TUNEL assay (Hassanen et al., 2019). Using the

Sperm Chromatin Structure Assay (SCSA), Saleh et al. (2002a) reported a median (IQR) for SDF of 23% (15 – 32) in 16 patients using sperm chromatin structure assay, while cut-off values of 30% (Bungum et al., 2007), 27% (Simon et al., 2014), 20% (Oleszczuk et al., 2016) were indicated using the SCSA. Furthermore, using the Halosperm G2 test kit, Zekiraj et al. (2018) found a mean SDF of 34.5 % in 113 infertile men and 14.9% in 80 fertile men. Also using the Halosperm technique, Cankut et al. (2019) found a mean SDF of 25% in a cross-sectional study involving 100 normozoospermic men, while Elbardisi et al. (2020) reported a mean SDF of 29.8% in a similar cohort than the current study involving 1,147 men residing in Middle East and consulting at Hamad Medical Center, Doha, Qatar. Using receiver operator characteristics (ROC) analysis based on SDF data from the TUNEL assay, the sperm chromatin dispersion test (SCD), the SCSA and the Comet assay, a meta-analysis of fertile (n = 2,883) and infertile (n = 1,294) males, an SDF threshold of 20% has been recommended (Santi et al., 2018). However, 20% is not formally accepted as an SDF cut-off threshold. In this context, the median for SDF reported in our retrospective study using the Halosperm G2 test kit was 25% (Table 3.1). However, the SDF data in this study is from 838 male residents in the MENA region. In the current study, low sperm viability and high SDF was found in men residing in the Middle East. Furthermore, a negative correlation between SDF and sperm viability (Table 3.8) was found in the current study. This is consistent with the literature which highlights a negative correlation between SDF and sperm viability (Arends et al., 1990; Brahem et al., 2012; Samplaski et al., 2015). Both high SDF and low sperm viability are correlated with impaired male fertility (Samplaski et al., 2015; Zeqiraj et al., 2018).

An imbalance between ROS production and the antioxidant network in favour of the former is referred to as oxidative stress (Haleng et al., 2007; Agarwal et al., 2013), a term introduced into biology by Helmut Sies in 1985 (Sies, 1985). Seminal oxidative stress (OS) is a major cause of male factor infertility and can be measured as oxidation–reduction potential (ORP) (Arafa et al., 2019). ORP is a reliable method in predicting poor sperm parameters in infertile men (Agarwal et al., 2014a; Agarwal et al., 2016d; Agarwal and Wang, 2017; Agarwal et al., 2018) and in prediction in successful ICSI cycle (Morris et al., 2019). The median normed sORP result in our

retrospective study (1.82 mV/10^6 sperm/mL) (Table 3.1) is higher than the normal recommended value (1.34 mV/10^6 sperm/mL) (Agarwal et al., 2019; Arafa et al., 2020). The sORP results shown in this study were obtained from Hamad Medical Center in Qatar and Androcryos Andrology Laboratory in South Africa. The ORP test was used as additional sperm test, mostly in cases of sperm abnormalities. Consequently, this result is mostly from infertile patients undergoing further investigations that may explain our results. This is further supported in the literature, where ORP levels are found to be significantly higher in men with abnormal sperm parameters (Agarwal and Wang. 2017).

4.2.1 Categorization of Sperm Parameters

Male contribution to couple primary and secondary infertility in Africa is reportedly high, with oligozoospermia and asthenozoospermia as the most common abnormal semen parameters reported from African countries (Ikechebelu et al., 2003; Nwajiaku et al., 2012; Jimoh et al., 2012; Gyasi-Sarpong et al., 2017; Abede et al., 2020). However, definitions of these terms vary based on the use of WHO (1999) or WHO (2010) criteria. Based on the WHO (2010) definition of abnormal semen parameters, most patient records from Africa and the Middle East in this study had normal semen volume (87.1%), normal sperm concentration (74.6%), and normal sperm morphology (74.3%). The prevalence of oligozoospermia was 20.3%, azoospermia was 3.6%, and polyzoospermia was 1.5% of 65,761 sperm concentration reports. Approximately half (49.2%) of the 10,808 sperm motility reports were asthenozoospermic, and 25.7% of the 24,133 morphology reports were teratozoospermic (Table 3.2).

A recent meta-analysis of male infertility cases in African countries that included 21 studies reported a higher prevalence of oligozoospermia (31%) and azoospermia (14%) in 2486 samples, and a much lower prevalence of teratozoospermia (8%) in 1,522 samples, (Abebe et al., 2020). Similar observation was made in Nigeria where the prevalence of oligozoospermia was also found to be higher than that for azoospermia (Owolabi et al., 2013; Ikyernum et al., 2019). However, in Sudan, a higher prevalence of azoospermia (26.4%) compared to oligozoospermia (15.9%), asthenozoospermia (17.9%), and teratozoospermia (5.3%) was found (Elhussein et

al., 2019). The current study (Africa and Middle East) reported a lower prevalence of olizoospermia (20.3% vs 31%) and azoospermia (3.6% vs 14%) than Abebe et al. (2020) study (Africa). However, the prevalence of teratozoospermia (25.7% vs 8%) in the current study is higher than the one reported by Abebe et al. (2020). Compared to studies performed outside Africa, the prevalence oligozoospermia (20.3%) and azoospermia (3.6%) were lower than previously reported prevalences in a 10 years retrospective study India (34.1% and 10.7%) (Kumar et al., 2015) and in Saudi Arabia (27.2% and 10%) (Alenezi et al., 2014)

The prevalence of asthenozoospermia obtained in the current study (49%) is higher than previously reported (19%) across multiple African countries (Abebe et al., 2020), and higher than the 18.7% reported in a large population analysis where it was a common abnormal semen analysis finding (Curi et al., 2003). Furthermore, the prevalence of asthenozoospermia reported in the current study is higher than the 20.7% reported in Ivory Cost (Ayekoue et al., 2018), the 19% globally reported in infertile men (Askari et al., 2019) and higher than the 19% reported in the Middle Anatolian Region (Öztekin et al., 2019). In Nigeria, asthenozoospermia and teratozoospermia were previously found to be the most prevalent abnormal sperm parameters in subferile and infertile men (Ugwuja et al., 2008). This is aligned to results from studies performed outside Africa. In Pakistan, the prevalence of asthenozoospemia (35%) was higher than that for oligozoospermia (23%) and teratozoospermia (2.4%) (Khan et al., 2011). Similarly, a greater prevalence of asthenozoospermia (26%) compared to azoospermia (15%) and teratozoospermia (3.3%) was further reported from infertile males in Pakistan (Butt and Akram, 2013). However, in Italian males, the prevalence of asthenozoospermia (26.5%) is similar to oligozoospermia (26.2%) (Catanzariti et al., 2013). The most common etiological factors contributing to decrease in sperm motility include reproductive tract infections (Diemer et al., 2000; Diemer et al., 2003; Rusz et al., 2012), varicoceles (Zuccarello et al., 2008; Baazeem et al., 2011; Öztekin et al., 2019), and some genetic abnormalities (Heidary et al., 2020; Tu et al., 2020). An annual prevalence of STIs by region showed that sub-Sharan Africa had a higher prevalence (11.9%) than Latin America and Caribbean (7.1%), Eastern Europe and central Asia (2.9%), Australia and New Zealand (2.7%), North Africa and Middle East (2.1%), Western Europe (1.9), North America (1.9%), and East Asia and Pacific (0.7%), (World Health

Organization, 2001). In 2018, a report from the WHO indicated that STIs are more prevalent in Asia, sub-Saharan Africa, and Latin America (WHO, 2018). In sub-Saharan Africa, *Neisseria gonorrhoeae* and *Chlamydia trachomatis* were found to be the most common reproductive tract infections (Brunham et al., 1993; Adachi et al., 2016). African region was found to have higher prevalence rates of gonorrhoea, syphilis and trichomoniasis infections than American region, Eastern Mediterranean region, European region, South East Asian region and Western Pacific region, and a higher prevalence rate of Chlamydia infections than European region and South East Asian region (Newman et al., 2015). Based on the literature, reproductive tract infections may contribute to the high prevalence of asthenozoospermia in Africa and Middle East, however, this would require further investigations.

In the current cohort study, comparison between the sperm concentration categories showed that polyzoospermic samples had a significantly higher percentage of progressive motile spermatozoa and morphologically normal spermatozoa (Table 3.3). This is consistent with literature that suggests males with polyzoospermia have a higher percentage of morphologically normal spermatozoa and progressive motility compared to patients displaying oligozoospermia and normal sperm concentration (Khan et al., 2011; Butt and Akram, 2013). This is also reflected in the correlations between semen parameters analysed in the current study, where sperm concentration positively correlated significantly with progressive motility (r^2 = 0.357) and normal morphology (r^2 = 0.504) (Table 3.8). Furthermore, a greater number of spermatozoa with abnormal morphology are reported in oligozoospermic samples compared to polyzoospermic samples and those with normal sperm concentration (Khan et al., 2011; Butt and Akram, 2013).

Zhylkova and colleagues (2012) demonstrated that oligozoospermic patients had higher SDF compared to normozoospermic patients (42.1% vs 14.3%, respectively). This is consistent with the results of this study, where oligozoospermic patients had a significantly higher SDF, sORP and normed sORP, and lower progressive motility, compared to normal sperm concentration (Table 3.3). Furthermore, SDF is reportedly higher in asthenozoospermic and oligoasthenospermic males compared to normozoospermic males in Iraqi infertile males (Fadhil et al., 2020). This is reflected in the results of this study, where SDF is significantly higher in oligo-,

astheno- and teratozoospermic males (Table 3.5). The clinical utility of SDF in patients with severe oligozoospermia remains unresolved due to the difficulty to accurately evaluate DNA integrity in semen samples having very low sperm concentration (Majzoub et al., 2017).

The significantly higher normed sORP results in oligozoospermic patients compared to patients with normal sperm concentration are further supported by the strong negative correlation found between sperm concentration and normed sORP (Table 3.8). An increase in sperm concentration is associated with a decrease in normed sORP as expected by norming of sORP values to sperm concentration using the MiOXSYS system (Ochsendorf et al., 1994; Pasqualotto et al., 2001; Agarwal et al., 2019). Furthermore, seminal ORP level was previously reported to be significantly higher in oligozoospermic men compared to asthenozoospermic and teratozoospermic men (Agarwal and Henkel, 2017), and ORP had the highest predictive value in detecting oligozoospermia compared to other sperm abnormalities (Agarwal et al., 2014b; Agarwal and Wang, 2017).

The current study demonstrated that oligozoospermic patients had a significantly lower progressive motility and normal morphology results compared to normal sperm concentration (Table 3.3). This can explain the positive correlation found between sperm concentration and progressive motility and between sperm concentration and normal morphology (Table 3.8). However, the current study found that sperm concentration positively correlated with SDF, nevertheless, the correlation was very weak (r^2 = 0.224). This is an uncertain result which contrasts previous findings indicating an increase in sperm concentration associated with a decrease in SDF (Shuai et al., 2019; Chopra et al., 2021).

The current study demonstrates that patients with normal sperm concentration had a significantly higher progressive motility, percentage spermatozoa with normal morphology and a greater percentage of live spermatozoa than the other sperm classification groups (Table 3.5). Asthenozoospermic patients were found to have a significantly higher semen volume than patients having normal sperm concentration and those displaying teratozoospermia (Table 3.5). Furthermore, sperm concentration, motility and morphology correlated positively in the results of this

study (Table 3.8). These results are similar to previously published findings by Khan et al. (2011) who indicated that the highest progressive motility and the lowest percentage of spermatozoa with abnormal forms were found in patients having normal sperm concentration when compared to teratozoospermic, asthenozoospemic and oligozoospermic patients. Samples displaying asthenozoospermia were found to have the highest semen volume, while teratozoospermic patients were reported to have the lowest semen volume (Khan et al., 2011).

The role of leukocytes in male infertility remains unclear (Fedder, 1996; Mongioì et al., 2020). The presence of leukocytes (≥1 × 10^6 WBC/mL) in semen is refered as leukocytospermia (Ford, 2010). Although, the prevalence rates of leukocytospermia between fertile men and infertile men is not yet well established in the literature, leukocytospermia in infertile men was indicated to vary from 10% to 20% (Ford, 2010). However, higher prevalences of leukocytospermia in infertile men were reported by Lackner et al. (2008) and Gambera et al. (2007) (21% and 30%, repectively) whereas a lower prevalence of leukocytospermia (7.9%) was found in infertile patients by Wang et al. (1994) in a study which additionally found a leukocytospermia prevalence of 0% in the fertile group of men. A prevalence of leukocytospermia of 10% was found in the current study which involves fertile and infertile men (Table 3.2). Although this prevalence is lower than previously found in infertile men (Lackner et al., 2008; Gambera et al., 2007), it is within the infertile prevalence range suggested in infertile men (Ford, 2010) and higher than the prevalence of leukocytospermia found in infertile men in China (Wang et al., 1994). Several reports have highlighted the negative effects of leukocytospermia on semen quality (Saleh et al., 2002c; Aziz et al., 2004; Moubasher et al., 2018; Derbel et al., 2021), and is specifically suggestive of male genital tract infection or inflammation (Chen and Haidl, 2009; Wolf et al., 1995; Agarwal et al., 2014b; Henkel, 2021; Sharma et al., 2021). Infection in the genitourinary tract contributes to 15% of male infertility (Jung et al., 2016). In the presence of a male genital tract infection, the body produces leukocytes such as neutrophils and macrophages as a first line defence mechanism at the site of infection. Leukocytes consequently generate ROS which in excess can result in oxidative stress (Ochsendorf, 1999; Villegas et al., 2005; Lobascios et al., 2015; Henkel et al., 2021). The prevalence of

leukocytospermia in the current study may contribute to the elevated normed sORP result observed in the study (Table 3.1). The presence of high ROS concentrations can have a negative impact on spermatozoa (Sharma et al., 2001; Agarwal et al., 2003; Aziz et al., 2004; Henkel et al., 2005). Furthermore, a positive association between leukocytes and sperm normal morphology as well as progressive motility was reported (Lackner et al., 2010). The clinical significance of leukocytospermia in men residing in Africa and Middle East can be an important point of future research.

The presence and impact of epithelial cells in semen is not well discussed in the literature (Phillips et al., 1978; Johanisson et al., 2000; Swidsisnski et al., 2010; Andrade-Rocha, 2011). Generally, epithelial cells in semen come from the prostate and seminal vesicles, although protate epithelial cells are rarely identified in semen (Johanisson et al., 2000). In the current study no epithelial cells in semen were reported in 86.4% in the patients' reports, while in 11.5% of the patients had some epithelial cells (1+; 5 - 15 epithelial cells per microscopic field), and in 2.1% of the patients 16 – 25 epithelial cells per microscopic field (2+). There is no generally accepted methodology suggested to report the presence of epithelial cells in semen. The classification of the presence of epithelial cells in this study was a single laboratory methodology. Other quantitative techniques to identify epithelial cells in semen consist of plating out semen samples and obtain epithelial culture (Phillips et al., 1978).

4.3 Geographical Distribution of Semen Characteristics in Africa and Middle East

4.3.1 Middle East and Africa

There is limited data comparing semen quality from men residing in the Middle East region with those residing in Africa. In the current study, patients residing in the Middle East region (n = 1,198) had significantly lower semen parameters compared to Southern Africa (n = 64,439) and Eastern Africa (n = 2,069) patients, except for sperm concentration which was lowest in Eastern Africa. However, compared to Northern Africa (n = 600) residents, Middle East residents had significantly lower sperm volume, concentration and total normal sperm count only (Table 3.15). These

results may reflect the high prevalence of male factors involvement in couple infertility reported in the Middle East (60 - 70%) compared to Africa (43%) and Sub-Saharan Africa (20 – 40%) (Agarwal et al., 2015). However, the combined number of patients residing in the MENA region (n = 1,858) was considerably lower than the data from sub-Saharan (Southern and Eastern Africa combined) regions (n = 66,508). Furthermore, data for the Middle East countries (n = 1198) mostly represented men residing in Qatar (n = 654; 55%), where infertility is considered a national priority due to a steady decline in fertility rates over the past 55 years (Zauner and Girardi, 2020), and an increasing incidence of primary infertility (Arafa et al., 2017).

The WHO (2014) reports that the Middle East have some of the highest rates of obesity globally, with Kuwait, Bahrain, Saudi Arabia and United Arab Emerits in the top 10 countries affected (ALNohair, 2014). This includes up to 30% of males and 55% of females in the region (ALNohair, 2014). Qatar has an obesity prevalence of 33.1% (Arab Human Development Report, 2009), including 19% of males (ALNohair, 2014), driven predominantely by rapid modernisation, reduced physical exercise, and changes in food consumption (Taheri and Al-Thani, 2021). As a complication of obesity, the Middle East and Qatar also has high prevalance and an increasing incidence of type-2 diabetes mellitus (Alyafei et al., 2018; Awad et al., 2018). Type-2 diabetes mellitus affects approximatly 17.9% of the male population in Qatar (Meo et al., 2019), well above the 6.2% global burden of disease for males and females (Khan et al., 2020). As both obesity and diabetes are known to negatively affect male fertility as well as increase SDF through seminal oxidative stress and inflammation (Bener et al. 2009; Leisegang et al., 2019; Pearce et al., 2019; Leisegang et al., 2021), The elevated prevalence rates of obesity and diabetes described in Qatar might possibly be associated with vitamin D deficiency (Haj Bakri, 2012), as a very high prevalence vitamin D deficiency was reported in Qatar (Badawi et al., 2012). These modern lifestyle pathologies may contribute to the poor semen parameters identified in the Middle East region in this study.

Furthermore, a 19.9% prevalence of unilateral or bilateral varicocele was found in patients residing in the MENA region (Table 3.2). However, this is lower than a prevalence of 43% of varicocele in a cohort of infertile men that was previously

reported in Qatar (Elbardisi et al., 2016), and 86% found in primary and secondary infertility patients in Kuwait (Al-Kandari et al., 2020). A cross-sectional analysis investigating varicocele in male athletes in Saudi Arabia also reported a higher varicocele prevalence of 46%, with a higher rate in those who exercised more than 3 times per week (Hariri et al., 2019). However, the prevalence of 19.9% varicocele found in the MENA region is similar to the one previously reported (19%) among African fertile and infertile men in a meta-analysis (Abebe et al., 2020). Globally, the prevalence of 19.9% in the MENA region found in this study is higher than the 11.7% prevalence reported in men with normal semen (WHO, 1992) and higher than the 15% prevalence reported in the general male population (Clarke, 1966; Dabaja and Goldstein, 2016), but lower than the 19 – 41% reported for infertile men (Pastuszak and Wang, 2015). Increased ROS in the reproductive tract is associated with varicocele, as part of a complex pathophysiology of varicocele mediated deterioration in sperm concentration, motility and morphology (Kavish et al., 2009; Abd-Elmoaty et al., 2010). Interestingly, the results of this study found that the normed sORP was significantly higher in MENA regions compared to Southern Africa, with no statistical difference between Northern Africa and Middle East regions (Table 3.15). ROS can damage sperm plasma membrane by lipid peroxidation, increase SDF and negatively impact sperm flagellar structure (Henkel, 2012).

Another argument that might explain the differences in semen quality between men residing in African regions compared to the Middle East is the reported increased heat observed in the Middle East region (Lelieveld et al., 2016; Cheng et al., 2017). An increase in temperature (a rate of 0.5 °C (0.72 °C) per decade) between 1979 and 2009 has been reported in Saudi Arabia, alongside reduced rainfall, for example (Almazroui et al., 2012). Furthermore, the negative impact of summer on male sperm parameters is reflected in this study for the sub-Saharan African countries located below the equator (Table 3.19). Spermatogenesis is a temperature-dependent process and can be affected if the scrotal temperature increases (Paul et al., 2009; Durairajanayagam et al., 2015). Increased testicular heat stress is known to decrease semen quality (Wang et al., 1997; Munkelwitz and Gilbert, 1998), partly through the production of ROS which leads to increased apoptosis of germ cells and SDF (Park and Rhee, 2013; Durairajanayagam et al., 2015; Shahat et al., 2020). Furthermore, heat stress as a result of varicocele is considered as one of the most

plausible causes of impaired spermatogenesis (Clavijo et al., 2017; Hassanin et al., 2018).

The last point of consideration that can possibly explain the results obtained in the current study is the environmental contamination caused by the rapid urbanization, oil and natural gas extraction (Zauner and Girardi, 2020). Air pollution was found to be associated with a decrease in semen volume, sperm concentration, normal morphology, and motility in Egypt (Zhang et al., 2020). Furthermore, air pollutants were found to cause defects during gametogenesis and impaired fertility (Carré et al., 2017). The MENA region is characterized by high energy and carbon concentrations. It was previously found that the MENA region has highers CO_2 emissions compared to non-MENA areas (Goel et al., 2013) as one third of the world's oil production is coming from the Middle East (Puri-Mirza, 2021). Chemicals such as petroleum hydrocarbons resulting from oil activity were found in Qatari air, waters, and sediments (Dahab et al., 1993; Javed et al., 2019). Occupational exposure to hydrocarbon was found to negatively affect spermatogenesis and leading to sperm abnormalities (De Celis et al., 2000).

4.3.2 African Regions

The prevalence of male factor infertility without female factors in North and West Africa (4.2% - 6.4%) is reportedly higher than the prevalence in sub-Saharan Africa (2.8% - 4.3%) (Agarwal et al., 2015). Decreasing trends in sperm concentration, progressive motility and normal morphology between 2013 and 2018 in men residing in North Africa have been reported in North African countries (Bahri et al., 2021). This reflects the results of this study where men residing in Northern Africa had a significantly lower sperm concentration, progressive motility and normal morphology than men residing in Southern African and Eastern African countries, and significantly higher sORP and normed sORP compared to those residing in Southern Africa (Table 3.15). However, the number of patients residing in the Northern African region (n = 660) and Eastern African region (n = 2,069) was considerably lower than the data from Southern African region (n = 64,439).

Compared to retrospective data from North African men undergoing semen evaluation from 2013 – 2018 (Bahri et al., 2021), the results of this study showed similar sperm concentration (median = 24.0 x 10^6/ml; Table 3.15) compared to males from Tunisia (median = 26.4 x 10^6/ml), Algeria (median = 25.6 x 10^6/ml) and Libya (median = 26.2 x 10^6/ml) (Bahri et al., 2021). However, there was a lower sperm concentration in Mauritanian (median = 18.7 x 10^6/ml) and Morrocan (median = 14.7 x 10^6/ml) men (Bahri et al., 2021). Progressive motility (median = 10%) was much lower in the North African men in this study (Table 3.15) compared to males in Tunisia, Algeria, Libya, Mauritania and Morroco (median = 38.2%, 42.6%, 38.2%, 23.8% and 29.4%, respectively) (Bahri et al., 2021). This was similar for normal morphology (median = 3%) in North African males (Table 3.15) compared to males in Tunisia, Algeria, Libya, Mauritania and Morroco (median = 12.1%, 12.2%, 13.1%, 8.8% and 16.3%, respectively) (Bahri et al., 2021). However, most of the Northern Africa data in our cohort study is from Egyptian men (79%; n = 520; not reported in the results), followed by Tunisian men (6%; n = 40) and Moroccan men (3%; n = 22). It was previously indicated that fertile Egyptian men had lower reference values for total sperm count than those recommended by the WHO (2010) (Zedan et al., 2018). Furthermore, oligozoospermia was found to be the most common semen abnormality with varicocele being the most common cause of male infertility in Moroccan men (Benbella et al. 2018).

In Egypt, hepatitis C has been reported to be endemic and a significant public health concern (Elgharably et al., 2016). Hepatitis C may negatively affect semen parameters, including decreased semen volume, sperm count, and progressive sperm motility (Hofny et al., 2011; Karamolahi, et al., 2019). Furthermore, Egypt has the second highest number of people living with diabetes in the MENA region (International Diabetes Federation, 2019). In Tunisia, a high prevalence of male genital infectious diseases was reported, with *Chlamydia trachomatis* being most prevalent in male partners of infertile couples (Feki et al. 2009). These factors may partly contribute to the results of this study showing recuded semen parameters in the North African residents compared to Eastern and Southern Africa.

Comparing African regions in Sub-Saharan Africa, patients from Southern African countries had significantly greater sperm concentration, progressive motility, total

progressively motile count and total normal sperm count in comparison to patients from Eastern African countries (Table 3.15). In addition, patients residing in Southern African countries had significantly lower sORP and normed sORP results than patients residing in Eastern Africa countries (Table 3.15). The Eastern African residents mostly originates from men residing in Uganda (40%; n = 833), Kenya (29%; n = 602) and Tanzania (24%; n = 497) which fall beyond the infertility belt in Africa, a geographical region characterized with high male infertility prevalence (Etuk et al., 2009). In Kenya, reproductive tract inflammation was found to be the main cause of infertility in a small cohort (n = 55) of males with infertility of unknown cause, followed by hypogonadism and idiopathic male infertility, and only 5% with varicocele (Muthuuri et al., 2005).

On review of the literature, there are no additional studies identified geographically comparing semen parameters obtained from patients residing in Northern Africa, Eastern Africa, and Southern Africa. Most studies in Africa are performed either between countries located within the same region, or different fertility centres located in one country or just involving one single centre, and mostly involving fertile men (Emokpae et al., 2007; Akande et al., 2011; Zedan et al., 2018; Bahri et al., 2021). Consequently, this study provides a novel insight into the geographical distribution of semen parameters from fertile and infertile men in Africa.

4.3.3 Southern African Countries

Significantly higher sperm concentration and total sperm count were observed in men residing in Mozambique (n = 703) compared to men residing in Zimbabwe (n = 1315), Zambia (n = 1043), Lesotho (n = 330), and Swaziland (n = 316) (Table 3.16). Men residing in South Africa (n = 60,732) and Mozambique had a significantly higher progressive motility compared to patients residing in Zambia and Swaziland. Normal sperm morphology was significant higher in patients residing in South Africa (Table 3.16). The lowest sperm concentration, total sperm count, and semen volume were found in men residing in Zambia. These men also had a higher number of morphologically small spermatozoa, duplicated spermatozoa and a higher red blood cell count (Table 3.16). Moreover, these men had lower semen parameters (volume, sperm concentration, progressive motility and normal morphology) than the full

cohort of men investigated in the current study. Therefore, considering the semen parameters broadly, men residing in Zambia had the lowest semen quality, while patients from Mozambique and South Africa had best semen profiles in Southern African countries.

These results can be explained by the differences in age between the investigated groups. Patients residing in Zambia had the highest average age of 46 (39 – 52) years, where increasing age is associated with a decreased semen quality (Sloter et al., 2006; Johnson et al., 2015; Gu et al., 2018).

Although age can possibly explain the current results, some other considerable factors that might impede semen quality in men residing in Zambia can be postulated. Some of the potential causes of male infertility in Zambia include sexual transmitted diseases (STDs) and schistosomiasis (Bowa et al., 2008; Patil et al., 1988). In Sub-Saharan Africa, about 46% of men have infertility related problems caused by sexually transmitted diseases (Gerais and Rushwan, 1992), whereas in Nigeria (Western Africa), factors such as sexually transmitted infections and genito-urinary tract infections/inflammations were found to be the most common causes of male infertility (Abarikwu, 2013). In comparison to South Africa and Mozambique, Zambia is in the "infertility belt" region, where higher clinical infertility prevalence compared to other countries located outside the infertility belt region is partly caused by increased reproductive tract infections (Howe et al., 2020). However, patients from Zambia did not have increased seminal leukocytes compared to other countries in the region in our cohort.

Environmental influences can be furher considered as another factor associated with low semen quality in Zambia compared to other investigated Southern African countries. Despite the fact that there is no data on the effects of copper on semen parameters in Zambia, the country is classified as the fourth largest copper producer worldwide. Zambia provides approximately 6% of the global copper reserves (Owuor, 2019), making it the largest producer of copper in the Southern African region. Copper was indicated to be an industrial pollutant in Zambia (Pure Earth, 2006). Exposure to high concentration of this element is associated with an increase in oxidative damage, decrease spermatogenesis and male infertility (Sakhaee et al.,

2012). Additionally, excess copper in seminal plasma is associated with a reduced sperm count, motility, vitality and morphology (Eidi et al., 2010).

There has been an increase in prevalence of schistosomiasis in Zambia in various provinces (Siziya and Mushanga, 1996; Chitsulo et al., 2000), making it a major public health issue (Kalinda et al., 2018). The increasing prevalence of haemospermia due to schistosomiasis in Zambia has also been reported, with a significant association between hemospermia and schistosomiasis found (Elem et al., 1987). The results of this retrospective study also show an increased concentration of red blood cells in Zambian patient's semen (Table 3.16). Schistosomiasis infections has been associated with a poor semen quality, such as reduced sperm concentration, progressive motility and sperm viability, in a case report (Chohan et al., 2020), and an inhibition of gonadal functions with hypogonadism, reduced LH and reduced estradiol (Saad et al., 1999). No comparative study on the prevalence of schistosomiasis in Southern African countries could be identified.

The current study demonstrates significant differences in semen parameters obtained from semen reports obtained from patients residing in Southern African countries. Although, patients from Zambia had the higher average age, low semen quality profiles were found in those patients. With infertility in Zambia being under-researched and the lack of available data (Sunil et al., 2002; Pantazis and Clark, 2014) more investigations on the impact of environmental in Zambia need to be performed.

4.3.4 South Africa

A consistant increase in the annual number of semen analysis reports was observed from 2005 (n = 549) to 2014 (n = 7 065), followed by a constant decline until 2019 (n = 4920), from the included andrology laboratories (Ampath, Androcryos, Lancet) located in South Africa (Figure 3.2 A). Such trends are difficult to explain. However, from 2014, an increasing number of fertility clinics started to perform semen analysis 'in-house' instead of referring patients to andrology laboratories. This has resulted in fewer patients being referred to andrology laboratory such as Ampath, Androcryos

and Lancet (P Loubser 2021, personal communication, 09 October 2021). The increasing number of semen analyses from 2005 to 2014 might be a result of an increasing number of doctors referring patients for fertility assessments. The average monthly reports were consistent each month but reduced in December (Figure 3.2 B). December time in South Africa is the festive season which is generally characterized by holidays, family travels and gathering, and many institutions slow down significantly with not essential work.

Reports on fertility parameters and male infertity in South Africa are scanty. Male factor infertility in South Africa contributed for 21% of all infertility cases in couples in attending King Edward VIII Hospital (Durban, South Africa) in a small study involving only 100 participants (Chigumadzi et al. 1998). In a population of suspected infertile black Africans (n = 1726) in Pretoria, Gauteng, South Africa, 9% of patients were azoospermic, 9% were oligozoospermic (< 20 x 10^6/ml), 5% had polyzoospermia, 9% had impaired sperm motility, and 19% had less than 15% normal morphological forms (Bornman et al., 1994). Furthermore, STDs and unfavourable lifestyle was prominent, where 36% had reported a history of urethral discharge, with 66% reporting alcohol use at least once per week and 47% smoked more than 20 cigarettes per day. Varicoceles, however, were identified in only 11% of cases (Bornman et al., 1994). In a cohort of obese and non-obese males in the Western Cape, South Africa, 26.2% had oligozoospermia, 42,9% asthenozoospermia, 78,6% teratozoospermia, and 42,9% SDF > 20% (Leisegang et al., 2014). Semen donors in the South African Lead Acid Battery workers study (East London, Eastern Cape, South Africa) showed higher rates of adverse semen parameters, including 20.6% oligozoospermia (< 20 x 10^6/ml), 22.7% with a sperm concentration of 20 – 40 x 10^6/ml, 22.7% asthenozoospermia (< 50%), 96.9% with normal morphological forms less than 15%, and 71.1% with normal morphological forms less than 5% (Robins et al., 1997). This is smilar to Limpopo male cohorts exposed to DDT, reporting 28% - 29% oligozoospermia, 26% - 32% asthenozoospermia, 84% morphological forms less than 5%, and 99.5% morphological forms less than 15% (Dalvie et al., 2004; Aneck-Hahn et al., 2007).

On review of the literature, comparative studies of semen parameters from men residing in different areas within South Africa are not identified. A few studies have

reported semen analysis results from males exposed to DDT as part of malaria control in Limpopo Provice, South Africa, showing impaired semen parameters and SDF through non-occupational environmental exposure. Median sperm concentration in these studies were similar to the 43 x 10^6/ml found in the South African cohort (Table 3.16), but lower than the 60 x 10^6/ml found in the Limpopo region (Table 3.17), specifically 36 x 10^6/ml (Dalvie et al., 2004), 38 x 10^6/ml (De Jager et al., 2009) and 39 x 10^6/ml (Aneck-Hahn et al., 2007). In a healthy control male cohort (n = 42) in the Western Cape, South Africa, the average sperm volume (2.6 ml) and sperm concentration (43.7 x 10^6/ml) was similar to the median values in the South African cohort (2.8 ml and 43 x 10^6/ml, respectively) (Table 3.16), with a lower mean progressive motility in the Western Cape (29.4%) compared to the South African cohort (40%). However, the median semen volume (3 ml) and sperm concentration (50 x 10^6/ml) in the Western Cape cohort (n = 3,690) of this study (Table 3.17) was higher than these healthy males residing in the Western Cape (Leisegang et al., 2016). In this study, Limpopo ranked 5th and the Western Cape ranked 8th out of 13 regions for the highest median sperm concentration, where the Gautheng regions generally had the highest sperm concentration (> 60 x 10^6/ml in Gauteng Central, North, West and Gauteng Pretoria) (Table 3.17).

The current study demonstrated that patients residing in the Free State (51 x 10^6/ml) and Mpumalanga (54.4 x 10^6/ml) provinces had lowest median sperm concentration (Table 3.17). Statistics from Statistics SA (2019) on the total fertility rate per province (2016 – 2021) indicated a total fertility rate (TFR) of 2.3% in the Free State, the third lowest TFR amongst the nine provinces after Gauteng province (1.9%) and Western Cape (2%). Furthermore, the Free State was the province having the lowest life expectancy at birth (Statistics SA, 2021). Although total fertility rate is not considered as a direct measurement of normal semen analysis test, it was used to assess male infertility levels (Schoumaker, 2013).

It was previously found that the Free State have one of the highest percentages of elderly population group aged 60 years and older (9.8%) in South Africa compared to other provinces such as Gauteng (8.5%), Limpopo (9.0%), KwaZulu-Natal (8.2%) (Statistics SA, 2021). In the current study, men residing in the Free State had the highest average age. The literature has demonstrated an inverse association of

semen quality with age (Kumar et al., 2017; Gu et al., 2018; Pino et al 2020). This is supported by results in this study that show the oldest age group (older than 60 years) had significant lower semen parameters, although this age group had the lowest sORP, compared to all other age groups (Tables 3.10 and 3.11). Therefore, age can be considered as a potential explanation of the decrease in semen quality in patients residing in Free State.

Mining activity in Mpumalanga accounts for 83% of the South African's coal production (Africa mining, 2019). In South Africa, mining and processing of minerals are highly associated with occupational exposure to toxic substances such as platinum, chromium, vanadium, manganese, mercury, cyanide, and diesel particulate (Utembe et al., 2015). South Africa has the second highest reserve of vanadium in the world (Raja, 2007). Vanadium is a trace element which is reportedly associated with impaired sperm function (Domingo, 1996).

The current study identified differences in semen quality amongst patients residing in South Africa, where the Free State and Mpumalanga provinces had the lowest semen concentration amongst the investigated provinces. Factors such as age and environment might have had an influence on semen quality of men residing in those provinces. These results suggest the need for further study in different South African regions.

4.4. Reproductive Hormones and Semen Parameters in the MENA Region

The median level of total testosterone obtained in the MENA cohort (16.4 nmol/L) (Table 3.1) from men aged 39 (35 – 45 years old) is within the harmonized reference ranges (9.2 nmol/L – 31.8 nmol/L) for total testosterone levels obtained from non-obese healthy males (18 – 39 years old) in USA and Europe (Travison et al., 2017). This is also above the lower limit for testosterone (12.1 nmol/L) obtained in males from the Framingham Heart Study Generation 3 (Bhasin et al., 2011). Furthermore, the median testosterone level in the MENA cohort is similar to total testosterone reported in 35 fertile males (17.4 nmol/L) (Babu et al., 2004).

The median testosterone level in the MENA cohort (Table 3.1) is lower than previously reported in males with azoospermia (17.1 nmol/L; n = 35), oligozoospermia (16.9 nmol/L; n = 35) and varicocele (17.6 IU/L; n = 11) (Babu et al., 2004). Furthermore, the median testosterone concentration in the normozoospermic group (12.2 nmol/L) (Table 3.6) was lower compared to testosterone previously reported in normozoospermic men (16.2 nmol/L) (Luboshitzky et al., 2002). Similarly, to these results, males with asthenozoospermia and oligozoospermia are reported to have higher testosterone levels compared to nomozoospermia males (Luboshitzky et al., 2002).

Low testosterone concentrations can be due to testicular, hypothalamic, or pituitary abnormalities (Kumar et al., 2010). Primary testicular failure is associated with a decrease in testosterone levels (Bhasin, 2007) and non-obstructive azoospermia (Cocuzza et al., 2013). Salama and Blgozah (2020) reported lowest levels of testosterone in males with low estradiol compared to normal and high estradiol groups of males with azoospermia. The highest testosterone levels were found in the oligozoospermic group, alongside the highest estradiol, FSH and LH, and second highest prolactin levels (next to normpzoospermic group) (Table 3.6). Similarly, Salama and Blgozah (2020) reported higher testosterone levels in males with high estradiol compared to normal and low estradiol groups. Although these were males with azoospermia, similar estradiol and testosterone levels were reported in fertile control males (Salama and Blgozah, 2020).

LH and FSH are gonadotropins that maintain testosterone production and spermatogenesis (Anawalt et al., 1996). The median FSH and LH levels in the MENA cohort (3.3 IU/L and 3.7 IU/L, respectively) (Table 3.1) are within the normal range previously provided for these gonadotropins (LH: 1.5 – 8 IU/L; FSH: 2 – 7 IU/L) in males (Raven et al., 2006). However, this is lower than FSH and LH reported in fertile males (8.5 IU/L and 7.9 IU/L, respectively) (Babu et al., 2004). Luboshitzky and colleagues (2002) further reported a higher level of LH (4.7 IU/L) and FSH (4.4 IU/L) in normozoospermic men compared to the medians in this study (3 IU/L, and 2.7 IU/L, respectively) (Table 3.6). Both LH and FSH are increased in asthenozoospermia and oligozoospermia patients (Luboshitzky et al., 2002) as also reported in this MENA cohort. Furthermore, the median FSH and level in the MENA

cohort is lower than males with azoospermia (12.6 IU/L and 12.4 IU/L, respectively; n = 35), oligozoospermia (12.9 IU/L and 15.0 IU/L, respectively; n = 35) and varicocele (20.3 IU/L and 12.9 IU/L, respectively; n = 11) as previously reported (Babu et al., 2004).

Estradiol contributes significantly to male reproductive physiology by exerting an inhibitory effect on the hypothalamus and anterior pituitary similar to testosterone. (Schulster et al., 2016; Gurung et al., 2021), The median estradiol level in the MENA cohort (97.0 pmol/L) (Table 3.1) is within the reference values been previously provided for estrodiol (50 – 200 pmol/L) (Raven et al., 2006). However, this is lower than estradioal reported in 35 fertile males (117 pmol/L) (Babu et al., 2004). Luboshitzky and colleagues (2002) further reported a higher level of estradiol (117 pmol/L) in normozoospermic men compared to the median in this study (72 pmol/L) (Table 3.6). Furthermore, estradiol is also reported to be increased in asthenozoospermia and oligozoospermia patients (Luboshitzky et al., 2002), as reported in this MENA cohort.

Prolactin acts as a trophic hormone (Dombrowicz et al., 1992; Hair et al., 2002) and is sensitizing the testis to LH (Bartkle and Dalterio, 1976). The median prolactin level in the MENA cohort (195.4 mIU/L) (Table 3.1) is within the normal reference range for men (58-419 mIU/L) (Whitehead et al., 2015).

In this study, LH positively correlated with FSH, testosterone, estradiol and prolactin. FSH positively correlated with testosterone. Furthermore, testosterone positively correlated with estradiol. These correlations are supported in a cohort of sub-fertile men, reporting a positive correlation for LH with FSH, and LH with total testosterone, total/free testosterone and total estradiol. (Zhao et al., 2020). In fertile Australian men, LH and FSH were positively correlated, as well as LH and testosterone (Stewart et al., 2009).

In the MENA cohort, there was a negative correlation found between testosterone, LH and FSH with sperm concentration and normal morphology, and FSH with progressive motility (Table 3.8 and Table 3.9). This is supported in the literature, where LH and FSH both negatively correlate with sperm concentration, motility and

normal morphology in males with fertility problems, although no correlation was found for testosterone (Kumanov at al., 2006). Furthermore, there are increased odds ratios for oligozoospermia, asthenozoospermia and teratozoospermia with high levels of FSH and LH compared to low and normal levels, although again no significant effect for testosterone on semen parameters was reported in the general population (Meeker et al., 2007). However, Zhao and colleagues (2020) reported negative correlations each for LH, FSH and total testosterone with sperm motility after ajustments for age, BMI, smoking and alcohol consumption. There were no significant correlations with these hormones and sperm concentration, and after adjusting for FSH, LH and total testosterone, only LH remained negatively correlated with motility. LH and FSH were also negatively correlated with normal sperm morphology (Zhao et al., 2020). In fertile Australian men, FSH and LH were found to negatively correlate with sperm concentration (Stewart et al., 2009). The negative correlation between LH, FSH and sperm concentration observed in the current study can be a result of the negative feedback by inhibin B on the anterior pituitary to inhibit FSH (Anawalt et al., 1996). Inhibin B levels are reportedly higher in men with normal sperm concentration than those with testicular dysfunction (Manzoor et al., 2012), and considered a more important biomarker of fertility status and spermatogenesis than FSH (Kumanov et al., 2006).

Estradiol negatively correlated with motility and normal morphology (Luo et al., 2021). Similar results were obtained in the current study where estradiol negatively correlated with progressive motility and normal morphology (Table 3.8). In the current study, no correlation was observed between estradiol and sperm concentration (Table 3.9). This result is similar to those previously reported by Wei et al. (2013) in patients with idiopathic or varicocele-related oligoasthenoteratozoospermia (OAT) syndrome. However, this result is different from the negative correlation between estradiol and sperm concentration found by Luo et al. (2021) in a cohort of infertile patients. The current study suggests that estradiol might not be associated with spermatogenesis. These results provide new perspectives on the current understanding of the role of estrogen in human spermatogenesis. The current cohort study showed that azoospermic men residing in the MENA region had the lowest estradiol concentrations and the lowest testosterone levels (Table 3.6). This is relevant in the current study, where men

residing in the MENA region were found to have a lower semen quality than men residing in sub-Sharan Africa.

Abnormally high levels of prolactin cause hypogonadism (De Rosa et al., 2003), potentially leading to a decrease sperm concentration. Oligozoospermic men were found to have higher prolactin concentration than normozoospermic men (Vandekerckhove et al., 2000). The negative correlation between prolactin and sperm concentration was found in the current study (Table 3.9). Furthermore, an increasing dose of prolactin was previously found to reduce sperm motility (Lübbert et al., 1992).

There are limited studies so far evaluating the relationship between the seminal ORP levels and reproductive hormones. Determining the association between seminal ORP and reproductive hormones is important as ORP was found to have a negative correlation with sperm parameters (Agarwal and Wang, 2017; Agarwal et al., 2017b). ORP is positively and significantly correlated with serum hormone levels, FSH and LH levels (Arafa et al., 2019). Similar results were obtained in the current study where FSH and LH positively correlated with normed sORP (Table 3.8). The current study found that estradiol and prolacting significantly positive correlated with normed ORP (Table 3.8). These results might be explained by the negative effect of oxidative stress on Sertoli cells. Sertoli cells contribute to the synthesis of estradiol (Dorrington, 1978). Oxidative stress causes degeneration of Sertoli cell with atrophy of the testis (Kasahara et al., 2002; Arafa et al., 2019). An upregulation of gene expression in Sertoli cells can lead to an increase in estradiol concentration (Cirelli et al., 2017). Furthermore, the negative effects of oxidative stress on Sertoli cells might results in a decrease secretion of inhibin caused by negative feedback on the hypothalamus. This could lead to an increase secretion of LH and FSH (Arafa et al. 2019). Consequently, increasing oxidative stress can be associated with an increase in LH and FSH as observed in the current study.

4.5. Age and Semen Quality in Africa and the Middle East

Investigating the effect of male age on semen quality and reproductive hormones is becoming a major public health issue, due to the increasing number of men who

decide to have their children at older ages (Ventura et al., 1997; Ford et al., 2000; Harris et al., 2011). Although, based on birth rate data in the USA, the peak age for fathers was reported as 25 – 29 years old, followed by the 30 – 34 years old group (Ventura et al., 1997). The median (IQR) age of the full cohort in our retrospective study was 38 years (34 – 43) (Table 3.1). This is reflected in the age distribution, with the largest percentage (34%) of patient records categorised as 35 – 40 years old, and the majority of patients (56%) categorised as 30 – 45 years old (Figure 3.3).

A steady decline in various semen parameters with increasing age has been proposed in the literature. In non-smoking males without known fertility concerns between 22 and 80 years of age, semen volume decreased by 0.03 ml per year, as did sperm total motility, progressive motility and total progressively motile sperm (0.7%, 3.1% and 4.7% per year, respectively). However, there was only a suggested decrease in sperm concentration with age (Eskenazi et al. 2003). A decline in sperm motility has been reported with age in healthy males aged 22 – 80 years of age (Sloter et al., 2006). In a meta-analysis of 90 studies and 93,839 males, age related declines are found for semen volume, progressive motility, total motility, normal morphology and unfragmented cells, independent of confounding variables. However, there was no marked decline in sperm concentration (Johnson et al., 2015).

This is reflected in the results of this retrospective study. The median semen volume was found to be significantly reduced in the age groups from 41 years. Sperm concentration peaked at 31 – 40 years and 41 - 50 years groups, declining from 51 years of age. A similar trend was seen for progressive motility, highest in ages 21 – 30 years and 31 – 40 years age groups before declining in the older age groups. Normal morphology also showed a downward treand in older age groups after peaking at 31 – 40 years of age (Table 3.10).

The trends found in this study are similar to the threshold age suggested by Levitas et al. (2007) based on 6022 semen samples, where the best sperm parameters were seen at age 30 – 35 years. This is supported in reports indicating that males older than 41 are more likely to have reduced sperm concentration, and those aged older than 31 are more likely to have reduced motility in 2678 men undergoing semen

evaluation (Pino et al., 2020). Furthermore, progressive motility began to decline from 28 years of age, earlier than sperm concentration that began to decline at 58 years of age (Li et al., 2019), similar to our results. However, in a Chinese population of infertile males (n = 71,623), there was a non-significant decline of sperm parameters with age. Furthermore, Boulegue et al. (1999) found no age-related decline between 20 and 60 years of age, although further concluding that ejaculate quality remains the same at all age groups until 50 years of age.

Although a peak age of 30 years was found for the progressive motility and normal morphology, a point to consider is the differences in sample size, a factor that might be a limitation in the current analysis. In fact, only 31 results for progressive motility were evaluated in the study, which is significantly less than the 10,706 results analysed for men aged more than 31 years old. Having comparable sample sizes might have shown an age-related constant decrease in progressive motility. Similar observation for sperm morphology can be reported. The current study only looked at 50 normal morphology results for men aged less than 30 years, against 23,952 morphology data for men aged above 30.

SDF was also found to increase steadily through each advancing age group (Table 3.11). As SDF data is from the MENA region only, it is limited in sample size particularly at the extremes of the age groups analysed. However, these results are supported by numerous studies that have reported a positive correlation between SDF and male age (Das et al., 2013; Gu et al., 2018; Petersen et al. 2018; Yang et al., 2019; Mettler et al., 2019; Rubes et al., 2021). Furthermore, men aged more than 50 years are 4.8 times more likely to present increased SDF compared to those aged between 21 and 30 years (Pino et al., 2020). SDF is associated with a longer time to conceive, impairement of embryo development and higher miscarriage rates (Morris et al., 2000; Evenson and Wixon, 2008; Robinson et al., 2012). Oxidative stress is one of the main factors triggering SDF (Albani et al., 2019).

Increasing evidence suggests that oxidative stress associated with advancing age negatively affects sperm parameters and SDF, in turn affecting fertility, pregnancy outcomes and the health of the offspring (Leisegang et al., 2017; Nago et al., 2021). Although normed sORP generally increased with advancing age groups in this study,

these changes were not generally significant. However, there was a non-significant reduction of normed sOPR in the over 60 years of age group compared to all groups analysed (Table 3.11). The sample size of normed sORP however is small, with 136 patient reports available for patients aged more than 50 years, compared to the 2,700 reports from patients aged less than or equal to 50 years. Agarwal and colleagues (2018) showed no correlation between age and ORP while Cocuzza et al. (2008), Koh et al. (2016) and Nago et al. (2021) suggested that male age could impact seminal oxidative stress. Cocuzza et al. (2008) suggested that older men have increased levels of ROS and/or decreased antioxidant capacities in semen. Mitochondrial disruption due to aging results in increased ROS production, decreased ATP production, and apoptosis (Gunes et al., 2016). This leads to a decrease in antioxidant capacity in the body, and potential oxidative damage to spermatic DNA (Pino et al., 2020).

There are several factors associated with a decrease in semen quality with increasing age that are proposed (Feldman et al., 1994; Feldman et al., 2002). Late-onset hypogonadism, characterized by a decrease in serum total testosterone levels with age, is amongst the main causative factors (Feldman et al., 1994; Feldman et al., 2002; Golan et al., 2015). Furthermore, the decline in serum total testosterone with age was suggested to be associated with alterations in the composition of the body, a decrease in energy, cognition, sexual function and muscle strength (Matsumotso, 2002). Another point of consideration can be the decline in free testosterone with age, which was associated with sex hormone binding globulin (SHBG) levels. SHBG is a protein produced in the liver which binds to free testosterone for transport in the blood. Consequently, a high level of SHBG can cause a decrease in availability of free testosterone (Selby, 1990). In males, LH and SHBG increase with age, while testosterone and free testosterone decrease with age (Liu et al., 2007; Zhou et al., 2020). Furthermore, anatomic changes (Kühnert and Nieschlag, 2004) such as reduced testicular volume are also associated with a decrease in Leydig cell and Sertoli cells numbers (Johnson et al., 1986; Mahmoud et al., 2003; Zirkin and Tenover, 2012; Golan et al., 2015). Additionally, age-related oxidative damage may contribute to a decrease in reproductive ability (Leisegang et al., 2017).

4.6. Age and Reproductive Hormones in the MENA Region

In 2020, the percentage of males aged 65 years and above in the Middle East was 5%, compared to 2.7% in the sub-Saharan region (World Bank, 2021). Longitudinal results have reported a decrease in serum testosterone and estrone, and an increase in prolactin levels, with icreasing age in males (Feldman et al. 2002). Late-onset hypogonadism is a clinical syndrome associated with advancing age that is biochemically characterized by a deficiency in serum testosterone levels (Mahmoud et al., 2006). The decrease in testosterone is associated with a decrease in estradiol (Decaroli and Rochira, 2017), and an increase in LH (Tajar et al., 2010) and FSH (Griffin et al., 2001) levels. Hypogonadism in males increases from 20% of males over 60 years to 50% of males over 80 years of age (Harman et al. 2001).

In this study, a constant age-related decline in testosterone and prolactin levels, and an increase in FSH, was found from 16 years to 60 years of age in men residing in the MENA region. However, both testosterone and prolactin were found to be increased again in the greater than 60 years age group compared to younger ages. (Table 3.12). These results are similar to previous findings by Feldman et al. (2002) in middle-aged men, reporting increases in FSH and decreases in testosterone levels with age. Although the level of testosterone seems to be higher for men aged 60 years and above in this study, the sample size of men aged 60 years and above (n = 62) is remarkably lower than the 51–60 years group (n = 312), the 41-50 years (n = 683), 31-40 years (n = 864) and the 21-30 years (n = 637). No significant influence of age on LH was observed in males in the MENA region (Table 3.12). This result is different from the significant increase in LH and FSH with age previously reported (Morley et al., 1997; Yu et al., 2017).

No significant influence of age on estradiol was observed in males in the MENA region (Table 3.12). However, increasing age is associated has been associated with a loss of estradiol (Horstman et al., 2012), which is important in spermatogenesis (O'Donnell et al., 2001). Furthermore, decreases in prolactin concentration were observed in each age category until 60 years old. The highest level (248.90 mIU/L / 11.66 ng/ml) found in the 16 – 20 years old group is less than the upper normal limit (20 ng/ml) suggested by Thapa et al. (2020). The lowest level (175.30 mIU/L / 8.23

ng/ml) found in the 61 – 60 years old group is higher than the normal average basal limit (5 ng/ml) reported by Thapa et al. (2020). Prolactin is a hormone that was found to significantly increase with age (Sawin et al., 1989; Kipp, 2007). Although in the current study, different trends were observed it is important to note that most results were not significant.

4.7. Abstinence Periods and Semen Quality in Africa and the Middle East

Numerous studies have been undertaken to deterimine the influence of ejaculatory abstinence on various semen parameters. Increases in semen volume, pH, viscocity, sperm concentration, total sperm count increase, total motile sperm and SDF are seen with recommended abstinence time (2 – 5 days) compared to shortened abstinence time, with further increases in these parameters with long abstiance compared to recommended 2 – 5 day period (Mayorga-Torres et al., 2016; Agarwal et al., 2016b; Borges et al., 2019). Based on a systematic review, the weight of evidence suggests reduced semen volume and sperm concentration with shorter abstinence, not all studies have the same conclusions and the relationships are complex (Ayad et al., 2018; Hanson et al., 2018).

The current study cohort evaluated the effects of different periods of abstinence (< 2 days; 2 – 5 days; >5 days) on basic semen parameters and functional tests. An increased duration of abstinence significantly increased semen volume, sperm concentration, progressive motility, normal morphology and SDF, with a decreased in normed sORP (Table 3.13 and Table 3.14). Similarly, in 2,458 fertile and infertile men, there was a significantly increased semen volume and sperm concentration with an abstinence period of more than 5 days compared to an abstinence period of 2 to 5 days and less than 2 days (Comar et al., 2017).

However, Dupesh and colleagues (2020) found only a reduction in semen volume with shorter abstinence periods, and no change for sperm concentration, motility or morphology. Furthermore, numerous studies have reported that shorter abstinence may be associated with increased sperm motility, morphology and SDF (Agarwal et al., 2016; Ayad et al., 2017; Comar et al., 2017; Borges et al., 2019; Dupesh et al., 2020; Okada et al., 2020; Dahan et al 2021; Sokol et al., 2021), questioning the

WHO (2010) recommentations for abstinence of 2 – 5 days (Sokol et al., 2021). The results of this cohort study are contrary to these reports, as progressive motility, normal morphology and SDF were significantly reduced with abstanance less than 2 days compared to abstiancne of 2 – 5 days (Table 3.13). Interestingly, the use of antioxidants was found to have lower SDF with shortened abstinence than short abstinence iwhtout antioxidant use (Dahan et al., 2021).

Although there was a downward trend in normed sORP with increasing abstinence in this study cohort, this was not significant. There is no published data on the impact of duration of abstinence on sORP and normed ORP identified in the literature. Therefore, the current study provides a novel in the investigation on the influence of ejaculatory abstinence on oxidative stress evaluated using the sORP.

4.8. Temporal Changes in Semen Quality (2005-2019) in the Sub-Saharan Region

In 1992, Carlsen and colleagues published a landmark article suggesting a decline in sperm parameters from previous data available on 14,947 men between 1938 and 1991 (Carlsen et al., 1992). Although this was associated with numerous critiques and comments (Olsen et al., 1995; Lerchl et al., 1996; Swan et al., 1997; Swan et al., 2000), numerous studies have further provided evidence for this decline (Irvine et al., 1996; Swan et al., 1997, Swan et al., 2000, Levine et al., 2017; Sengupta et al., 2017b). Several reports have suggested a global decline in semen quality in America (Younglai et al., 1998), Asia (Bahk et al., 2010; Tang et al., 2015; Huang et al., 2017; Sengupta et al., 2018a; Liu et al., 2020), Europe (Bendvold et al., 1991; Auger et al., 1995; Irvine et al., 1996; Van Waeleghem et al., 1996; Sengupta et al., 2018 b), Africa (Osegbe et al., 1986; Sengupta et al., 2017b), and in the MENA region (Bahri et al., 2021). Conversely, some reports indicated no decline in semen quality over years (Bujan et al., 1996; Lipshultz, 1996; Saidi et al., 1999; Costello et al., 2002).

While semen quality seems to have declined dramatically in recent times, the data available on male fertility status and semen quality in sub-Saharan Africa are limited. The reported decrease in semen quality in Africa is generally limited to Northern African countries (Bahri et al., 2021) or Western Africa (Nigeria only) and mainly

focus on sperm concentration (Sengupta et al., 2017b). This retrospective data available in the sub-Saharan Africa region over a 15-year period (2005 – 2019) has shown significant trends during this time. Significant decreases in semen volume and sperm concentration were found (Table 3.18). It was previously indicated that sperm normal morphology decreases over time (Bahri et al., 2021; Rosa-Villagrán et al., 2021). Sperm morphology from men residing in sub-Sahara Africa significantly declined alongside the percentage of small spermatozoa, but an increased percentage of elongated spermatozoa was found over time (Table 3.18). An increase in the percentage of elongated spermatozoa was associated with testicular stress caused by male urogenital tract infection (Menkveld et al., 2011).

The findings of the current study align sub-Saharan African region to the global decreasing trends in semen quality over years observed in other regions (Younglai et al., 1998; Sengupta et al., 2018b; Liu et al., 2020). As opposed to other sperm parameters, progressive motility was found to increase over years (Costello et al., 2002). This was observed in the current study, where an increase in progressive motility and total progressively motile sperm were found (Table 3.18), although there is a lack of these variables available for 2013 - 2018 (Table 3.18). This comprehensive retrospective study concisely presents the evidence of decreased sperm concentration, morphology, semen volume, in men residing in sub-Sahara Africa over the past 15 years (2005 – 2019).

4.9. Seasonal Variation in Semen Parameters in sub-Saharan Africa below the Equator

The possible changes in semen parameters according to seasonal variations have been investigated in the literature (Levine 1991; Levine, 1999; Henkel et al., 2001; Zhang et al., 2013), with conflicting results. Some reports highlighted changes in semen parameters such as semen volume (De Giorgi et al., 2015), sperm concentration and total sperm count (Gyllenborg et al., 1999; Zhang et al., 2013), progressive motility (Zhang et al., 2013; De Giorgi et al., 2015), normal morphology (Zhang et al., 2013) and chromatin condensation (Henkel et al., 2001), while others reported no seasonal variations in semen parameters (Carlsen et al., 2004).

However, there has not been any data about seasonal changes in semen parameters in sub-Sahara Africa.

The current study found that sperm concentration and total sperm count were significantly higher in winter compared to summer and autumn, with the lowest sperm concentration reported in summer (Table 3.19). It was previously found that sperm concentration was highest in winter in United States and in Asia (Chen et al., 2003; Yogev et al., 2004), and the lowest semen volume in summer in China (Zhang et al., 2013).

In the current study, no significant seasonal changes in semen pH, progressive motility and normal morphology were found (Table 3.19). Although the results for semen pH were not significant, a higher semen pH was observed during spring (Table 3.19). This is consistent with previous findings by De Giorgi et al. (2015). Semen volume was found to be higher in spring compared top winter and autumn (Luo et al., 2018). This was observed in the current study (Table 3.19).

In the current study, sperm abnormalities (elongated and duplicated) were found to be higher in summer and spring than in winter. Testicular heat stress has frequently been associated with a decrease semen quality (Levin et al., 1990; Durairajanayagam, et al., 2014). Sperm elongation is well recognized as a stress-induced sperm morphology aberration (Menkveld et al., 2011).

Data on possible changes in semen ORP is limited, and almost non-existent. In the current study, significantly higher normed sORP was found in winter, although the sample size was extremely very low (n = 8) (Table 3.19). To our knowledge this is the first study to analyze the seasonal variations of ORP in sub-Saharan Africa. The current study suggests evident seasonal changes in semen parameters with respects to semen volume, sperm concentration, total sperm count, and normed sORP obtained from men residing in sub-Saharan Africa.

4.10. Global Comparison of Semen Parameters from Africa and the Middle East in the Context of WHO (2010) Guidelines

Semen analysis is the corner stone of the evaluation of male infertility (Esteves, 2014). The WHO periodically releases universal guidelines to help the practitioners

and andrologists to make decisions in evaluating semen samples and to categorize them as normal or abnormal. Several studies have quantified the shift from one guideline to another in order to reclassify previous semen analysis parameters and provide a definition of patients with male factor infertility (Murray et al., 2012; Alshahrani et al., 2018). It was previously found that the WHO (2010) recommendations result in some infertile men being reclassified as fertile if status is based on semen analysis alone (Murray et al., 2012). The current study found that 11% of the patients, having normal semen volume following the WHO (2010), would have been considered abnormal according to the WHO 1999 classification (WHO, 1999). Similar observations were made for sperm concentration where 4.3% of patients who were previously classified as having abnormal sperm concentration following the WHO (1999), are having normal sperm concentration following the WHO (2010) (Table 3.7).

The analysis performed by the WHO (2010) to determine the lower reference limits to differentiate for normal and abnormal semen parameters did not include any data from Africa (Esteves et al., 2012). The more recent WHO (2021) guideline is based mostly on data originating from Chinese men (more than 60%) and only included one study from Northern Africa (Kandil et al., 2021). This might not provide a global reference values for semen parameters. The current study compared data obtained from a cohort of men residing in Africa and Middle East to data from Cooper (2010) and Campbell (2021). Cooper (2010) data refers to published data by Cooper et al. (2010) which was used to formulate the WHO (2010) reference intervals for semen parameters, while Campbell (2021) data refers to published data by Campbell et al. (2021) and used to formulate the WHO (2021) decision limits.

Semen parameters (volume, sperm concentration, progressive motility, total progressively motile count, normal morphology, and total normal sperm count) obtained from Africa and Middle East were significantly lower than the values reported in the Cooper (2010) and the Campbell (2021) data (Table 3.25). The percentage of sperm viability was significant lower in the African and Middle East data compared to the Campbell (2021) data (Table 3.25). These results could be explained by the inclusion and exclusion criteria within the data set. Data from Africa and the Middle East was obtained from fertile and infertile men while the Cooper

(2010) and Campbell (2021) data included semen analysis results obtained from fertile men in partnerships with time to pregnancy (TTP) of 12 months. Therefore, data from Africa and the Middle East is more likely to be lower than the Cooper (2010) and the Campbell (2021) data. The differences in data set (fertile and infertile) require the results to be interpreted with caution. The difference in age between the data sets could provide further explaination of the results observed in the current study. The average age in the African and Middle East data (38.00 (34-43) years) was higher than in the Cooper (2010) data (31.00 (28 - 36) years). The effect of aging on semen parameters was evaluated in the current study. Significant negative correlations were found between increasing age and semen parameters (Table 3.10), sperm functional tests (Table 3.11) and reproductive hormones (Table 3.12).

Geographical differences in semen quality are well documented in the literature, with most studies highlighting differences between countries (Jensen et al., 2000; Jorgensen et al. 2001; Palani et al., 2020) and provinces within the same country (Swan et al., 2003; Glina et al., 2010). The current study evaluated the differences in semen parameters between patients residing in Africa (from the cohort study) and previously reported semen parameters results from patients residing in America, Asia, Australia and Europe (from the Cooper (2010) data). Men residing in African countries had a significant lower semen volume than those residing in America, Australia and Europe (Table 3.29). Furthermore, men residing in Africa were found to have significant lower sperm concentration, total sperm count, progressive motility, total progressively motile count, normal morphology and total normal sperm count (Table 3.29). The differences in semen quality amongst the different regions observed in the current study could be explained the inclusion and exclusion criteria of data as previously discussed. The current study is the first to our knowledge to provide an attempt in comparing data from 5 continents. Sperm concentration results obtained in the current study for men residing in Africa is lower than those previously reported in America (Younglai et al., 1998; Redmon et al., 2013; Junior et al., 2015), Asia (Wang et al., 2017; Lu et al., 2018; Liu et al., 2020), and Europe (Irvin et al., 1996; Sripada et al., 2007; Rahban et al., 2019).

Various explanations have been put forward in the literature for the observed differences in semen between men residing in different continents. General lifestyle

factors such as regular consumption of alcohol and tobacco products were indicated to be the main causes of the decrease in sperm concentration in Africa (Okonofua et al., 2005; Sengupta et al., 2017). Most African data in the current study is from South Africa, which was classified as the country with the highest consumption of alcohol per year in Africa and ranked 8th in the world in 2021 (WHO, 2021). South Africa has the 3rd highest prevalence of tobacco consumption in Africa after Lesotho and Morocco (Conway, 2020). Non-communicable diseases such as obesity, diabetes mellitus, hypertension, were highlighted as important socio-economic determinants impacting African mens' health (Heyns and Bornman, 2008; WHO, 2016). A controlled male cohort by Leisegang et al. (2014) highlighted the negative effect of obesity on male reproductive function in South Africa. Sexual transmitted diseases and infections may play a crucial role in the differences. *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infections are the most common genital tract infections in Africa and are found to be major causes of infertility in the region (Brunham et al., 1993; Benbella et al., 2018). Genital tract infections were found to be the cause of male infertility in 34.7% of male in Nigeria (Ekwere et al. 2007). In 2019, the highest prevalence of HIV infections was found in Africa (25 million of people) compared to America (3.7 million), Europe (2.6 million) and Southeast Asia (3.7 million) (WHO, 2020). HIV infection was found to alters decrease semen quality may be one of the factors that causing Male infertility (Dondero et al., 1996; Leruez-Ville et al., 2002; Keerthana and Ramakrishnan, 2018).

Ethnic differences may be implicated to some extent. Previous studies have reported low semen parameters in African men compared to Caucasian, Asian and Hispano/Latino men (Redmon et al., 2013; Khandwala et al., 2017; McCray et al., 2020). Caucasian were found to have significant lower ORP than African and Central South Asian while African men and Central/South Asian men had significant higher number of spermatozoa with fragmented DNA than Caucasian, and South-East Asian (Henkel et al., 2019). Additionally, significant higher numbers of live spermatozoa were observed in Caucasian men compared to African, Central/South Asian and South-East Asian (Henkel et al., 2019). The investigation of the variations of reproductive hormones according to ethnicity was previously explored by Henkel et al. (2019) who showed that African men had a significantly lower testosterone than Caucasian men and higher estradiol concentrations than Caucasian (99.23

pmol/L) and Central/South Asian men. A more recent study by Punjani et al. (2020) showed a higher FSH levels in African men compared to Caucasian men. The same study indicated that Caucasian men had a higher testosterone concentration compared to Asian men.

The current study demonstrated that semen parameters data from Africa and Middle East is significantly lower than data used by the WHO to determine the lower reference values (WHO (2010) and the decision limits (WHO (2021). Additionally, the current study found that semen quality from men residing in Africa is significantly lower than the one from America, Asia, Australia and Europe. The current study provides novel insights into the validity of the WHO (2010) and WHO (2021) guidelines for semen assessment in which data from Africa was underrepresented. Furthermore, this study suggests that the investigation for male infertility should not only consist of the evaluation of semen analysis, but must also consider epidemiological factors such as age, seasonal variations, ethnicity, and geographical location. These factors will provide a better understanding of patient's endocrine, genetic variations, and environmental factors.

4.1.2. Limitations

The key limitations of the study should not be overlooked. Firstly, in a study design, the current study analyzed retrospectively a large sample size of data. As such the study is subjected to random errors, inherent biases and confounding. Secondly, the current study mostly involves patients consulting for fertility screening, generally with suspected sub-fertility. Extrapolated results from these suspected sub-fertile men to the general male community might be scientifically incorrect. Thirdly, information about patient's lifestyle behaviours (e.g. smoking, nutritional patterns, and drinking habits), occupational and environmental exposures and comorbidities (e.g. obesity and diabetes mellitus) have not been available as potentially confounding variables. Fourthly, there is no regression analysis to estimate the relationship between dependent variables such as semen parameters and hormones and independent variables such as age, abstinence periods. Fifthly, data available from African males is mostly from men residing in South Africa, and do not include data from Central

and West Africa. Similar observation was made for the Middle East data which consisted largely of data from men residing in Qatar. Lastly, there was a limited data for progressive motility compared to other evaluated semen parameters and no reproductive hormones for men residing in sub-Sahara Africa.

4.11. Conclusions

This study provides retrospective statistical data for semen parameters in men residing in Africa and the Middle East. In the full cohort, median ejaculate volume, sperm concentration, total sperm count, progressive motility and normal morphology were within normal thresholds recommended by WHO (2010). The prevalence of oligozoospermia was 20.3% and azoospermia was 3.6% in Africa and the Middle East. However, vitality, limited to the MENA region, was found to be below the recommended threshold, with a high percentage of asthenozoospermia. Similarly, the median SDF in the MENA cohort was higher than current recommended thresholds. Compared to Southern and Eastern Africa, the MENA region had generally worse semen parameters, most notably in the Middle East region. In Southern Africa, Mozambique and Zimbabwe were found to have the highest semen parameters, with Zambia having the lowest. In South Africa, the Free-State and Mpumalanga provinces had the lowest median sperm concentration. Age was found to negatively influence semen parameters in Africa and the Middle East, specifically affecting sperm concentration, progressive motility and normal morphology, with increasing SDF and normed sORP. In the MENA region, an age-related decline in testosterone and prolactin, and increase in FSH was found, with no significant changes for LH and estradiol with age. An increased sexual abstinence period positively impacted semen volume, sperm concentration and progressive motility, where reduced abstinence improved SDF. Furthermore, a temporal decline in semen parameters was found between 2005 and 2019 in males from sub-Sahara Africa. Seasonally, sperm concentration and total sperm count were higher in winter compared to summer and autumn, with the lowest sperm concentration reported in summer in males from sub-Sahara Africa below the equator. Finally, the results showed reduced semen quality in men residing in Africa compared to those living in America, Asia, Australia and Europe when comparing to global data from Cooper et al. (2010) and Campbell et al. (2011).

4.12. Future Recommendations

There are a number of gaps around the distribution of semen parameters of men residing in Africa and the Middle East that follow from these findings, and would benefit from further research studies. Future recommendations include:

1. Research comparing semen quality of homogeneous populations, either between fertile or between infertile men from African and Middle East regions is necessary to support the current study results
2. Since most data from Africa in this study originates from men residing in South Africa, and do not include men residing in Central and West Africa, further evaluations using comparable sample sizes between regions and including all 4 African regions (North, South, East and West Africa) are necessary to support the findings
3. The Middle East data in the current study are mainly from men residing in Qatar, it would be important to have a study involving more Middle East countries
4. Although the study highlighted an increasing trend in progressive motility in sub-Saharan Africa, there was limited amount data for progressive motility. Consequently, research analysing more consistent data for progressive motility in sub-Sahara Africa need to be established to validate the current findings
5. Possible environmental, biological and lifestyle differences need to be assessed in order to explain the geographical differences in semen parameters observed in the current study
6. The current study compared semen parameters of fertile and infertile men residing in Africa to semen parameters of fertile men residing in America, Asia, Australia and Europe. Further similar studies focusing only on fertile men in Africa need to be done in order to support the current study findings

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