IMPACT OF MICRO-NUTRIENT SUPPLEMENTATION ON SEMEN PARAMETERS

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DECLARATION

I declare that the thesis hereby submitted by me for the PhD degree at the University of the Free State is my own independent work and has not previously been submitted by me to another university/faculty. I further cede copy right of this research report in favour of the University of the Free State.

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LIST OF ABBREVIATIONS

DHA docosahexaenoic acid

DNA deoxyribonucleic acid

EPA eicosapentaenoic acid

FSH follicle-stimulating hormone

FT free testosterone

HC hip circumference

LH luteinizing hormone

MET Metabolic Equivalent of Task

MUFA monounsaturated fatty acids

NC neck circumference

PUFA polyunsaturated fatty acids

RNA ribonucleic acid

ROS reactive oxygen species

TT total testosterone

SFA saturated fatty acids

SHBG sex hormone-binding globulin

WC waist circumference

Keywords: semen parameters, micro-nutrient supplementation, lifestyle, environment, anthropometry

SUMMARY

The health of parents determine the development of their children and a link between paternal diet, metabolic health, body weight and semen parameters have been shown. Various factors may influence semen parameters and in this study the effect of micro-nutrient and omega-3 supplementation on semen parameters was investigated by evaluating semen parameters and fatty acid composition of intact semen at baseline and three months after intervention. The study also investigated the effect of age, environmental-, lifestyle-, anthropometric and dietary factors on semen parameters.

A placebo controlled intervention study on 50 apparently healthy volunteers between the ages of 18 and 45 years was conducted and data collection took place at the Faculty of Health Sciences, University of the Free State. Participants completed a self-reporting questionnaire to report on age, environmental-, lifestyle- and dietary factors. Standard techniques were used to obtain anthropometric measures and physical activity was determined using the self-administered short International Physical Activity Questionnaires (IPAQ). Two semen samples were collected and the average used to provide a representative reflection of sperm parameters. Semen analysis included semen volume, sperm concentration, -morphology, quantitative and qualitative motility, pH as well as fatty acid analysis. Descriptive statistics were used to describe the sample and Chi-squared tests or Fisher exact tests were used to determine associations between variables and two tailed Pearson's or Spearman's correlations, as well as analysis of variance were used to describe correlations.

A relation between aging and sperm parameters are described in literature, however in this younger study sample with a median age of 24 years, no correlation was found between age and semen parameters, probably because age related changes are only expected later.

According to body mass index classification the majority of participants were overweight/ obese and according to neck circumference measurements a large percentage of participants were overweight/obese, but none of the anthropometric measures showed an association with semen concentration, -motility or morphology. In literature, the number of sitting hours per day is linked to semen quality and in this study a weak correlation was found between sperm morphology and the number of hours per day spent sitting. Reported high activity

levels did not show an association with sperm parameters. More than half of participants spent more than four hours per day using electronic devices connected to Wi-Fi. A significant association between using electronic devices connected to Wi-Fi for four hours or more per day and a lower sperm motility was found. No statistically significant association between where the cellular phone is carried and normal or abnormal sperm parameters were shown. Although more than half of participants in this study took hot baths, no significant association was found between the use of hot baths and below reference limits for sperm parameters. More than a third of participants wore tight fitting underwear or trousers, which may contribute to an elevation in scrotal temperature and consequently poor semen quality, however no association was found between wearing tight fitting clothing and poor sperm parameters.

A healthy prudent diet has been proposed as an economical and safe way to improve sperm function. Although the intake of vegetables and fruit were inadequate and a cause for concern in this study, no association with poor semen quality was found. Alcohol intake of more than five units per week however was significant associated with lower sperm concentration.

Supplementing a healthy group of young men with a multi vitamin-mineral and omega-3 supplement over a period of 90 days did not influence the fatty acid composition of their semen or most of the sperm parameters, but showed an improvement in the percentage of sperm with normal forms.

For future studies, it is recommended that a larger sample be included if more resources are available and that other geographic areas in South Africa be included, especially as habitual food intake can differ considerably. This study provided valuable information about the possible negative effects of alcohol and use of electronic devices on sperm parameters and the potential of nutrient supplementation to improve sperm morphology. These results can be used when advising males about reproductive health, in order to optimise sperm parameters, which could influence the health of future generations.

OPSOMMING

Die gesondheid van ouers bepaal die ontwikkeling van hul kinders en ‘n verband tussen die dieet van die pa, metaboliese gesondheid, liggaamsmassa en semenparameters word geïmpliseer. Verskeie faktore mag semenparameters beïnvloed en in hierdie studie is die effek van mikrovoedingstof- en omega-3-supplemente ondersoek deur semenparameters en die vetsuursamestelling van intakte semen by basislyn en na drie maande van supplementasie te ontleed. Hierdie studie het ook die effek van ouderdom, omgewings-, leefstyl-, antropometriese- en dieetfaktore op semenparameters ondersoek.

‘n Plasebo-gekontroleerde intervensiestudie, wat 50 oënskynlik gesonde vrywilligers tussen die ouderdom van 18 en 45 jaar ingesluit het, is uitgevoer en data-insameling het by die Fakulteit Gesondheidswetenskappe, Universiteit van die Vrystaat geskied. Deelnemers het self ‘n vraelys voltooi om inligting oor demografiese-, omgewings-, leefstyl- en dieetfaktore te verskaf. Standaardtegnieke is gebruik om antropometriese metings te neem en fisieke aktiwiteit is bepaal deur die verkorte International Physical Activity Questionnaire (IPAQ) te gebruik. Twee semenmonsters is versamel en die gemiddelde waarde gebruik om ‘n verteenwoordigende aanduiding van spermparameters te verskaf. Semenanalise het semenvolume, spermkonsentrasie, -morfologie, kwalitatiewe en kwantitatiewe motiliteit, pH sowel as vetsuuranalise ingesluit. Beskrywende statistiek is gebruik om die steekproef te beskryf en die Chi-kwadraattoets of Fisher eksakte toets is gebruik om verbande tussen veranderlikes te toets en tweesydige Pearson’s of Spearman’s korrelasies sowel as analise van variansie ontledings gebruik om korrelasies te beskryf.

‘n Verband tussen veroudering en spermparameters word in die literatuur beskryf, alhoewel daar in hierdie jonger steekproef met ’n mediaan ouderdom van 24 jaar geen korrelasie tussen ouderdom en semenparameters gevind is nie; waarskynlik aangesien ouderdomverwante veranderinge eers by ’n later ouderdom verwag word.

Volgens liggaamsmassa-indeks klassifikasie, was die meerderheid van deelnemers oormassa/ vetsugtig en volgens nekomtrekmetings was ’n groot persentasie van deelnemers oormassa/ vetsugtig, maar geen van die antropometriese metings het ’n verband met semenkonsentrasie, -motiliteit of -morfologie getoon nie. In die literatuur word die aantal ure

per dag wat ’n persoon sittend deurbring in verband gebring met semenkwaliteit en in hierdie studie is ’n swak korrelasie tussen spermmorfologie en die aantal uur wat per dag sittend deurgebring word, gevind. Hoë aktiwiteitsvlakke is gerapporteer wat nie ’n verband met spermparameters getoon het nie. Meer as die helfte van die deelnemers het meer as vier ure per dag elektroniese toerusting gebruik wat aan Wi-Fi gekoppel is. ’n Betekenisvolle verband tussen die gebruik van elektroniese toerusting gekoppel aan Wi-Fi vir vier ure of meer per dag en laer spermmotiliteit is gevind. Geen statisties betekenisvolle verband tussen waar sellulêre telefone gedra word en normale of abnormale spermparameters is aangedui nie. Alhoewel meer as die helfte van die deelnemers aan hierdie studie van ’n warm bad gebruik gemaak het, is geen betekenisvolle verband gevind tussen die gebruik van ’n warm bad en spermparameters laer as die verwysingswaardes nie. Meer as ’n derde van die deelnemers het stywe onderklere of broeke gedra, wat kon bydra tot ’n toename in skrotale temperatuur en tot swakker semenkwaliteit kan lei. Geen verband is egter gevind tussen die dra van stywe klere en swak spermparameters nie.

’n Gesonde, gebalanseerde dieet word voorgestel as ’n ekonomiese manier om spermfunksie te verbeter. Alhoewel die inname van groente en vrugte in hierdie studie onvoldoende was en ’n rede tot kommer, is geen verband met semenkwaliteit gevind nie. Alkoholinname van meer as vyf eenhede per week is egter betekenisvol in verband gebring met ’n laer spermkonsentrasie. Supplementasie met ’n multi-vitamien-mineraal en omega-3-supplement oor ’n tydperk van 90 dae in ’n gesonde groep jong mans het nie die vetsuursamestelling van hul semen of meeste van die spermparameters beïnvloed nie, maar het ’n verbetering in die persentasie sperm met normale vorms tot gevolg gehad.

Vir toekomstige studies, word aanbeveel dat ’n groter steekproef ingesluit word, indien meer hulpbronne beskikbaar is en dat ander geografiese areas van Suid-Afrika ingesluit word, veral aangesien tipiese voedselinname aansienlik kan verskil. Hierdie studie het waardevolle inligting verskaf oor die moontlike negatiewe effek van alkohol en die gebruik van elektroniese toerusting op spermparameters; asook die moontlikheid dat voedingsupplementering spermmorfologie mag verbeter. Hierdie resultate kan gebruik word wanneer mans oor reproduktiewe gesondheid geadviseer word, ten einde spermparameters te verbeter, wat weer die gesondheid van toekomstige generasies kan beïnvloed.

Chapter 1 ORIENTATION AND MOTIVATION

1.1 Introduction

Health and lifestyle of parents influence the health and development of their offspring, with maternal health being especially significant (Black et al 2013:427; Black et al 2008:243; Ferguson-Smith and Patti 2011:115; Levy et al 2005:182). Animal and human studies further suggest a link between paternal diet, metabolic health, body weight and semen parameters (Bakos et al 2010:402,408,409, Ferguson-Smith and Patti 2011:115,116), as well as pregnancy health and embryo development of the offspring (Binder et al 2012:e52304). Wu and Suzuki (2006:201) suggested that the paternal diet in humans, even before intrauterine development takes place, can affect body fat accumulation in their offspring. The researchers added that paternal diet may even impact on the lifelong health of the offspring.

Sperm parameters provide an indication of the general health of males (Jensen et al 2009:559). Various factors may influence semen parameters. Age, environmental-, and lifestyle factors as well as anthropometric measures, dietary factors and nutrient intake have been described as factors contributing to sperm health.

Stewart and Kim (2011:496,498,499) reported in their review that the majority of research proposes that an increase in paternal age, as a demographic factor, has genetic risk implications for the offspring. The age at which the risk develops and the extent of the risk are however not clear.

Various environmental, lifestyle, and psychological factors may impact negatively on the general health and fertility of males (Begum et al 2009:18, Braga et al 2012:53,56,57,58, Campagne 2013:214,220, Homan et al 2007:209). These factors are sometimes reversible and include amongst others smoking, alcohol consumption, caffeine intake, recreational drug use, psychological stress, excessive exercise, body weight and dietary intake (Braga et al 2012:53,56,57,58, Campagne 2013:214,220, Homan et al 2007:209). There is strong indication that smoking and body weight have a negative influence on general health and contribute to sperm disorders and therefore fertility. The underlying mechanisms and the extent to which these factors may influence fertility however needs further investigation (Homan et al 2007:209,217,219, Du Plessis et al 2010:153,159).

The impact of obesity on animal fertility has been described. Animal studies showed that obesity impacts on sperm quality and may reduce male fertility (Fernandez et al 2011:2, Ghanayem et al 2010:96,103, Palmer et al 2012:259), possibly due to oxidative stress and lower testosterone levels (Erdemir et al 2012:153,157,158). These factors may impact on testicular function and the authors speculated that obesity may be an important contributing factor towards the etiology of male infertility (Erdemir et al 2012:153,157).

Research on obese male rats indicated that an improvement in metabolic (lipids, glucose and insulin sensitivity) and reproductive parameters (sperm motility and morphology) can improve the reproductive health of the next generation (McPherson et al 2014:865,868,872). The improvement in metabolic and reproductive parameters, as well as that of sperm function can be attained through simple dietary changes and exercise (McPherson et al 2014:865,868,872, Palmer et al 2012:259). Male obesity should therefore be prevented or treated as it impacts negatively on the health of the offspring (Palmer et al 2012:259).

The prevalence of obesity in humans is on the increase (Ghanayem et al 2010:96, WHO 2015:Online). A preliminary study in humans suggested that a high pre-conception body mass index (BMI) in males may negatively impact the semen quality of their offspring (Ramlau-Hansen et al 2007:2758,2762), possibly due to a decrease in Sertoli cell numbers and sperm count (Sharpe 2010:1697, Winters et al 2006:560). Obesity also has an impact on paternal sperm quality (Tsao et al 2015:10) by increasing the risk of deoxyribonucleic acid (DNA) damage in sperm (Dupont et al 2013:622,624). It is however not clear whether weight loss will prevent further DNA damage (Dupont et al 2013:622,624). More research in this regard is needed.

Various dietary factors influence general health (Anderson et al 2010:9, Jensen et al 2012:411,414,416, Katz and Meller 2014:83, Kefer et al 2009:453), the reproductive system (Anderson et al 2010:9) and semen quality (Gaskins et al 2012:2899). A high intake of saturated fats, trans fatty acids, full-fat dairy products, protein-rich foods, meat and processed meats, as well as sweets may negatively impact sperm parameters (Afeiche et al 2013:2265,2269,2272, Attaman et al 2012:1, Chavarro et al 2014:429,432,435,437,438, Eslamian et al 2012:3331,3333,3334, Mendiola et al 2010:1128,1131, Mendiola et al 2009:812,816). Although a prudent dietary pattern is associated with higher progressive

sperm motility, a Western dietary pattern does not seem to affect semen quality (Gaskins et al 2012:2899,2907). It must however be noted that a low intake of vegetables and fruit, which is typical in a Western dietary pattern, may negatively impact sperm parameters (Eslamian et al 2012:3331,3333,3334, Gaskins et al 2012:2899,2907, Wong et al 2003:51,53). A low intake of vegetables and fruit results in a low antioxidant intake (Mendiola et al 2010:1128,1131, Mendiola et al 2009:812,816, Mínguez-Alarcón et al 2012:2807,2811,2812).

A Danish cohort of 43 277 men showed lower mortality rates in males (with and without children) with good semen quality, due to a lower incidence of a wide range of diseases. This reduction in mortality could not be ascribed to lifestyle and/or social factors (Jensen et al 2009:559), leading Jensen et al (2009:559) to label semen quality as a fundamental biomarker of overall male health.

Decreased fertility, due to poor semen quality, amongst younger cohorts of otherwise normal men is described, and may partly explain the observed decline in conception rates. This may act as an early warning sign of reproductive health problems and lower fertility in future (Jensen et al 2008:81).

1.2 Problem statement

Healthy motile and morphologically normal sperm is essential for fertilization to take place (Dott and Glover 1999:49). It is expected that younger males would have sperm parameters of a high quality (Ng et al 2004:1812,1813), however, a high incidence of sub-optimal semen quality was described in 20 year old males who were submitted to compulsory medical examinations in the military service in Denmark (Andersen et al 2000:366,368,369). This early prevalence of sub-optimal semen parameters is concerning, especially in the light of the influence on the health of future generations, as well as the global change in dietary and lifestyle factors that may influence sperm parameters.

In South Africa, as in the rest of the world, dietary patterns has changed during the last 20 years. The total vegetable intake has decreased (Ronquest- Ross et al 2015:4), but the use of frozen vegetables, packaged fats and oils, sweet and savoury snacks as well as soft drinks (Ronquest- Ross et al 2015:4,5,7,8,10) have increased. It does seem that a modern lifestyle of convenience negatively impacts on dietary quality.

Dietary supplements can be of value to improve the diet of individuals with an inadequate intake. Clinical studies suggest that antioxidant supplements are beneficial to improve sperm function, DNA integrity (Zini and Al-Hathal 2011:374,377,379) and semen quality (Agarwal et al 2008c:5). Antioxidant intake reduces oxidative stress (Agarwal et al 2006:883, Agarwal et al 2008c:5, Showell et al 2012:1) and dietary lipids, especially omega-3, help to maintain the fluidity and flexibility of sperm; and are thus necessary for successful fertilization (Hammadeh et al 2009:87, Wathes et al 2007:190,197).

The optimal nutrient supplement (Zini and Al-Hathal 2011:374), combination of nutrients (Cheah and Yang 2011:188, Gharagozloo and Aitken 2011:1636, Tremellen 2008:253) dose of antioxidant nutrients (Gharagozloo and Aitken 2011:1636, Tremellen 2008:253) and ingredients (Gharagozloo and Aitken 2011:1636) that provide sperm with optimal protection against oxidative stress (Tremellen 2008:253) or may assist in the treatment of infertility (Cheah and Yang 2011:188) has not been established. A combination of antioxidants should be more efficient than a single antioxidant as oxidative stress is a non-localised heterogeneous occurrence (Gharagozloo and Aitken 2011:1636) and a combination of antioxidants seems to offer a better solution (Lanzafame et al 2009:638). However, more is not better, as large doses of antioxidants may cause negative effects like disrupting the redox balance or homeostasis (balance between oxidation and antioxidation) (Bouayed and Bohn 2010:234, Valko et al 2007:44) and influence the number of motile sperm (Hawkes and Turek 2001:768,770). On the other hand, it does seem that the side effect profile of antioxidant supplements is low (Showell et al 2012:43). Furthermore, due to the outstanding safety profile of omega-3 fatty acid supplements, it has been suggested to be used as nutraceuticals to improve semen quality (Safarinejad et al 2010:101).

At present the impact of nutrient supplementation on semen parameters in males has not been determined in South Africa. The consequences of poor health and the treatment of infertility is expensive (Showell et al 2012:6) whilst nutrient supplements are easily obtained and relatively cheap in comparison (Safarinejad et al 2010:101, Showell et al 2012:6). Nutrient supplements have the potential to address some of the root problems of poor semen parameters and may offer a substantial contribution in improving semen parameters and male fertility (Cheah and Yang 2011:189). If a supplement is found to be effective in improving semen parameters, nutrient supplementation will offer a simple, cost effective solution to an

expensive problem including infertility and the cost to treat health and developmental problems in future generations.

It is important to investigate age, environmental-, and lifestyle factors, as well as anthropometric measures and food and nutrient intake, to determine how these factors affect the ability of the male to produce healthy sperm and to investigate whether nutrient supplementation can address or correct the possible impact of these factors. This study may be able to fill the research gap and provide important information on the future treatment of males with poor semen parameters, especially with reference to the South African context.

1.3 Aim and Objectives

1.3.1 Aim

The main aim of this study was to investigate the impact of micro-nutrient and omega-3 supplementation on semen parameters.

1.3.2 Objectives

The following objectives were set to reach the aim of this study and to provide data to describe various practices, which in literature have been proposed as possible factors that could influence semen parameters.

To describe age, environmental-, lifestyle-, anthropometric- and dietary factors in the study sample that may impact semen parameters at baseline;
To determine anthropometric measures of the study sample before and after intervention;
To determine baseline semen parameters including semen volume, sperm concentration, sperm motility, sperm morphology, pH and fatty acid composition;
To determine post intervention semen parameters including semen volume, sperm concentration, sperm motility, sperm morphology, pH and fatty acid composition examining the effect of the nutrient supplements;
To determine the associations between age, environmental-, lifestyle-, anthropometric- and dietary factors on semen parameters.

1.4 Structure of this thesis

This thesis is divided into eight chapters. Chapter 1 includes the background and motivation for the study. The problem statement, aim and objectives of the study, as well as the structure of the thesis is discussed.

Chapter 2 consists of a literature review to provide background on male fertility and provides an overview of the male reproductive system, concept clarifications of fertility, subfertility and infertility as well as possible causes of infertility. Various aspects influencing semen parameters are also discussed, including age, environmental, lifestyle-, anthropometric- and dietary factors.

Chapter 3, the methodology chapter, describes the study design, time frames, ethical considerations and data collection process. In this chapter, the measurements used and statistical analysis performed are also explained.

Chapters 4 to 7 are written in article format, as approved by the University of the Free State. The articles are written according to the instructions to the author for the specific journal to which it will be submitted.

Chapter 4 includes an article prepared for the South African Journal of Clinical Nutrition to report on age, environmental-, lifestyle factors, body composition and dietary factors in this study that may have an influence on semen parameters.

Chapter 5 reports on the association of anthropometric measures on various semen parameters in this sample. This article is prepared for submission to South African Family Practice.

Chapter 6 consists of an article prepared for the South African Journal of Obstetrics and Gynaecology and provides a baseline profile of the semen parameters and reports on the impact of nutrient supplementation on sperm parameters in this study

The article in Chapter 7 reports on the impact of nutrient supplementation on the fatty acid composition of human semen and will be submitted to Andrologia.

In each article that is presented in Chapters 4 to 7, the applicable methods, results and discussion of results are presented and results compared with other research findings. Each article also contains a conclusion and recommendations section.

Chapter 8 covers an overview of the conclusions and recommendations made according to the results obtained from this study. The research significance and recommendations for implementation are argued and recommendations for future research provided.

Chapter 2 LITERATURE REVIEW

2.1 Introduction

This chapter provides an overview of the male reproductive system. A discussion on male fertility and infertility, as well as factors influencing male fertility is also included. Special reference is made to the effect of age, environmental-, lifestyle-, and dietary factors, as well as specific nutrients on semen parameters.

2.2 The male reproductive system

In order to gain insight on male fertility, an overview of the physiology of the male reproductive system will be discussed in the following sections.

2.2.1 Development of the male reproductive system during the embryonic phase

During the first six weeks of embryonic development the external genitalia of males and female are fundamentally the same (Fox 2013:703,705, Sherwood 2013:778). The genitalia share a urogenital sinus, genital tubercle, urethral folds and two labioscrotal or genital swellings (Fox 2013:705, Sherwood 2013:778). Secretions by the testes at this early age masculinize these structures to form the penis, spongy urethra, prostate and scrotum (Fox 2013:704, Sherwood 2013:778). Testosterone is responsible for the stimulation of the wolffian duct derivatives, namely the epididymis, ductus or vas deferens, ejaculatory duct, and seminal vesicles (Fox 2013:706, Sherwood 2013:779).

During the embryonic phase the testes develop from the gonadal ridge situated in the back of the abdominal cavity (Cohen and Wood 2000:418, Sherwood 2013:781). In the last months of the foetal phase, the testes commence with a slow descent, moving from the abdominal cavity through the inguinal canal into the scrotum, where the testes drop into the pockets of the scrotum sac (Cohen and Wood 2000:418, Fox 2013:704, Sherwood 2013:781-782). The descent of the testes into the scrotum is initiated by testosterone secreted by the foetal testes (Sherwood 2013:782) and the descent is usually complete by the seventh month of gestation (Sherwood 2013:782), at birth (Sharpe 2010:1703), shortly after birth (Fox 2013:704) or at least before puberty (Sherwood 2013:782). Each testis contains a spermatic cord, that runs

through the inguinal canal, which contains blood vessels, lymphatic vessels, nerves and ductus deferens (Cohen and Wood 2000:418). The ductus deferens transports the sperm from the testis (Cohen and Wood 2000:418).

2.2.2 The production of testosterone

The testes is located on the outside of the body, suspended in the scrotum that is situated between the thighs (Cohen and Wood 2000:418). The size of the testes or male gonads in adults is approximately 3.7-5.0 x 2.5 cm (Cohen and Wood 2000:417,418, Iammarrone et al 2003:212) or consists of a volume of 20.7 ± 5 ml (Jensen et al 2004), with the left testis slightly smaller (23.9 ± 1.3 cm^3) than the right testis (24:3 ± 1:2 cm^3) (Simmons et al 2004:297).

The testes are responsible for production of sperm and testosterone (Agarwal et al 2011:455, Fox 2013:704, Sherwood 2013:782, 779) and comprise of two parts, namely the seminiferous tubules where spermatogenesis takes place and the interstitial tissue which contain Leydig cells, responsible for testosterone secretion (Cohen and Wood 2000:418, Fox 2013:711, Karavolos et al 2013:2, Sherwood 2013:782). The two parts of the testes are structurally and functionally distinct (Sherwood 2013:782), but interact in intricate ways (Fox 2013:712,714). Seminiferous tubules contribute to 80-90 percent of the weight of testes in adults (Fox 2013:711, Sherwood 2013:782). The interstitial tissue is a thin web of connective tissue that fills the spaces between the seminiferous tubules (Fox 2013:711, Sherwood 2013:782). Leydig cells are the most abundant cells in the interstitial tissue, but this tissue is also rich in blood and lymphatic capillaries that transport testicular hormones (Fox 2013:711).

Luteinizing hormone (LH) stimulates secretion of testosterone by the Leydig cells, while follicle-stimulating hormone (FSH) stimulates spermatogenesis in the tubules (Fox 2013:712,717). LH in males is also known as interstitial cell stimulating hormone (ICSH) (Fox 2013:708). FSH binds to Sertoli cells and is responsible for the increase in spermatogonial numbers and maturation of the spermatocytes, but cannot complete spermatogenesis on its own (Karavolos et al 2013:2). LH also plays a vital role in maturation of sperm (Karavolos et al 2013:2).

Newly produced testosterone enter the lumen of the seminiferous tubules, where it assists with sperm production (Sherwood 2013:782). For sperm production testosterone binds with

androgen receptors in the cytoplasm of target cells (Sherwood 2013:782). The androgen-receptor complex moves to the nucleus, where it binds with the androgen response element on DNA (Sherwood 2013:782). This process leads to transcription of genes that direct the synthesis of new proteins, responsible for the cellular response (Sherwood 2013:782).

Testosterone is derived from a cholesterol precursor molecule (Sherwood 2013:782). After production of testosterone, some testosterone is secreted into the circulatory system, mainly bound to plasma protein and transported to the target sites of action (Cohen and Wood 2000:418, Sherwood 2013:782).

2.2.3 Control of gonadotropin secretion

One of the functions of FSH is to stimulate spermatogenesis (Sherwood 2013:787). Spermatogenesis is governed by a negative feedback loop, with testosterone acting as feedback component that slows LH and FSH secretions (Iammarrone et al 2003:216, Quallich 2006:277). This feedback system can be overruled by the use of exogenous testosterone, or medication such as luteinizing hormone-releasing hormone antagonists (Quallich 2006:277). Exogenous testosterone and luteinizing hormone-releasing hormone antagonists will stop the body's own production of testosterone, resulting in cessation of spermatogenesis (Quallich 2006:277).

Inhibin also inhibits FSH secretion selectively and is secreted by the Sertoli cells and seminiferous tubules in the testes (Fox 2013:708, Iammarrone et al 2003:216, Quallich 2006:277, Sherwood 2013:787). Inhibin works directly on the anterior pituitary to inhibit FSH secretion (Sherwood 2013:787).

The negative feedback of testosterone and inhibin help to sustain a reasonably constant secretion of gonadotropins (Fox 2013:708).

2.2.4 The effect of age on testosterone and male reproductive tract secretions

The reason for the decrease in androgen secretion with aging is unknown (Fox 2013:713). The decline in testosterone secretion is most likely not due to a decrease in gonadotropin secretions as gonadotropin concentrations remain elevated despite the decline in testosterone (Fox 2013:713).

The drop in testosterone secretion and spermatogenesis begin as early as the age of 20 years, with this process continuing through the lifecycle (Cohen and Wood 2000:422, Fox 2013:713). A hypogonadal state (<3.2ng/mL) can be expected at the start of the eight decade (Cohen and Wood 2000:422, Fox 2013:713). However, in a small percentage of men (less than 10%), sperm is still produced by 80 years of age (Cohen and Wood 2000:422). Physical inactivity, obesity and certain drugs or medication also contribute to the reduction in testosterone secretion, resulting in a loss of lean muscle and bone mass (Fox 2013:713).

A reduction in secretions from the prostate and seminal vesicles also occur in the aging male, resulting in less viscous secretions (Cohen and Wood 2000:422).

2.2.5 Effects of testosterone in the male

The main androgen secreted by the testes is testosterone (De Souza and Hallak 2011:1860). Testosterone has various effects on the male body as indicated in Table 2.1. Androgens are also called anabolic steroids due to their effect on muscle growth (Fox 2013:713) and stimulate not only the growth of muscles, but also bone, other organs (including the larynx) as well as haemoglobin (Fox 2013:714, Sherwood 2013:784), seminal vesicles and the prostate during adolescence. In this way testosterone contributes to the typical male pattern of hair growth including beard and chest hair, a deep and lower voice (growth of the larynx and thickening of the vocal folds), thick skin and the male shape of broad shoulders, and heavy arm and leg musculature as a result of protein disposition (Cohen and Wood 2000:419, De Souza and Hallak 2011:1860,1861, Fox 2013:713, Sherwood 2013:784).

Table 2.1 Effects of testosterone in the male (Adapted from De Souza and Hallak 2011:1860,1861,1862, Fox 2013:713, Sherwood 2013:784)

Effects before birth
Sex determination • Growth and development of wolffian ducts into epididymis, ductus deferens, seminal vesicles, and ejaculatory ducts • Development of urogenital sinus into prostate • Masculinize the reproductive tract and external genitalia (penis and scrotum) • Promotes descent of the testes into the scrotum
Effects after birth
At puberty • Completion of meiotic division and early maturation of spermatids • Promote growth and maturation of the reproductive system **After puberty** • Is essential for spermatogenesis • Maintains the reproductive tract throughout adulthood
Other reproduction-related effects
• Develops the sex drive at puberty • Control gonadotropin secretion
Effects on secondary sexual characteristics
• Growth and maintenance of accessory sex organs • Growth of penis • Facial and axillary hair growth (male pattern of hair growth) • Growth of body hair • Vocal folds thicken (voice deepen) • Promotes muscle growth responsible for the male body shape
Non-reproductive actions
• Exerts a protein anabolic effect and contribute to muscle growth • Promotes bone growth (at puberty) • Closes the epiphyseal plates after being transformed to oestrogen by aromatase • Promotes growth of other organs, including the larynx • Promotes erythropoiesis (formation of red blood cells) • Contributes toward aggressive behaviour

2.2.6 Temperature regulation

The temperature of testes needs to be closely regulated, as spermatogenesis is temperature sensitive (Jung and Schuppe 2007:203, Sherwood 2013:782), although it still seems that temperature control of the testes and epididymis is not a significant contributing factor in male fertility for most individuals (Dott and Glover 1999:44). The average temperature within the scrotum is lower than the core or normal body or abdominal cavity temperature and the testes thus descent into the cooler environment of the scrotum as spermatogenesis cannot take place at core temperature (Cohen and Wood 2000:418, Ivell 2007:Online, Sherwood 2013:782). It is required for the testes to descent towards the bottom of the scrotum, rather than being positioned at the top, where proximity to the body is likely to influence temperature control of the testes (Sharpe 2010:1703).

Testicular temperature is regulated by a spinal reflex mechanism that adjusts the position of the scrotum in relation to the abdominal cavity (Sherwood 2013:782). In a cold environment reflex contraction of scrotal muscles brings the scrotal sac closer to the warmer abdomen (Sherwood 2013:782). The opposite happens in a hot environment when relaxation of the muscles allows the scrotal sac to move away from the body (Sherwood 2013:782). The testes does not function at a specific temperature only, but within a physiological range of temperatures (Mieusset and Bujan 1995:175).

2.2.7 Spermatogenesis

Sperm is small, specialized cells and the average semen ejaculate contains at least 200 million sperm (Cohen and Wood 2000:421) or between 60 and 150 million per ml (Fox 2013:721). Each sperm consists of a head, midpiece and tail. The oval head contains the nucleus with chromosomes and is covered by a cap or acrosome, which is a modified lysosome that contains enzymes to assist the sperm to penetrate the ovum (Cohen and Wood 2000:421, Fox 2013:716, Sherwood 2013:786). Attached to the head is the midpiece, which provides energy, supplied by the mitochondria (Cohen and Wood 2000:421, Fox 2013:716, Sherwood 2013:786). Movement results from the whip like motion of the tail or flagellum of the sperm (Fox 2013:716, Sherwood 2013:786,812).

The basis for spermatogenesis is laid during the foetal growth period and adverse events at this stage may consequently impact on the scale or quality of sperm production in later life (Sharpe 2010:1697). However, sperm is not produced until puberty (Quallich 2006:277, Sharpe 2010:1697), when testosterone begins to wield its influence on general development and growth in males (Quallich 2006:277). Spermatogenesis is then maintained during the rest of the lifecycle in normal men (Sharpe 2010:1697). Testosterone produced by the Leidig cells of the testes initiate spermatogenesis and maintains sperm function (Agarwal et al 2011:445, Fox 2013:704,705, Quallich 2006:277). The anterior pituitary release LH and FSH that start the process of sperm development (spermatogonia, spermatocyte, spermatid, sperm (Agarwal et al 2011:445, Quallich 2006:277). FSH levels tend to increase with age (Pasqualotto et al 2005:1087,1088,1090).

The sperm producing seminiferous tubules is about 250m long (Sherwood 2013:784). Germ cells and Sertoli cells are found in the tubules (Sherwood 2013:784). Germ cells are in various stages of sperm development, while the function of Sertoli cells is to provide support and hormonal signals for the development of the germ cells (Battista et al 2008:84) as well as providing nutrients to the sperm (Battista et al 2008:84, Cohen and Wood 2000:421, Sherwood 2013:784). Another function of the Sertoli cells is the production of oestrogens in males, where the oestrogens control spermatogenesis and contribute to male heterosexuality (Sherwood 2013:784). Oestrogen receptors have been found in the testes, prostate, bones and other parts of the male body (Sherwood 2013:784).

Spermatogenesis is a complex process where fairly undifferentiated primordial germ cells, the spermatogonia multiply and are transformed into specialised motile sperm (Fox 2013:714,715, Sherwood 2013:784-785). Spermatogonia are diploid cells with 46 chromosomes which multiply and give rise to mature haploid gametes each with a set of 23 chromosomes (Sherwood 2013:785). Each of the 23 chromosomes comprises of two strands or chromatids of matching DNA (Fox 2013:714). Sperm development takes about 72-74 days to complete with an additional 12 days for final maturation as the sperm navigate through the seminiferous tubules and the length of the epididymis (Iammarrone et al 2003:214–216, Wong et al 2000:440, 2002:492). At any particular time, different seminiferous tubules are in diverse stages of spermatogenesis (Sherwood 2013:785). Sperm maturation is an intricate process and several hundred million sperm reach maturity daily (Iammarrone et al 2003:214,

Sherwood 2013:785). Sperm acquire mobility and develop acrosome functionality during maturation. Increased membrane fluidity is needed for optimal acrosome reaction (Toshimori 1998:177).

Only one of the approximately 165 million sperm in the ejaculate, if any, will fertilize the ovum (Cohen and Wood 2000:421, Sherwood 2013:813). The life expectancy of the remainder of the sperm is a few hours up to a maximum of 3 days (Cohen and Wood 2000:421). Although only one sperm fertilizes the ovum, enough sperm is needed to dissolve the barriers around the ovum (Cohen and Wood 2000:421, Fox 2013:734, Sherwood 2013:813). Each sperm has a large, enzyme-filled vesicle (acrosome) above its nucleus (Fox 2013:734). During the acrosomal reaction, acrosomal enzymes are released which allow the sperm to digest a path through the zona pellucida to an ovum (Fox 2013:734, Sherwood 2013:813,814).

As spermatogenesis is sustained during the lifecycle the spermatogenic process itself is continuously susceptible to adverse effects of the work environment, lifestyle factors and/or exposure to toxic agents in the environment during this time period (Sharpe 2010:1697).

2.2.8 Male accessory sex organs

Sperm is produced from germinal cells (spermatogonia) in the seminiferous tubules that are linked at both ends to a tubular network namely the rete testis (Fox 2013:717, Karavolos et al 2013:2, Sherwood 2013:789). Tubular fluids are secreted by the Sertoli cells (Sherwood 2013:789) and are transferred to the rete testis combined with sperm; and are drained via the efferent ductules into the epididymis (Fox 2013:717). The epididymis is lightly attached to the back surface of each testis (Sherwood 2013:789) as depicted in Figure 2-1.

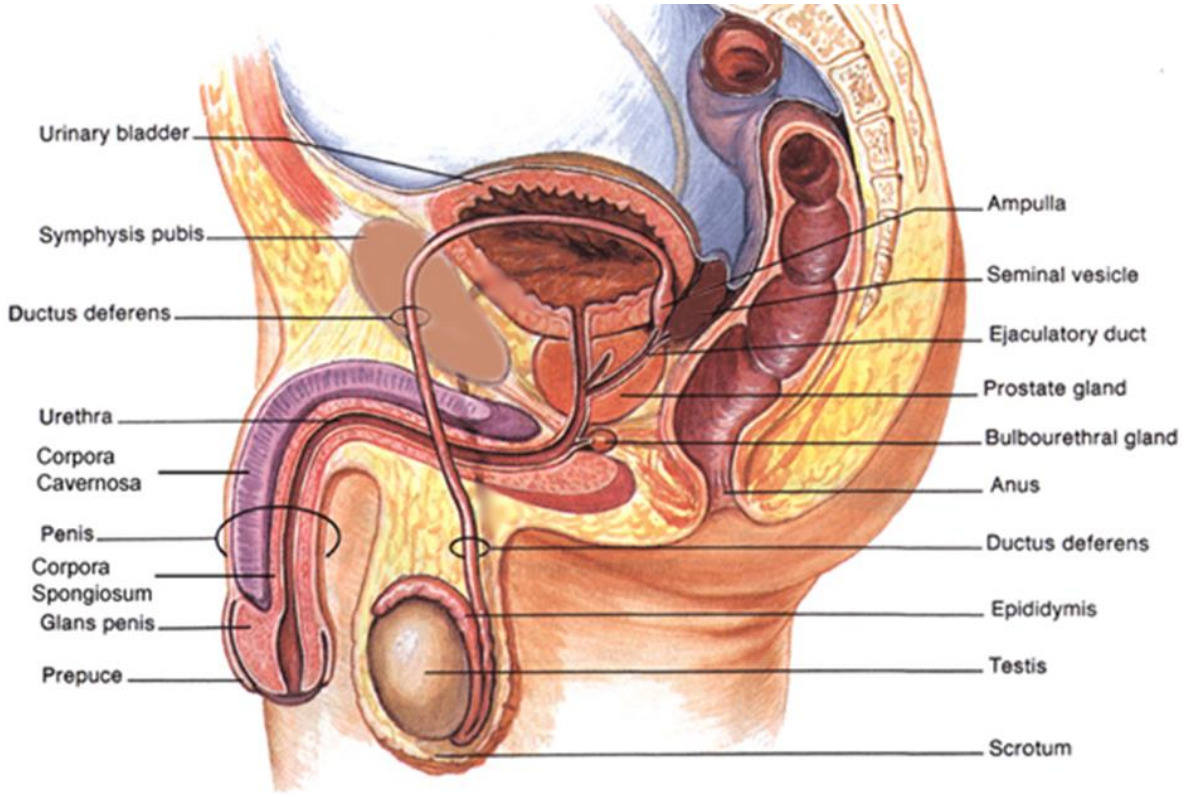

Figure 2.1 Male reproductive system (http://www.thinksciencemaurer.com/wp-content/uploads/2015/05/Male-Reproductive-system-Diagram.jpg)

The epididymis is a tightly wound structure of approximately five to six meters if uncoiled that receives the tubular products (Cohen and Wood 2000:419, Fox 2013:717). Sperm enter at the head of the epididymis and exit from its tail through a single, thick-walled tube namely the ductus deferens (Fox 2013:717, Sherwood 2013:789). The ductus deferens passes up and out of the scrotal sac and runs back into the abdominal cavity where it finally empties into the urethra (Sherwood 2013:789).

Sperm that leave the testis enter the head of the epididymis, but are not capable of moving or fertilizing (Fox 2013:717, Sherwood 2013:789). This inability to move or fertilize is partly due to the low pH of the fluid in the epididymis and ductus deferens (Fox 2013:717). The low pH is caused by the bicarbonate that is reabsorbed and the secretion of hydrogen by active transport through Adenosine Tri phospate (ATP)ase pumps (Fox 2013:717). During passage through the epididymis, maturational changes stimulated by testosterone make sperm more

resistant to changes in pH and temperature (Fox 2013:717, Sherwood 2013:789). Sperm is concentrated hundred fold by the epididymis through absorption of fluid from the seminiferous tubules (Sherwood 2013:789). Rhythmic contractions of smooth muscles in the epididymis further cause maturing sperm to move into the ductus deferens (Sherwood 2013:789). The ductus deferens stores the sperm for up to 2 months (Cohen and Wood 2000:418, Sherwood 2013:789). The epididymis matures and stores sperm between ejaculations (Fox 2013:717). The tightly packed sperm are reasonably inactive and their metabolic needs are low (Sherwood 2013:789). Their nutrition comes from simple sugars (mainly fructose) in the tubular secretions / seminal vesicles secretions (Agarwal et al 2008a:165, Cohen and Wood 2000:420, Fox 2013:718, Sherwood 2013:789). The seminal fluid contributes to about 60 percent of the volume of semen and act as the transport medium (Agarwal et al 2008a:165, Cohen and Wood, 2000:420, Fox 2013:718, Krausz 2011:275). The prostate also supply fluids that contain citric acid, calcium, coagulation proteins (Fox 2013:718), free zinc and zinc bound to citrate (De Jonge et al 2004:64). The prostatic fluid, rich in zinc, will retain the endogenous chromatin zinc of sperm, and therefore chromatin stability is likely to be maintained (De Jonge et al 2004:64).

During ejaculation the urethra carries the sperm out of the penis (Sherwood 2013:789). The low pH is neutralized by the alkaline prostatic fluid, resulting in fully motile sperm that is capable of fertilizing an ovum (Cohen and Wood 2000:420, Fox 2013:717). Secretions of the female reproductive tract further neutralize the acidity and enhance the motility of sperm and capacity of the sperm to fertilize ovum (Cohen and Wood 2000:420, Sherwood 2013:789). This enhancement of the sperm's capacity is known as capacitation (Sherwood 2013:789).

2.2.9 Erection, emission, and ejaculation

The male sex act is characterized by erection and ejaculation (Sherwood 2013:792). During erection the erectile tissue of the penis is filled with blood, as a result of parasympathetically nerve-induced vasodilation of the arterioles in the penis (Fox 2013:718,719, Sherwood 2013:792). The penis practically consists of erectile tissue made up of three columns of sponge like vascular spaces through the length of the penis (Sherwood 2013:792). As the erectile tissue becomes enlarged with blood the penis becomes turgid as the erection is supported by the partially occluded blood flow (Fox 2013:719). Without sexual stimulation

and excitation the erectile tissues contain little blood because the arterioles are constricted and the penis remains small and flaccid (Sherwood 2013:792).

In the course of ejaculation during the emission phase the semen and sex-gland secretions is expulsed into the urethra due to the sympathetically induced contraction of the smooth muscle in the reproductive ducts and accessory sex glands (Cohen and Wood 2000:420, Fox 2013:719, Sherwood 2013:792,811). During the expulsion phase when the muscles in the pelvic floor contract, semen is expulsed by force, which is accomplished by motor neuron-induced contraction of the skeletal muscles in the base of the penis (Cohen and Wood 2000:420,421, Fox 2013:719, Sherwood 2013:792). During this process the involuntary sphincter at the neck of the bladder prevents the release of urine or the semen from entering the bladder (Cohen and Wood 2000:421, Sherwood 2013:792). Sympathetic nerves stimulate emission and ejaculation (Fox 2013:719, Cohen and Wood 2000:420). The synergistic action of the parasympathetic and sympathetic systems is needed for sexual function in males (Fox 2013:720, Sherwood 2013:793).

2.3 Male fertility

Semen analysis remains one of the first-line investigations in the assessment of male fertility (Karavolos et al 2013:4,7, Krausz 2011:273, Pacey 2012:739). Semen analysis is performed on fresh ejaculate by a trained technician, using laboratory methods as described by the World Health Organization (WHO) (Karavolos et al 2013:273, Pacey 2012:739). In clinical practice it is suggested that at least two semen samples be analysed to assess semen quality (Carlsen et al 2004:363, Quallich 2006:277, WHO 2010:8). Although measurements are done on all the sperm in the ejaculate, the measurements cannot predict the fertilizing ability of the sperm that reach the fertilization site, but semen analysis offer vital information on the clinical status of the male (Karavolos et al 2013:5, WHO 2010:8–9). Collection and analysis of semen must be done by using standardized techniques to ensure valid and useful data (WHO 2010:9).

Semen volume and sperm count or concentration depend on the length of time between ejaculations, with the higher volume seen after periods of abstinence (De Jonge et al 2004:57, Karavolos et al 2013:5, Sherwood 2013:761,794). Semen volume varies between 1.5 and 6.0 ml per ejaculate (Fox 2013:720, Sherwood 2013:761,794). The lowest reference limit

according to the WHO (2010:224) is 1.5 (1.4–1.7) ml that indicates the 5th centile with a 95% confidence interval (Table 2.2) (WHO 2010:224). The seminal vessels produce the bulk of the semen (45-80%), while the prostate add 15 to 30 percent (Fox 2013:720). According to Fox (2013:721) a total sperm count below 40 x 10^6 per ejaculate may be of clinical significance in contributing towards male infertility. According to the WHO (2010:224) the lower reference limit for total sperm number is 39 (33-46) x 10^6 per ejaculate and for sperm concentration 15 (12-16) x 10^6 per ml.

Table 2.2 Lower reference limits for semen characteristics (WHO 2010:224)

Parameter	**Lower reference limit** (5th centiles and their 95% confidence intervals)
Semen volume (ml)	1.5 (1.4–1.7)
Total sperm number (10^6 per ejaculate)	39 (33–46)
Sperm concentration (10^6 per ml)	15 (12–16)
Total motility (PR + NP, %)	40 (38–42)
Progressive motility (PR, %)	32 (31–34)
Vitality (live spermatozoa, %)	58 (55–63)
Sperm morphology (normal forms, %)	4 (3.0–4.0)
Other consensus threshold values	
pH	≥7.2
Peroxidase-positive leukocytes (10^6 per ml)	<1.0
MAR test (motile spermatozoa with bound particles, %)	<50
Immunobead test (motile spermatozoa with bound beads, %)	<50
Seminal zinc (*u*mol/ejaculate)	≥2.4
Seminal fructose (*u*mol/ejaculate)	≥13
Seminal neutral glucosidase (mU/ejaculate)	≥20

The limits as suggested by the WHO are not absolute limits and should at all times be interpreted by taking the relevant clinical information of an individual into consideration (Karavolos et al 2013:4, WHO 2010:224). Although routine semen analysis provides information about spermatogenesis and sperm supply, it provides little information about the functional ability of the sperm (Karavolos et al 2013:5).

2.3.1 Subfertility

Subfertility is a condition commonly described as any form of reduced fertility over an extended time period resulting in no conception (Gnoth et al 2005:1144). When sperm morphology, motility and concentration in fertile and infertile males were described the subfertile ranges included less than 9 percent sperm with normal morphology, less than 32

percent motile sperm and a sperm concentration of less than 13.5 x 10^6 per ml (Guzick et al 2001:1388). It is estimated that 10–15% of couples in western countries experience subfertility (Evers 2002:151) with the male partner being responsible for 30–50% of these cases (Chow and Cheung 2006:149, Pacey 2009:42).

2.3.2 Infertility

Globally 8–15% of couples of reproductive age are affected by infertility (Ombelet et al 2008:607,616, Quallich 2006:277, Sharma et al 2013:16, Thomas and Bishop 2007:324) or more than 70 million couples (Ombelet et al 2008:607) or one out of six couples (Brugo Olmedo, 2000:6053).

The majority of these couples are from developing countries (Ombelet et al 2008:605), although reports on the prevalence of infertility in these countries are limited (Ombelet et al 2008:607). The reason for this higher prevalence rate in developing countries is ascribed to sexually transmitted infections, unsafe abortion practices, post-partum pelvic infections and female genital mutilations (Nachtigall 2006:871, Ombelet et al 2008:607–608,617), that are not always detected and treated as in developed countries (Nachtigall 2006:871, Nwajiaku et al 2012:19). According to a retrospective panel data analysis, South Africa has the lowest total fertility rates on the African continent (Rossouw et al 2012:18). It seems that primary infertility is mainly experienced in the developed world, while secondary infertility is more prominent in the developing countries (Lunenfeld and Van Steirteghem 2004:317). Primary infertility is when a woman or a couple, who was never pregnant before is unable to conceive for one or two years. Secondary infertility is referred to in couples who meet the criteria for primary infertility, but who have been pregnant in the past (Lunenfeld and Van Steirteghem 2004:318). As mentioned, secondary infertility in developing countries, is typically caused by sexually transmitted infections and post-partum complications (Lunenfeld and Van Steirteghem 2004:324). Human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS) further impacts on this issue (Lunenfeld and Van Steirteghem 2004:324).

According to Karavolos et al (2013:1) it is difficult to assess the incidence of male infertility in the general population, but it is estimated that male factor infertility affects approximately

seven percent of men in the general population (Krausz 2011:271). Male infertility is a significant reproductive problem with the global prevalence estimated at ten percent of couples of reproductive age (Shefi and Turek 2006:385); or 12 percent of males in the United States of America (Louis et al 2013:4,6) or one male in every 20 in Australia (McLachlan and de Kretser 2001:116) or the reason for up to half of all couples needing fertility treatment (Dohle et al 2005:703, Eisenberg et al 2013a:1030, Practice Committee of the American Society for Reproductive Medicine 2012:341). In South East Nigeria on the African continent the prevalence of male factor infertility was reported as 25 percent (Nwajiaku et al 2012:16,18).

Possible causes of impaired sperm production and function, or male (factor) infertility can be linked to factors acting at pre-testicular, testicular and post-testicular level (Karavolos et al 2013:2, Krausz 2011:271). According to Meachem et al (2007:2064) causes for male infertility can also be divided into medical or surgical causes.

2.3.2.1 Concept clarifications

i Infertility

Infertility is the failure of a sexually active couple, not using contraceptives to fall pregnant in one year or more in women younger than 35 years of age and after six months in women 35 years and older if efforts were made to time sexual intercourse with ovulation (Practice Committee of the American Society for Reproductive Medicine 2015:e23, Quallich 2006:277). Initial evaluation and treatment may commence earlier if either physical findings or medical history warrant it (Practice Committee of the American Society for Reproductive Medicine 2015:e23).

If sperm concentration falls below 20 x 10^6/ml semen, an individual is considered clinically infertile (Sherwood 2013:795).

ii Idiopathic male infertility

Idiopathic male infertility is when no cause for the abnormal semen analysis can be identified (Iammarrone et al 2003:214, Practice Committee of the American Society for Reproductive Medicine 2015:e23). Usually only the semen analysis is abnormal with no related history or

physical and endocrine abnormalities (Dohle et al 2005:703,708, Krausz 2011:271,281,282, Practice Committee of the American Society for Reproductive Medicine 2015:e23). Idiopathic infertility is the cause of 40-60% of infertility cases (Dohle et al 2005:703,708, Krausz 2011:271,281,282, Practice Committee of the American Society for Reproductive Medicine 2015:e23).

iii Azoospermia

Azoospermia is when no sperm is present in the ejaculate (given as the limit of quantification for the assessment method used) (Agarwal et al 2008a:159, Krausz 2011:275, Quallich 2006:279, WHO 2010:226). Two to twenty percent of infertile males are affected by azoospermia (Iammarrone et al 2003:219, Jarow et al 1989:62).

Azoospermia can further be classified as obstructive or non-obstructive (Kefer and French 2011:23, Iammarrone et al 2003:219). Obstruction can occur in the ductus deferens, epididymis and / or rete testis (Iammarrone et al 2003:220). Non-obstructive azoospermia, also known as secretory azoospermia, is due to pituitary insufficiency or primary testicular failure (Iammarrone et al 2003:220). If pathophysiology is considered, azoospermia can also be classified as pre-testicular (LH-FSH deficiency), testicular (seminiferous failure) or post-testicular (obstructive azoospermia) (Iammarrone et al 2003:220).

Reasons for azoospermia may include abnormal spermatogenesis, ejaculatory dysfunction, obstruction, hypogonadism, and iatrogenic causes, for example loss of part of the sample, chemotherapy or idiopatic factors, most probably of genetic origin (Agarwal et al 2008a:159, Agarwal and Said 2011:17).

iv Oligospermia

Oligospermia or oligozoospermia is characterized by a reduced number of sperm or low sperm concentration (Cohen and Wood 2000:422, Dohle et al 2005:703, Isidori et al 2005:314, Quallich 2006:279) or when the total sperm count or concentration of sperm is below the lower reference limit (WHO 2010:226). According to table 2-2 the lower reference limit for total sperm number is 39 (33–46) x 10^6 per ejaculate and for sperm concentration 15 (12–16)

x 10^6 per ml (WHO 2010:224). It is recommended that total sperm number rather than concentration should be used, as sperm number takes precedence (WHO 2010:226).

Various factors including heat from a hot bath or sauna, certain medications, poisoning (lead and arsenic), as well as recreational drugs for example marijuana, cocaine and anabolic steroids may cause temporary or permanent oligospermia or even azoospermia (Agarwal and Said 2011:17, Fox 2013:721, Quallich 2006:279). Loss of a portion of ejaculate as a possible cause should be investigated (Agarwal and Said 2011:17). Other reasons may include partial obstruction of the genital tract or genetic abnormalities (Agarwal and Said 2011:17, Dohle et al 2005:706).

v Asthenospermia

Asthenospermia (asthenozoospermia) points to decreased motility (Agarwal and Said 2011:17, Dohle et al 2005:703, Isidori et al 2005:314) or when the percentage of progressively motile (PR) sperm is below the lower reference limit of 32 (31–34)% (Table 2-2) (WHO 2010:226).

Possible reasons for asthenospermia may include long periods of abstinence; long periods before samples are examined, sample containers that are possibly toxic to sperm, or exposure of the sample to extreme temperatures or sunlight (Agarwal and Said 2011:17, Quallich 2006:281). Other causes may include sperm axonemal deformities, excessive leucocytes and idiopathic factors (Agarwal and Said 2011:17). Asthenospermia is also frequently seen with antisperm antibodies (immunologic infertility) (Agarwal and Said 2011:17, Quallich 2006:286). Sperm clumping with low sperm motility is an additional indication of antisperm antibodies (Agarwal and Said 2011:17).

Asthenospermia was the most common anomaly of semen observed in a study by Gaur et al (2010:35). According to the authors it may be an early pointer of a decrease in the semen quality, which sometimes gets overlooked if the semen sample has a satisfactory sperm count and normal morphology (Gaur et al 2010:40).

vi Teratospermia

Teratospermia (teratozoospermia) is indicative of a large percentage of sperm with abnormal morphological forms (Dohle et al 2005:703, Isidori et al 2005:314) or when the percentage of morphologically normal sperm is below the lower reference limit of 4 (3.0–4.0)% (5th centile with a 95% confidence interval)(Table 2.2) (WHO 2010:224,226).

2.3.2.2 Association with cancer and other diseases

Male factor infertility may be an early and easy identifiable risk factor for high-grade prostate cancer (Walsh et al 2010:2140,2146) and testicular cancer (Walsh et al 2009:351) as males with azoospermia may have a bigger risk for developing cancer (Eisenberg et al 2013b:681,685). Peng et al (2009:e5591) showed in a systematic review an association between subfertility and an increased risk for testicular cancer. The link between semen quality and cancer has also been referred to by other authors (Jørgensen et al 2011:e37, Rives et al 2012:1394 Baker et al 2005:295).

An association between impaired semen parameters and risk for overall mortality was shown in a cohort study including 43 277 men (Jensen et al 2009:559). Jensen et al. (2009:559) stated that semen quality may thus be an important biomarker of overall male health.

Furthermore, Kolettis and Sabanegh (2001:168) discovered in their research significant medical pathology in six percent of infertile men and Honig et al (1994:1028) found that one percent of all men that had been referred to an infertility clinic, had serious medical pathology diagnosed after evaluation, which included genetic disorders, endocrine disease and tumours.

2.3.2.3 Factors influencing prognosis of infertility

Factors influencing the prognosis of infertility include duration of infertility, age and fertility status of the female partner, primary or secondary infertility and results of semen analysis (Dohle et al 2005:703).

Dohle et al (2005:703) reported a conception rate of only 1.5% per month in couples trying to conceive, who had unprotected intercourse for longer than four years. Females tend to

finish their education and start a career before considering pregnancy (Dohle et al 2005:704). However by the age of 35 years the fertility potential of a woman is only 50% compared to her potential at the age of 25 years (Dohle et al 2005:704). By the time she is 38 years old her fertility potential is only 25% of the potential before the age of 30 years (Te Velde and Pearson 2002:142) and when she is older than 40 years it is less than five percent (Dohle et al 2005:704).

2.4 Factors influencing semen parameters

2.4.1 Age

Semen parameters can be affected from a young age as seen in a study performed amongst an unbiased population of young males (18 years of age), who were enrolled to compulsory military service in Denmark. Some of these young men already displayed sub-optimal semen quality that might impact on fertility (Andersen et al 2000:366,368,371).

In a review article, Stewart and Kim (2011:498,499) reports that reproductive function progressively deteriorates as an individual ages. Aging has a significant effect on semen parameters especially semen volume, sperm motility, total motile sperm, concentration, total sperm count, morphology and reactive oxygen species (ROS) content (Chen et al 2003:226,229, Cocuzza et al 2008:490, Cooke and Nelson 2011:167, Eskandar et al 2012:3, Eskenazi et al 2003:447,449, Kidd et al 2001:237, Li et al 2011a:116,119,120, Paasch et al 2010:2899, Pasqualotto et al 2005:1087–1090, Stewart and Kim 2011:498, Zitzmann 2013:617,625), partly due to a decrease in testosterone levels, contributing to hypogonadism (Sharma et al 2013:66, Stewart and Kim 2011:496, Travison et al 2007:594) and a decline in the testicular histological structure (Zitzmann 2013:617). Increased sperm DNA damage in older men has also been reported (Schmid et al 2007:180,184, Varshini et al 2012:642,647).

Research shows that age of the male partner has an influence on the ability of couples to conceive within 12 cycles. The percentage of couples unable to conceive within 12 cycles increased from 18 to 28 percent as age of the male increased from 35 to 40 years (Dunson et al 2004:51). Paternal age of more than 40 years has also been linked to miscarriage when controlled for maternal age (Kleinhaus et al 2006:369,373,376). Maconochie et al (2007:170,180,182) however report that overall paternal age does not extensively influence

the odds of miscarriage, although the odds may increase if paternal age increase above the age of 45 years. Paternal age has also been linked to pre-term birth, low birth weight, autosomal dominant diseases, chromosomal damage and (epi-)genetic problems, including neurocognitive disorders (autism, schizophrenia and bipolar disorders) and metabolic dyshomeostasis (Varshini et al 2012:642, Zitzmann 2013:625).

According to Zitzmann (2013:625) the incidence of the implicated diseases due to paternal age is still few, therefore the incidence should be compared to the individual risk of a couple seeking guidance. He added that individual counselling is needed, but that the challenge does not lie with the future parents, but in the trend that is observed within the general population.

2.4.2 Environmental factors

Optimal spermatogenesis requires a temperature of approximately 34°C which is 1 - 2^{o}C lower than core abdominal temperature (±36-37°C) (Agarwal et al 2008b:550, Ivell 2007:Online). Increased intrascrotal temperature may influence semen parameters negatively (Agarwal et al 2008b:550, Ivell 2007:Online, Kefer et al 2009:452, Thonneau et al 1998:2122,2124).

A work environment, where individuals are sedentary for long periods of time (truck or taxi drivers) (Thonneau et al 1998:2124) or exposed to heat sources (welders, bakers, foundry workers or working with laptop computers) (Sheynkin et al 2005:452,453,454, Thonneau et al 1998:2124) contributes to an increase in scrotal temperature and decrease in semen parameters (Bonde 1992:508, Ivell 2007, Jung and Schuppe 2007, Magnusdottir et al 2005:208, Sharpe 2010:1697,1703, Sheiner et al 2003:60). Additionally, if a male is seated, air does not circulate effectively around the scrotum resulting in less effective cooling (Sharpe 2010:1703). Therefore the duration of sitting during a work day is positively linked to daytime scrotal temperatures which impact negatively on semen quality (Jung and Schuppe 2007:203,205,212, Magnusdottir et al 2005:208).

Other factors that may also cause an increase in scrotal temperature include hot baths (Dohle et al 2005:709) or long use of saunas (Dohle et al 2005:709, Sharpe 2010:1703). Sharpe (2010:1703) expressed his opinion that exposure to hot showers or saunas will have a small effect as the scrotum is not immersed in water and can consequently still regulate the intrascrotal temperature.

Thermal or tight-fitting underwear may also contribute to an increase in scrotal temperature and poor semen quality (Dohle et al 2005:709). Jung et al (2005:1022) suggested that men wearing loose fitting underwear or no underwear have lower scrotal and thus testicular temperatures than those wearing tight-fitting underwear. A more recent study also showed an association between low-motile sperm concentration and wearing tight-fitting underwear (Povey et al 2012:2799). Nevertheless, it is important to note that clothes in general cause an increase in scrotal temperature irrespective of the position (supine, standing, sitting with legs crossed or uncrossed) compared to scrotal temperatures taken when men are undressed (Mieusset et al 2007:170,171,172,173).

Any factor that has an influence on the normal cooling of the testes or scrotum has a negative effect on spermatogenesis (Sharpe 2010:1704). It is therefore prudent to advice all those attempting to have children, especially those with low sperm counts or motility to reduce scrotal heating to the minimum (Sharpe 2010:1704).

It is not only increased scrotal temperature that seems to have an influence on semen parameters, but modern technology may also have an effect. The non-thermal effect of a wireless internet-connected laptop after a four hour exposure have shown to cause a significant reduction in sperm motility and an increase in sperm DNA fragmentation (Avendaño et al 2012:39,41,44).

Another technological consideration that should be kept in mind is the use of cellular phones, as cellular phones may cause oxidative stress due to the emission of radiofrequency electromagnetic waves (RF-EMW) (Agarwal et al 2009:1318,1324, 2011:440) and may impact on certain sperm parameters (Adams et al 2014:106,111). The mechanisms of exactly how RF-EMW affect sperm is currently still unknown (Agarwal et al 2011:449). Merhi (2012:297) however cautioned that a difference in effect may exist between in vitro studies and what happens in the human body, when investigating the impact of RF-EMW on sperm. In humans the cell phone and reproductive organs are separated by multiple tissue layers, which is not the case in a laboratory set-up. More research in this regard is thus needed.

Kilgallon and Simmons (2005:254) found that the position on the body where a cellular phone is worn may impact sperm motility. Cellular phones worn on the belt or in hip pockets were

more likely to result in decreased sperm motility compared to carrying no phone or carrying cellular phones elsewhere on the body (Agarwal et al 2009:1324, Kilgallon and Simmons 2005:254). Cellular phones is mostly reported to decrease sperm motility, but have also shown a negative effect on sperm count, viability, morphology and an increase in ROS and a decrease in reactive oxygen species-total antioxidant capacity (ROS-TAC) score (Adams et al 2014:106,111, Agarwal et al 2009:1318, 2011:442, Agarwal et al 2008b:552, Jurewicz et al 2009:305,320). Agarwal et al (2011:432,446), in their review, pointed out that at this stage no conclusive evidence exists to link cellular phone use with an effect on sperm parameters, as study design (especially inclusion of a control group), ethical considerations and reproducibility of studies must first be standardized. Further research is needed in men with sub-fertility as well as in the general population (Adams et al 2014:111).

Sharma et al (2013:75) expressed concern about the effect of increased use of text messaging, as it is often used more than phone calls, while Argarwal et al (2009:1318,1324) is concerned about the use of Bluetooth while cellular phones are kept in trouser pockets as this type of technology expose the testes to high-power-density radiation compared to cellular phones in standby mode. In a review on the effect of modern technology on semen parameters Agarwal et al (2011:449) therefore recommends that the increased use of smartphones should be investigated. As modern technology are widely used on a daily basis, further research is definitely warranted.

Another work environmental factor that was studied was sedentary work. Sedentary work and obesity were associated with poor semen parameters when males with poor semen quality were compared with males of normal semen quality, but with idiopathic male subfertility (Magnusdottir et al 2005:208). Lifestyle changes including a more sedentary lifestyle with high energy intake and the increased incidence of obesity may play a role in the decreased semen parameters (Magnusdottir et al 2005:208,214). The authors recommend that more research should be done to determine whether sedentary lifestyle and obesity are underlying factors of poor semen parameters (Magnusdottir et al 2005:208,214). Sharpe (2010:1697) indicated that a Western lifestyle with sedentary work or lifestyle and obesity has a potential impact on semen parameters.

2.4.3 Lifestyle factors

Lifestyle factors impact on general health and fertility potential and are described as behaviours and circumstances that are modifiable (Campagne 2013:214, Homan et al 2007:209,219).

Regular exercise is recommended at part of a healthy lifestyle (Haskell et al 2007:1081) and may contribute to general health and wellbeing (Homan et al 2007:213). Exercise may most likely provide some protection against lifestyle diseases like obesity, cardiovascular disease, hypertension, diabetes, osteoporosis and psychological stress (Homan et al 2007:213). It does seem that the general public in South African is not physically active enough to improve their general health status(Botha et al 2013:S18).

Although exercise should form part of a healthy lifestyle, extreme sports like marathon running and strength training may contribute towards poor semen quality (Dohle et al 2005:708); and pressure on the perineal area during long distance cycling may influence erectile function (Quallich 2006:279). There is also a possible link between lean and underweight men who train vigorously and reduced fertility (Sharma et al 2013:76).

The effect of alcohol intake should also be investigated, as it negatively impacts on semen parameters (Practice Committee of the American Society for Reproductive Medicine 2015:e19) especially on sperm concentration and motility (Braga et al 2012:53,56,58) as well as semen volume (Li et al 2011). Testosterone concentrations tend to increase and sex hormone binding globulin (SHBG) concentrations decrease with alcohol intake (Jensen et al 2014:Online). Excessive alcohol consumption (alcohol dependency) may negatively influence spermatogenesis, semen volume, sperm count, sperm motility and sperm morphology (Gaur et al 2010:35, Muthusami and Chinnaswamy 2005:919,922).

According to a review it is not clear which level of alcohol consumption affects fertility (Anderson et al 2010:10). But Jensen et al (2014:Online) suggested that a moderate intake of more than 5 units of alcohol per week already negatively impacts on semen quality. As alcohol intake increases to more than 25 units per week the association became more prominent (Jensen et al 2014:Online). Povey et al (2012:2799) on the other hand found no significant association between alcohol consumption and low-motile sperm concentration. Jensen et al

(2014:Online) recommended that young men should not drink alcohol regularly, but according to Anderson et al (2010:10) individuals trying to conceive should abstain from alcohol.

Gaur et al (2010:38) label smoking as a lifestyle hazard for active and passive smokers, that affects sperm count, concentration, motility and/or morphology (Anderson et al 2010, Braga et al 2012, Campagne 2013, Gaur et al 2010, Li et al 2011, Pasqualotto et al 2008). The effect that smoking has on the decline in semen parameters (Practice Committee of the American Society for Reproductive Medicine 2015:e19), including DNA and ribonucleic acid (RNA) damage appears to be directly related to the number of cigarettes smoked (Gaur et al 2010:39,40, Selit et al 2013:35). Smoking reduces the free radical scavenging ability of antioxidants, especially vitamin C and E in seminal fluid and sperm (Anderson et al 2010:15, Pasqualotto et al 2008:282) resulting in oxidative stress (Anderson et al 2010:15). According to Karavolos et al (2013:3) men with suboptimal semen quality should be strongly encouraged to quit smoking.

Povey et al (2012:2799) however failed to find an association between smoking as a lifestyle factor that results in low-motile sperm concentration. De Jong et al (2014:112) also did not find a significant association between smoking combined with drinking and semen parameters. Wong et al (2003:53) likewise did not find an association between smoking and male factor sub-fertility.

Marijuana is regarded as the most universally used recreational drug (Battista et al 2008:82). These drugs are often used during reproductive years (Fronczak et al 2012:525) and as they have an impact on hormonal axis, spermatogenesis and sperm parameters (Fronczak et al 2012:525,526, Safarinejad et al 2013:18,21) it is associated with reduced fertility (Anderson et al 2010:13, Battista et al 2008:84, Campagne 2013:216, Practice Committee of the American Society for Reproductive Medicine 2015:e19). Some authors (Anderson et al 2010:11, Fronczak et al 2012:525, Safarinejad et al 2013:23) are concerned that individuals sometimes use more than one drug. Povey et al (2012:2799) however found no significant association between recreational drug use and low-motile sperm concentration.

Underreporting of recreational drug use (Anderson et al 2010:11) and because only retrospective studies can be used (Fronczak et al 2012:525), limited knowledge on the true effect of drugs on reproductive health is available. According to Anderson et al (2010:11) research on the influence of recreational drugs has therefore mostly been done on animals.

Studies in humans have the potential to be biased as drug users often have inadequate prenatal care, are of a low socioeconomic status and use more than the recommended amount of alcohol (Anderson et al 2010:11). Another substance that may contribute to poor semen quality and infertility is the use of anabolic steroids (Campagne 2013:216, Dohle et al 2005:708, Karavolos et al 2013:3, Practice Committee of the American Society for Reproductive Medicine 2015:e19) Anabolic steroids affects libido and cause erectile dysfunction secondary to low testosterone concentrations (Gazvani et al 1997:1706,1707,1708, De Souza and Hallak 2011:1860,1865). Anabolic steroids may cause azoospermia which is sometimes reversible mostly in non-heavy users (Gazvani et al 1997:1706,1707,1708, De Souza and Hallak 2011:1860,1865).

Evidence on the effect of psychological stress on a daily basis on semen quality seems mostly small or lacking (Hall and Burt 2012, Hjollund et al 2004, Li et al 2011). Nevertheless, stress alone can potentially reduce testosterone concentrations and spermatogenesis (Hall and Burt 2012:434). A high incidence of mood and anxiety disorders has been described in infertile males undergoing assessment and treatment (Hall and Burt 2012:434,438). Investigations have not yet provided clear answers on the association between psychological stress and male fertility (Hall and Burt 2012, Homan et al 2007, Li et al 2011) but Li et al (Li et al 2011) indicated that psychological stress impacts on semen quality, including sperm density and progressive motility.

It is also realistic to accept that the general health benefits obtained by healthy living including moderate exercise and a balanced diet may similarly apply to fertility (Homan et al 2007:214). Lifestyle practices are theoretically modifiable and changes should be recommended to couples trying to conceive (Homan et al 2007:214,220). Small lifestyle changes can therefore have a positive effect on spermatogenesis (Sharpe 2010:1704).

Sharma et al (2013:66) concluded that the growing attention and amount of research on impact of lifestyle factors (smoking, recreational drug use, alcohol intake, obesity, exercise, psychological factors, dietary factors and age when starting a family) on fertility suggests that these factors have a significant impact. Present lifestyle habits and amenities are a challenge as it probably impairs reproductive ability to a certain extent (Ivell 2007:Online, Sharma et al 2013:66,77). According to Campagne (2013:221) there are no commonly appropriate 'safe' and 'unsafe' thresholds for lifestyle, environmental and psychological factors that may impact negatively on semen parameters. He further suggests that assessment and recommendations should be individualised.

Clear evidence on all factors influencing spermatogenesis is still pending, therefore research should be done to convincingly provide evidence or contest it (Sharma et al 2013:66,77).

2.4.4 Weight

According to Qin et al (2007:827) and Jensen et al (2004:863) being underweight has a negative impact on semen parameters. On the other hand various factors may contribute to the effect of obesity on sperm production and infertility as indicated in a review by Hammoud et al (2008a:900,902). Teerds et al (2011:667) reported that infertility is not a significant problem in most overweight and obese men, even with lower testosterone and sex hormone binding globulin (SHBG) concentrations as indicated in a systematic review with a meta-analysis (MacDonald et al 2010:293, Zhang et al 2014:1861,1863), but that infertility in overweight / obese males may be influenced by leptin insensitivity (Teerds et al 2011:667). Changes in reproductive hormone concentrations, due to overweight or obesity do not automatically cause a reduction in the reproductive potential in these men (Chavarro et al 2010:2222). This opinion of a small effect of overweight / obesity on semen quality is supported by a number of researchers (Aggerholm et al 2008:619,625, Chavarro et al 2010:2222,2225–2227,2230, Duits et al 2010:1356–1358, Hammoud et al 2008a:902).

Stewart et al (2009:1561) however disagree and indicated that obesity, even in fertile Australian men, appear to reduce testicular function. Several other researchers also reported that overweight and obese men tend to have a higher risk for poor semen parameters (Kay and Barratt 2009:237, Magnusdottir et al 2005:208, Martini et al 2010:1739,1741,1742),

sexual dysfunction and subfertility (Du Plessis et al 2010:159). Typical changes in semen parameters reported include low semen volume, smaller numbers of morphologically normal sperm when compared to men with normal BMI (Shayeb et al 2011:717,722), decreased sperm concentration, motility, progressively motile sperm count (Braga et al 2012:56, Hammoud et al 2008b:2222, Hofny et al 2010:581, Jensen et al 2004:863,866, Kasturi et al 2008:257, Martini et al 2010:1739,1741, Stewart et al 2009:1563,1566), total sperm count (Hammoud 2006:624, Jensen et al 2004:863,866) and increased DNA damage (Kasturi et al 2008:257). Eisenberg et al (2014:193,198–199, 2015:493,494) evaluated the impact of body size, including BMI and waist circumference (WC), of 501 fertile males on semen parameters. Both measures showed a negative effect on semen parameters. A meta-analysis of 21 studies also showed an increased prevalence of azoospermia or oligospermia in overweight and obese men (Sermondade et al 2012:2).

An elevated BMI is associated with a sex hormone imbalance that can increase the risk of subfertility and infertility in men (Aggerholm et al 2008:619,622,624,625, Jensen et al 2004:863,866,868, Kay and Barratt 2009:237, Nguyen et al 2007:2488,2492, Du Plessis et al 2010:159). Hormonal changes, including increased oestrogen and reduced androgen, total testosterone (TT), free testosterone (FT), SHBG (Aggerholm et al 2008:619, Hammoud et al 2008a:897,900,902, Jensen et al 2004:863, Kay and Barratt 2009:237,240, MacDonald et al 2010:308, Pasquali 2006:363,369, Du Plessis et al 2010:159), as well as increased insulin resistance, and reduced inhibin B concentrations, associated with obesity, may play an important role in semen parameters, male subfertility and infertility (Aggerholm et al 2008:619, Hammoud et al 2008a:897,900,902, Jensen et al 2004:863, Kay and Barratt 2009:237,240, MacDonald et al 2010:308, Pasquali 2006:363,369, Du Plessis et al 2010:159). The increase in oestrogen concentration due to aromatization in fatty tissue has been indicated as a possible mechanism for the hypoandrogenemia and changes in sperm parameters (Hammoud et al 2008a:902). It must also be noted that central obesity is predominantly linked to lower TT, FT and SHBG (Mah and Wittert 2010:180, Pasquali 2006:363,369). A greater decline in TT and FT is observed as men get older when compared to lean men (Mah and Wittert 2010:180).

Hormonal changes seen in obese men, shows that dysregulation of the hypothalamic-pituitary-gonadal (HPG) axis and endocrine dysregulation might explain the increased risk of

poor semen parameters and infertility (Hammoud et al 2008a:897, Du Plessis et al 2010:159). Excessive adipose tissue may also contribute to a higher conversion rate of testosterone to estradiol, which may contribute to secondary hypogonadism during reproductive (hypothalamus-pituitary-gonadal) axis suppression (Michalakis et al 2013:458,471). Overweight and obesity also cause excessive suprapubic and thigh fat, which cause an increase in scrotal temperature (Kasturi et al 2008:251,253,257, Kay and Barratt 2009:238,239,240).

Some authors however found no significant correlation between BMI and semen parameters (Aggerholm et al 2008:619, Eskandar et al 2012:1,2), even in a systematic review with meta-analysis (MacDonald et al 2010:293) or low-motile sperm concentration (Povey et al 2012:2799).

2.4.5 Dietary intake

Poor, unbalanced diets, combined with a high alcohol intake, has been linked to increased seminal oxidative stress (Kefer et al 2009:453,454, Koch et al 2004:191).

Vegetables, fruits, nuts and whole grain products are food sources rich in antioxidants including vitamin A, ß-carotene vitamin E and vitamin C (Al-Azemi et al 2009:584, Gallagher 2012:60,71,84,88,89), which assist to reduce oxidative damage in sperm, maintain the integrity of sperm cells (Cheah and Yang 2011:182, Sharma et al 2013:67, Wong et al 2003:53) and improve semen parameters (Mendiola et al 2010:1128,1132). According to Showell (2011:6) dietary intake of antioxidants is vital for optimal semen quality.

Wong et al (2003:53) named a low intake of vegetables and fruits a risk factor for male factor sub-fertility. Braga et al (2012:53,55,56) showed a positive link between sperm motility and fruit intake. The phytochemical, lycopene found in vegetables like tomatoes, has been linked to improved total motile sperm count and semen parameters. (Mendiola et al 2010:1128,1130,1131, Mínguez-Alarcón et al 2012:2807,2810–2811). When considering sperm quality, including sperm motility Braga et al (2012:53,55,56) showed improvement with increased intake of fruits, but found no association between sperm morphology and any specific food or food group. A higher intake of vitamin C rich fruits and vegetables was

associated with increased semen volume in students (Mínguez-Alarcón et al 2012:2807,2810–2813).

Gaskins et al (2012:2899) reported that a prudent dietary pattern in young men (fish, chicken, vegetables, fruit, legumes and whole grains) favourably affected progressively motile sperm.

A high cereal (carbohydrate) and fibre intake have also been linked positively to semen parameters (Braga et al 2012:55,56, Mendiola et al 2010:1128,1130,1131). Unrefined cereals / carbohydrates, vegetables and fruits are good sources of fibre.

Total fat intake was negatively related to total sperm count and concentration. These associations appeared to be driven primarily by the intake of saturated fat (Attaman et al 2012:6). Diets with a low polyunsaturated and high saturated fat content have been associated with lower semen quality (Attaman et al 2012:5,8, Jensen et al 2013:415,417) with a dose-response reported for lower sperm concentration and lower total sperm count (Jensen et al 2013:417, Olsen and Ramlau-Hansen 2012:511) even in the general population (Jensen et al 2013:417). The intake of trans-fatty acids, mainly from hydrogenated oils in chips (French fries) and commercially baked food items, may also contribute to lower sperm count (Chavarro et al 2014:429,431,433–434,436–438). However the impact of this modifiable factor on fertility is still unknown (Chavarro et al 2014:429,432,434,437,438).

Attaman et al (2012:6) indicated in a large study on the effect of dietary fats on male infertility, that a high intake of processed meats that contain considerable amounts of saturated fats, was associated with poorer semen parameters. It can therefore be concluded that a lower intake of fats and protein may have a positive effect on semen quality (Mendiola et al 2010:1128,1130,1131). A higher intake of omega-3 fatty acids as recommended by the Academy of Nutrition and Dietetics (2014:136), showed a significant positive association with normal sperm morphology (Attaman et al 2012:6, Olsen and Ramlau-Hansen 2012:511) and is associated with a significant lower number of sperm head defects (Attaman et al 2012:6, Olsen and Ramlau-Hansen 2012:511).

The intake of full fat dairy products had a negative impact on progressive motility and morphology of sperm in physically active men (Afeiche et al 2013:2265,2267–2270,2273).

This association was mainly linked to the consumption of cheese and not related to overall dietary patterns (Afeiche et al 2013:2265,2267–2270,2273).

2.4.5.1 Specific nutrients

According to Cheah and Yang (2011:182) various nutrients are involved in spermatogenesis, sperm maturation and development of the reproductive system, but have not been studied in depth. Antioxidants protect sperm from oxidative damage (Agarwal et al 2008c:2, Cheah and Yang 2011:182, Sikka 2004:5) and several nutrients and antioxidants have been proven beneficial to improve fertilization and male infertility (Agarwal and Sekhon 2010:217, Begum et al 2009:16). Dietary intakes of cryptoxanthin, vitamin C and ß-carotene were positively associated with total motile sperm count (Mínguez-Alarcón et al 2012:2807,2810–2813).

In the following section, nutrients affecting sperm parameters will be discussed as water soluble vitamins, fat soluble vitamins, minerals and combinations, as well as fatty acids.

Water soluble vitamin C supports normal functioning of the male reproductive system, genetic integrity of sperm (Begum et al 2009:17, Cheah and Yang 2011:186) and protects cells from oxidative stress (Gallagher 2012:88, Cheah and Yang 2011:196). Vitamin C intake is closely linked to sperm number and concentration in healthy men (Eskenazi et al 2005:1006) and sperm motility and sperm number with normal morphology in infertile men (with oligospermia) (Akmal et al 2006:440). The Recommended Dietary Allowance (RDA) for vitamin C for males between the ages of 18-45 years is 90 mg/day with a Tolerable Upper Intake Level (UL) of 2 000 mg/day (IOM 2000:Online). According to Mínguez-Alarcón et al (2012:2807,2813) the present recommendations of vitamin C intake may not be sufficient to be of benefit to semen quality.

An intake of more than 400 mg vitamin C per day in healthy men showed a positive link with sperm count (Eskenazi et al 2005:1006,1010). Akmal et al, (2006:440) showed that vitamin C supplementation of 2000 mg/day to infertile men for two months might improve sperm count, sperm motility, and sperm morphology and may be used to improve semen parameters. Furthermore Agarwal and Sekhon (2010:217) stated in a review article that vitamin C, vitamin E and carnitine are effective in the treatment of male infertility and may be regarded as a first line treatment. In contrast Rolf et al (1999:1028,1030) found no

improvement in sperm parameters in a randomized controlled trail after infertile men were supplemented for 56 days with high doses of vitamin C (1 000 mg/day) and vitamin E (800 mg/day). The supplementation period however was too short as sperm development takes 72-74 days to complete (Quallich 2006:277). A randomized controlled trial with Vitamin C (1 000 mg/day) and Vitamin A (1 000 mg/day) supplemented for 60 days showed a significant reduction in sperm DNA damage (Greco et al 2005a:349, Greco et al 2005b:2590). This reduction in sperm DNA damage was also seen in a study where 400 mg vitamin C, 400 mg vitamin E, 18 mg β-carotene, 500 μmol zinc and 1 μmol selenium were supplemented for 90 days, but where an unforeseen negative result, increased sperm decondensation, was reported (Ménézo et al 2007:418,419).

Folic acid (Vitamin B_9) improved sperm concentration in subfertile human males when a dose of 5 mg combined with 66 mg zinc sulphate was given for 26 weeks (Wong et al 2002:491). The Adequate Intake (AI) for folate for males (18-45 years) is 400 μg/day, while the UL for the same age group is 1 000 μg/day (IOM 1998: Online). Both folate and zinc are involved in the production of DNA and RNA (Wong et al 2000:435).

Vitamin B_{12} in humans is involved in cellular division, especially in DNA and RNA synthesis (Gallagher 2012:86, Begum et al 2009:17). An UL for Vitamin B_{12} for human males has not been determined, but the RDA for males between the ages of 18-45 years has been set at 2.4 μg/day (IOM 1998: Online).

Fat soluble vitamin A is needed for spermatogenesis (Gallagher 2012:60). A dietary intake of more than 3 970 μg/day of β-carotene in healthy men showed a positive correlation with sperm concentration and progressive motility (Eskenazi et al 2005:1006,1010). The RDA for vitamin A for males (18-45 years) is 900 μg/day with a UL of 3 000 μg/day (IOM 2001: Online).

Vitamin E is involved in the functioning of reproductive systems (Gallagher 2012:113) and protects cell membranes from oxidative degradation from ROS (Gallagher 2012:70,72, Suleiman et al 1996:530). Vitamin E can also restore incomplete acrosomal membranes which are needed for successful fertilization (Cheah and Yang 2011:194). In healthy men a high dietary intake of vitamin E has been linked to improved progressive sperm motility (Eskenazi et al 2005:1006,1011). Males between the ages of 18-45 years should consume 15 mg vitamin

E/day (RDA) and their intake should not exceed 800 to 1 000 mg/day depending on their age (UL) (IOM 2000:Online). Miller et al (2005:37) however stated that the daily intake of vitamin E should not exceed 400 IU/day, as a meta-analysis has shown that higher dosages have been linked to all-cause mortality. An International Unit of vitamin E equals 0.67 mg of RRR-α-tocopherol or 1 mg all-rac- α-tocopherol (Dietary Supplement Ingredient Database, 2015: Online).

Low serum concentrations of vitamin A and E have been linked to poor semen parameters when men with normal sperm were compared with men with oligospermia and asthenospermia (Al-Azemi et al 2009:584,588).

Vitamin E and selenium may reinforce each other in their antioxidant function to reduce oxidative damage (Gallagher 2012:120). The RDA for selenium in males 18-45 years is 55 μg/day and the UL is set at 400 μg/day (IOM 2000: Online). Keskes-Ammar et al (2003:83,86) showed that sperm motility significantly improved and sperm malondialdehyde (MDA) concentration was reduced with an intake of 400 mg vitamin E per day and 225 μg selenium per day compared to a placebo over a three month period in an age range of 23-67 years. A significant reduction in MDA concentration was also found after supplementation with only vitamin E in a dose of 100 mg three times per day in asthenospermic males (Suleiman et al 1996:530,533). However a combination of vitamin E (500 mg, twice a day) and vitamin C (500 mg, twice a day) over a two month period provided to infertile men with more than 15 percent fragmented DNA (Greco et al 2005a:349, Greco et al 2005b:2590) did not improve sperm morphology, but significantly reduced sperm DNA damage, as seen in the reduced percentage of damaged sperm (Greco et al 2005a:349,352, Greco et al 2005b:2590).

Selenium is an ultra-trace element, which acts as a potent antioxidant and is essential to human health (Gallagher 2012:117,120, Beckett and Arthur 2005:455, Rayman 2012:1256,1257). The RDA for selenium in males 18-45 years is 55 μg/day and the UL is set at 400 μg/day (IOM 2000:Online). A study by Safarinejad and Safarinejad (2009:741,745), showed that selenium (200 μg/day) and/or N-acetyl-cysteine (600 mg/day) supplementation over a six month period improved semen parameters significantly in males with idiopathic oligo-asthenoteratospermia. Agarwal and Sekhon (2010:217) considered selenium, based on a few well conducted studies as a possible second line treatment for male infertility. If the

selenium content of selenoproteins in animals is low, sperm may be more susceptible to oxidative stress which may decrease the possibility of fertilization (Beckett and Arthur 2005:455).

Zinc, a trace mineral, is indispensable for normal functioning of the male reproductive system, including spermatogenesis, androgen production, proliferative activity of germ cells and capacitation of sperm (Begum et al 2009:17, Tikkiwal 1987 et al:30,33, Wong et al 2000:435). An inadequate intake of zinc may impede testicle development and may contribute to termination of spermatogenesis (Cheah and Yang 2011:183). Zinc deficiency has been linked to decreased spermatogenesis and impaired male fertility in sub-fertile males (Wong et al 2000:435). Low zinc concentrations have been linked to decreased testosterone levels and sperm count in oligospermic males (Tikkiwal 1987 et al:30,32).

Zinc and selenium are needed for morphological integrity of sperm, particularly midpiece formation (Cheah and Yang 2011:194). If midpiece formation is abnormal, the connection of the sperm head and tail to this piece will be affected, which in turn will negatively impact on fertilization (Cheah and Yang 2011:194). The RDA for zinc in males (18-45 years) is 11 mg/day and the UL is 40 mg/day (IOM 2000: Online). When infertile males in France were supplemented with 400 mg vitamin C, 400 mg vitamin E, 18 mg β-carotene, 500 µmol zinc and 1 µmol selenium for 90 days, a decrease in sperm DNA fragmentation, but also a negative increase in sperm decondensation was found (Ménézo et al 2007:418,419).

Zinc sulfate (66 mg/day) has also been used with folic acid (5 mg/day) in subfertile men in a placebo controlled trial (Wong et al 2002:491,493). The zinc and folic acid combination significantly increased sperm concentration, but also caused a small increase (4%) in abnormal sperm, despite normal blood folic acid or zinc levels (Wong et al 2002:491,496). Similar trends regarding sperm concentration were seen in fertile males (Wong et al 2002:491,496). The authors speculated that lower, physiological doses of micronutrients may have a more beneficial effect as the lower doses have a stronger influence on absorption, transport and metabolic processes (Wong et al 2002:497). It is even suggested that the beneficial effect of supplementation may be larger if lower doses of folic acid or zinc sulfate are used (Wong et al 2002:497).

Lipids form part of the structure of sperm membranes and are involved in the functional activity of sperm (Gallagher 2012:41,44,45, Mandal et al 2014:1 Tavilani et al 2007:45, Wathes et al 2007:190). Sperm have a high polyunsaturated fatty acid (PUFA) content (Hammadeh et al 2009:87, Ménézo et al 2007:418) and one of the functions of PUFA's in the cell membrane is to sustain the properties of the lipid bilayer (Farooqui et al 2000:3). PUFA's are sources of antioxidants (Showell et al 2012:5). The fatty acid composition and amount of PUFA's in sperm seems to determine the physiological features, morphology and function of the cells (Lenzi et al 2000:230, Safarinejad et al 2010:103). The lipids, especially docosahexaenoic acid (DHA; 22:6n-3) in the sperm membrane helps to maintain the fluidity and flexibility of sperm, and is thus necessary for successful fertilization (Aksoy et al 2006:75, Safarinejad et al 2010:100,101, Wathes et al 2007:190,197). A significant correlation is described between DHA levels and sperm concentration, motility, morphology and antioxidant activity of seminal plasma (Aksoy et al 2006:75,79, Safarinejad et al 2010:104). According to Safarinejad et al (2010:100) fatty acid composition of sperm may be a significant predictor of fertility.

Infertile males have lower concentrations of DHA in sperm and males with asthenospermia, oligospermia, oligoasthenospermia or oligoasthenoteratospermia have significantly higher omega-6: omega-3 ratios (Aksoy et al 2006:75,77, Conquer et al 1999:795,798, Safarinejad et al 2010:100,104), and a lower omega-3 index [eicosapentaenoic acid (EPA) + DHA] (Safarinejad et al 2010:100,104) than normospermic males. Lower concentrations of PUFA's, DHA and higher concentrations of saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) are found in sperm of males with asthenospermia, oligospermia and/or oligoasthenospermia compared to normospermic males (Aksoy et al 2006:75,78, Conquer et al 1999:793,798, Tavilani et al 2007:45,48,49). Tavilani and co-authors proposed that small variations in fatty acid concentrations and compositions could have a negative impact on sperm, with declines in sperm motility and fertility in men with asthenospermia (Tavilani et al 2007:49). It has been suggested that supplementation with PUFA's could improve semen quality in males with oligospermia, asthenospermia and/or oligoasthenospermia (Aksoy et al 2006:75,79) or idiopathic oligoasthenoteratozspermia (Safarinejad et al 2010:104).

According to Wathes et al (2007:198) PUFA's are a two-edged sword. PUFA's are vital, but high levels are potentially detrimental to the cell. The high levels of PUFA's needed for fluidity of sperm and eventual fertilization, makes the sperm susceptible to attack by ROS. The

amount of PUFA's that should be consumed for optimum fertility, remains unclear (Wathes et al 2007:190,198).

An optimal plasma DHA response can be achieved with a dose of ≈2 gram DHA /day (Arterburn et al 2006:1467S,1470S). According to the IOM (2005:Online) an adequate intake of DHA for men is 1.6 gram/day and a dose of more than 3 g / day should be avoided (Opperman 2013:9).

Most of the recommendations for omega-3 fatty acid intake are approximately 500 mg/day and consumers should be advised to use supplements containing these amounts (Opperman 2013:7,9). Furthermore, supplement labels should clearly indicate the fish oil supplement content, the EPA and DHA contents, as well as the source of fish oil (Opperman 2013:9).

Supplements that have not been exposed to excessive light and are packaged in opaque containers should be bought, since exposure to light may speed up the rancidity process (Opperman 2013:9). Supplements beyond the expiry date should not be used (Opperman 2013:9). The most expensive supplements do not always epitomise optimum quality (Opperman 2013:9).

Overall, fish oil supplements are seen as a harmless and easy way to accomplish adequate omega-3 fatty acid intakes (Opperman 2013:10). If omega-3 fatty acid supplements are consumed with a fat-containing meal it may assist the absorption of the fatty acids (Opperman 2013:10).

Due to the outstanding safety profile of omega-3 fatty acid supplements, it has been suggested that it can be used as nutraceuticals to improve semen quality (Safarinejad et al 2010:101).

2.5 Conclusion

Various demographic-, environmental-, lifestyle- and dietary factors, as well as nutrients and anthropometric indices may impact on the general health and fertility of males. As these factors are mostly modifiable, all these factors should be considered when a couple plans to have children, as each factor might contribute to better health and possibly improved fertility. Although sperm parameters provide an indication of the general health of males, males with

a low sperm count can still fertilize an ovum and have children, while those with normal sperm counts could be unable to have children (Campagne 2013:215, Safarinejad et al 2013:23).

Chapter 3 METHODOLOGY

3.1 Introduction

In this chapter the study design, study population, sample selection, data collection process and time frames, measurements and techniques used, validity and reliability of measurements, ethical consideration, statistical analysis and limitations of the study are discussed.

3.2 Study design

A single blind placebo controlled intervention study was conducted between February and November 2015.

3.2.1 Study population

The study population consisted of males between the ages of 18 and 45 years from three ethnic groups (white, black and coloured) in the Bloemfontein area.

3.2.2 Sample selection

A convenience sample of 25 healthy, male volunteers per group is deemed to be adequate to achieve 80% power to detect a difference of 0.72 using a two-side hypothesis test at a significance level of 0.05 (Zar, 1984, Guenther, 1977, Graybill, 1961). A sample of 56 males were recruited by means of advertisements at the National Defence Force, the campus of the University of the Free State, personal recruitment and by word of mouth. The results of 50 males were used for final analysis as 6 participants did not provide all four semen samples.

3.2.3 Inclusion Criteria

Males between 18 and 45 years, that reacted on advertisements and provided consent to participate in the study.

3.2.4 Exclusion criteria

Participants were excluded from the study in case of:

a history of cancer treatment;

previous testes surgery;

use of medication known to limit sperm production in the last three months; and/or

the daily use of a dietary supplement during the previous three months.

3.3 Study procedures

The study was conducted as follows:

The protocol was submitted and approved by an evaluation committee of the Faculty of Health Sciences on 22 November 2013.

Ethical approval was obtained from the Health Research Ethics Committee (ECUFS NR 08/2014), Faculty of Health Sciences, University of the Free State in February 2014.

To recruit participants, the study was advertised in the Faculty of Health Sciences and the National Defence Force by means of electronic advertisements and presentations. Approval was later obtained to advertise among students on the campus of the University of the Free State (Addendum B) as well.

All volunteers were assigned a three digit study number and listed on a participant register.

A pilot study was conducted on five participants that were randomly selected from the participant register by Department of Biostatistics to test logistics, measurement procedures and techniques as well as the questionnaires. As no changes were made after the pilot study, data obtained during this process were included in the final results.

At the start of the study, participants were orientated by the researcher about the aims, goals and procedures of the study. Each participant received an information document and completed a consent form in the language of his choice, English, Afrikaans or Sesotho (Addendum C). Sesotho documents were available but were not used by any participant

Two baseline appointments were scheduled with participants, who were requested to report at the Department of Obstetrics and Gynaecology, Faculty of Health Sciences, University of the Free State.

During the first visit, a self-administered questionnaire (Addendum D) was completed and data on age, environmental-, lifestyle- and dietary factors collected. The researcher was available to answer questions while participants completed the questionnaire and ensured that all questions were answered.

Anthropometric measurements were taken by the researcher in a private room and recorded on the questionnaire. Anthropometric measurements included weight and height as well as neck-, waist- and hip circumferences. Standard measurement techniques were used, as described later in this chapter.

Two semen samples were required from participants and a private room was available for collection. Participants were requested to have sexual intercourse or masturbate; and then abstain for three days before each sample was provided for the study. A second sample was provided three days after the first. Samples were delivered by the researcher to the laboratory within 30 minutes of collection.

Participants were randomized in two groups, one receiving nutrient supplements (vitamin-mineral and omega-3) and the other receiving a placebo. Randomization tables were used to allocate participants to either the supplementation or the placebo group. Nutrient supplementation included Centrum® Adult and Centrum® My Nutrients™ Omega 3 and the placebo containing glucose and fructose. The placebo consisted of a white tablet, manufactured by Acuvet Pharma, South Africa. The nutrient analysis of the supplements is shown in Addendum E. Participants were instructed to take the vitamin mineral supplement twice daily, one Omega-3 mini-gel in the morning and two Omega-3 mini-gels in the evening. The placebo group was instructed to take the placebo twice daily. Participants took the nutrient supplements or placebos daily for a period of three months. Compliance was sought by requesting participants to return empty containers after 90 days. Although participants were randomly assigned to groups, final group sizes differed as a result of participants not providing all semen samples/ withdrawing from the study.

Semen parameters were determined by the Department of Obstetrics and Gynaecology, using standard techniques. Analysis of fatty acid composition of sperm membranes was performed by the Department of Microbial, Biochemical and Food Biotechnology.

Baseline data were captured as soon as questionnaires were completed and pre- intervention semen analysis were available. Data were captured by the researcher on two separate Excel sheets and electronically compared, to detect input errors. If an input error was detected, the researcher referred back to the original data sheets of the individual. Data were cleaned before analysis was done. Respondent numbers were used on data sheets and only the researcher and biostatistician had access to data in a secure environment.

After the intervention period of three months, anthropometric measurements were repeated and two semen samples provided three days apart. These post-intervention results were captured as soon as semen analysis were available. The study treatment was revealed at completion of the study and the placebo group then received their nutrient supplements.

Statistical analysis was performed by the Department of Biostatistics, University of the Free State and interpreted.

Written feedback was provided to participants who requested feedback on their results.

Study results are presented in the form of a thesis in the article format as approved by the University of the Free State.

3.4 Time frames

The study was planned and executed according to the time frame as indicated in Table 3.1.

Table 3.1 Time frames of study

Activity	Date
Protocol	January to November 2013
Evaluation committee	November 2013
Obtain ethical approval	February 2014
Obtain international sponsorship	June 2014 to February 2015
Pilot study and adaptations	February to June 2015
Baseline semen analysis Literature review continue	June to August 2015
Administer nutrient supplement and post-supplementation semen analysis	September to November 2015
Data analysis	September to November 2015
Writing of thesis	January to December 2015
Editing of thesis Submit completed thesis Submission of report to Department Obstetrics and Gynaecology	January 2016
Publication of articles	February to December 2016

3.5 Measurements and description of techniques

In this study age, environmental-, lifestyle- and dietary factors, as well as anthropometric measurements and semen parameters were determined according to the objectives set for this study.

3.5.1 Age

As only males were included, age was the only other demographic factor obtained in the self-reported questionnaire (Addendum D).

3.5.2 Environmental Factors

Environmental factors recorded in the self-reported questionnaire (Addendum D) included work environment, use of modern technology, use of saunas and hot baths, as well as wearing of tight-fitting underwear/ clothes. For categorising purposes the affirmative or not was used for use of laptop on lap, tight underwear/ trousers and use of hot bath. For the same purpose the use of electronic devices connected to Wi-Fi was split into 0-3 hours and ≥4 hours, place where cell phone is worn into belt/ hip pocket and other, as well as self-reported work stress as low or high.

3.5.3 Lifestyle Factors

Lifestyle factors referred to physical activity level, alcohol intake, smoking, recreational drug use, supplement use and psychological stress (Addendum D), and were also obtained by means of the self-reported questionnaire.

Physical activity was determined using the validated last seven day, self-administered short International Physical Activity Questionnaires (IPAQ) (Craig et al 2003:1381,1388) (Addendum D). One Metabolic Equivalent of Task (MET) is defined as the amount of energy required at rest and is equal to 3.5 ml O_2 per kg body weight x min (Jetté et al 1990:555).

Activity was categorised as low, moderate and high:

Category 1 (low): participants who do not meet criteria for Categories 2 or 3 are considered to have a ‘low’ physical activity level.

Category 2 (moderate):

Three or more days of vigorous-intensity activity of at least 20 minutes per day; or

Five or more days of moderate-intensity activity and/or walking of at least 30minutes per day; or

Five or more days of any combination of walking, moderate-intensity or vigorous intensity activities achieving a minimum total physical activity of at least 600 Metabolic Equivalent of Task (MET)-minutes/week.

Individuals meeting at least one of the above criteria were defined as accumulating a minimum level of activity and therefore were classified as 'moderate'.

Category 3 (high):

Vigorous-intensity activity on at least 3 days achieving a minimum total physical activity of at least 1500 MET-minutes/week; or

Seven days of any combination of walking, moderate-intensity or vigorous-intensity activities achieving a minimum total physical activity of at least 3000 MET-minutes/week.

Results were evaluated according to current recommendations for adults for activity which is 150 minutes (2 hours and 30 minutes) per week of moderate-intensity, or 75 minutes (1 hour and 15 minutes) per week of vigorous-intensity aerobic physical activity (Lambert et al 2001:S12, USDA 2010:18).

Alcohol intake was assessed by recording the number of alcohol units a participant consumes per week (Addendum D). If alcohol is consumed, the recommended intake for males is two or less units/day, which is considered a moderate intake (USDA 2010:31). The supporting documents for the first South African Food Based Dietary Guidelines (SAFBDG's), published in 2001, indicate low risk drinking for men as consuming less than four alcohol units/day, with at least 2 alcohol-free days per week (van Heerden and Parry 2001:S71). In the current SAFBDG's, the guideline for alcohol is no longer included, due to the health risks associated with alcohol consumption outweighing its health benefits (Jacobs and Steyn 2013:S119, Vorster et al 2013a:S7). In this study habitual alcohol intake was recorded and for association purposes was categorised as <5 portions per week and ≥5 portions per week as Jensen et al (2014:Online) suggested that a moderate intake of more than 5 units of alcohol per week negatively impacts on semen quality.

Questions on smoking assessed if participants smoked or not, the number of cigarettes /day, the number of packs smoked per week, as well as years of smoking (Addendum D). Recreational drug use and supplement use were also assessed (Addendum D). In this study, supplements referred to all non-food substances consumed to supplement the diet.

Psychological stress refers to any work-related stress or stress experienced at home, and was indicated as self-reported stress, on a Likert scale from one to ten (Addendum D).

3.5.4 Dietary factors

For the purpose of this study dietary factors referred to usual intake of specified foods including vegetables and fruits, starchy foods, fibre, dairy products, fatty foods, meats and processed meats, as well as beverages. Intake was reported by means of the self-administered questionnaire (Addendum D) to provide a quantitative indication and frequency of intake.

A food frequency questionnaire is defined as "A questionnaire in which the respondent is presented with a list of foods and is required to say how often each is eaten in broad terms such as x times per day / per week / per month, etc. Foods chosen are usually chosen for the specific purposes of a study and may not assess total diet." (Cade et al 2002:567).

Intake of vegetables and fruit, starchy foods, fibre, dairy products, fatty foods, meat and processed meat, beverages and alcohol was interpreted using recommendations from the South African Food Based Dietary Guidelines (SAFBDG) for adults (Vorster et al 2013a:S5–S12, Vorster 2001:S1-S6), the Dietary Guidelines for Americans (USDA 2010:1–54) and Guidelines for Healthy Eating (2012:Online). The SAFBDG represents a prudent dietary pattern for South Africans. Frequency of intake was used to describe intake as higher versus lower intakes, and also to examine interrelationships between dietary intake and other variables (e.g. semen volume, sperm concentration, sperm motility and sperm morphology as well as fatty acid composition of semen).

Vegetable and fruit intake was assessed with the self-reported questionnaire (Addendum D), as these are good sources of vitamins especially vitamins A, C, and folate (Gallagher 2012:63-66). The SAFBDG recommends vegetable and fruit consumption equivalent to 400g (approximately five portions) / day (Love and Sayed 2001:S24,S29, WHO 2003:56). Fruit and vegetable intake was described according to frequency of consumption and for association purposes was categorised as one vegetable and one fruit portion per day.

Intake of fibre was determined in the structured questionnaire (Addendum D) with questions on consumption of fruit and vegetables, whole grains, fortified cereals and legumes (USDA 2010:35,36,52,88). The recommended fibre intake for males is 38g/day which can be achieved by eating a variety of fibre rich foods daily (IOM 2002:Online, Love and Sayed 2001:S26,S27, USDA 2010:41, Venter and van Eyssen 2001:S32). Fibre consumption practices were described according to frequency of consumption.

The SAFBDG recommends that fat should be used sparingly and that vegetable oils and unsaturated fats rather than hard fats should be used, as high fat diets have an adverse effect on health (Smuts and Wolmarans 2013:S87, Wolmarans and Oosthuizen 2001:S48). The SAFBDG recommends that total fat intake should be less than 30% of total energy (Smuts and Wolmarans 2013:S87, Wolmarans and Oosthuizen 2001:S48). It does seem that the quality rather than the quantity of fat is important, especially to prevent coronary heart disease (Rossouw 2015:38,42). However, high saturated fat intake is linked to a negative impact on sperm concentration (Attaman et al 2012:1,3,4,5,8). Fat intake was described according to the frequency of consumption.

Less than 560g meat (red meat, fish and poultry) per week is recommended by the 2001 SAFBDG (Scholtz et al 2001:S39,S46), but this recommendation was refined to an intake of 90g lean meat (beef, lamb, pork, chicken) per day (Schonfeldt et al 2013:S75). The USDA recommends an intake of 45 g poultry (chicken and turkey) and 36 g fish per day to achieve an intake of 165 g protein rich foods from animal and plant sources (USDA 2010:53). The SAFBDG recommends two to three servings of fish per week (Scholtz et al 2001:S46, Schonfeldt et al 2013:S69,S75) and the USDA food guidelines recommends consuming 240g or more seafood per week (USDA 2010:52). Poultry and meat intake of study participants were described according to the frequency of consumption (Addendum D) and the intake of seafood was categorised for association purposes as <2 portions and ≥2 portions per week.

Egg consumption was described according to number of eggs consumed per week. The SAFBDG recommends up to four eggs per week (Scholtz et al 2001:S45, Schonfeldt et al 2013:S75).

An intake of 400 - 500 ml of low-fat milk, maas or yoghurt, per day is advised (Vorster et al 2013b:S58–S59). Milk, maas or yoghurt consumption was categorised in less than 500 ml and ≥500 ml per day.

The SAFBDG recommend that salt and foods high in salt should be used sparingly, if at all and includes salt used at the table, in the preparation of meals, as well as processed foods (Charlton and Jooste 2001:S55, Wentzel-Viljoen et al 2001:S105). Processed meats are those preserved by smoking, curing, or salting, or by the addition of preservatives and questions regarding the consumption may give a qualitative indication of sodium and fat intake (Addendum D). Processed meats such as biltong, ham, bacon, polony, salami, sausages and viennas were included in the questionnaire (Charlton et al 2007:86, USDA 2010:67). Consumption of added salt and processed meat was described according to the frequency of intake.

For categorising purposes the use of take-away foods was split into ≤1 and >1 per week.

3.5.5 Anthropometric measures

In this study anthropometric measures included weight and height, as well as neck- (NC), WC and hip circumference (HC) (Addendum D). Weight and height were used to calculate BMI (WHO 2006: Online, WHO 1995:326–327, 2000:8,9). WC and height were used to calculate waist-to-height ratio (WHtR) (Ashwell et al 2012:284, Ashwell and Hsieh 2005:303). HC and height were used to calculate body adiposity index (BAI) (Bergman et al 2011:1083). All measurements were taken in a private room (WHO 2005:3-4).

3.5.5.1 Weight

Body weight is described as the total weight of the whole body including bones, muscles, adipose tissue, organs, and body fluids (Lysen and Israel, 2012:463).

A platform electronic Soehnle Professional scale, was used to measure weight. Before each session, a known weight was placed on the scale and used to calibrate the scale before weight was measured. Each participant removed outer clothing and shoes (Gibson 2005:247,253, NHANES 2007:3–3) and stood still in the middle of the scale's platform (Lee and Nieman 2013:170). Feet were close together, arms at the side (Gibson 2005:247, NHANES 2007:3–3)

and body weight evenly distributed (Lee and Nieman 2013:170). Weight was only recorded when the participant was correctly positioned and the digital reading was stable (NHANES 2004:3–3). If two weights taken in direct succession varied more than 100g (Lee and Nieman 2013:170) a third weight was taken and the average of the three was calculated.

3.5.5.2 Height

Height or stature is the standing height or maximum vertical length of an individual (Lee and Nieman 2013:168, NHANES 2007:3–7).

To measure the standing height, a Seca stadiometer with a vertical height of two meters was used. Participants were measured without shoes and stood with their heels together, arms to the side, legs straight, shoulders relaxed and head in the Frankfort horizontal plane (looking straight ahead) (Lee and Nieman 2013:168, NHANES 2007:3–7,3–8,3–9). The participant's heels, buttocks, scapulae, and the back of the head pressed against the vertical surface of the stadiometer. Ideally two or three of the four points should be against the vertical surface (Lee and Nieman 2013:168, NHANES 2007:3–7,3–9). Before the measurement was taken, the individual inhaled deeply, held their breath and maintained an upright position while the sliding-headpiece was lowered to the highest point of the head (NHANES 2004:3–7). Enough pressure was used to compress the hair (Gibson 2005:247,248, Lee and Nieman 2013:168, NHANES 2007:3–7,3–8). The deep breath allowed the spine to straighten, to obtain a more reliable and reproducible standing height measurement (NHANES 2004:3–8). The reading was taken to the nearest 0.1 cm, with the researcher's eyes level to the headboard (Lee and Nieman 2013:168).

3.5.5.3 Body Mass Index

BMI is used to determine if an individual is underweight, normal weight, overweight or obese as it relates to percentage body fat (Gibson 2005:318, Hammond and Litchford 2012:166). BMI however, can be influenced by muscle mass (Kay & Barratt 2009:239). BMI was calculated by dividing a participant's weight in kilogram by the square of the height in meters (kg/m^2) (Lee and Nieman 2013:181, WHO 1995:329,364, 2000:8–9, 2014:Online).

This value was used to categorise the participant as underweight, normal weight, overweight or obese (Table 3.2) (WHO 2006:Online, WHO 1995:329,364, 2000:8–9, 2004). For association purposes BMI was categorised as ≤24.9 and ≥25 kg/m^2.

Table 3.2 International classification of body mass index (BMI) (WHO 2006:Online, WHO 2000:8–9, 2004) and (WHO 1995:329,364)

Classification	BMI(kg/m^2)	
	Principal cut-off points	**Additional cut-off points**
Underweight	<18.50	<18.50
Severe thinness	<16.00	<16.00
Moderate thinness	16.00- 16.99	16.00- 16.99
Mild thinness	17.00- 18.49	17.00- 18.49
Normal range	18.50-24.99	18.50- 22.99
		23.00- 24.99
Overweight	≥25.00	≥25.00
Pre-obese	25.00- 29.99	25.00- 27.49
		27.50- 29.99
Obese	≥30.00	≥30.00
Obese, class I	30-34.99	30.00- 32.49
		32.50- 34.99
Obese, class II	35.00-39.99	35.00- 37.49
		37.50- 39.99
Obese, class III	≥40.00	≥40.00

3.5.5.4 Neck Circumference

According to Ben-Noun et al (2001:470) NC is a simple screening tool to identify overweight and obese individuals. The measurement is easy to use, non-invasive, not time consuming, cost-effective and correlates well with other standard anthropometric parameters (Adamu et al 2013:82, Aswathappa et al 2013:28,31, Ben-Noun et al 2001:470). NC as an indicator of upper-body subcutaneous adipose tissue distribution (Preis et al 2010:3701) has been assessed in relation to cardiovascular risk factors (Ben-Noun and Laor 2006:14,16,18, Preis et al 2010:3701,3709) in severely obese individuals (Sjöström et al 1995:9), insulin resistance (Aswathappa et al 2013:28), metabolic syndrome in men and obstructive sleep apnea syndrome (Hoffstein and Mateika 1992:377,380, Onat et al 2009:46). Akin et al (2014:963,965,969) proposed that a NC of equal or more than 35 cm in men with metabolic syndrome may predict erectile dysfunction.

A study on 3182 Chinese men showed that a NC of equal or more than 39 cm is an appropriate cut-off point to indicate metabolic syndrome (Yang et al 2010:2465,2467). A NC of equal or

more than 38 cm is regarded as the best cut-off point to identify overweight and a NC equal or more than 37 cm as the best cut-off point to identify central obesity (Yang et al 2010:2465,2467). Aswathappa et al (2013:28) showed that NC more than 36 cm in males with diabetes and more than 37 cm in males without diabetes was the best cut-off value to indicate central obesity in India. Ben-Noun et al (2001:470,477) recommends that males with a NC of equal or more than 37 cm need further evaluation of their overweight or obesity status.

NC was measured with a non-stretch Seca 201 tape measure in the midway of the neck between the mid cervical spine and mid anterior neck, to the nearest 1 mm (Aswathappa et al 2013:29, Ben-Noun and Laor 2006:15, Ben-Noun et al 2001:473). Participants stood upright, head erect with the shoulders in a relaxed position (not hunched), and looked straight ahead towards the researcher (Aswathappa et al 2013:29, Ben-Noun and Laor 2006:15, Ben-Noun et al 2001:473). The measuring tape was placed just below the laryngeal prominence / Adam's apple / thyroid cartilage (Aswathappa et al 2013:29, Ben-Noun et al 2001:473, Hingorjo et al 2012:37). The measuring tape was placed parallel with the floor, ensuring that the tape in front was at the same height as the tape at the back of the neck (Hingorjo et al 2012:37). The researcher made sure that the shoulder / neck muscles (trapezius) were not included in the measurement (Aswathappa et al 2013:29).

Neck circumference in this study was divided into <38 cm and ≥38 cm for categorising purposes.

3.5.5.5 Waist Circumference

WC is an appropriate and simple anthropometric measurement for assessment of abdominal fat (Larsson et al 1984:1401, WHO 2011:1,20) to determine disease risk (Larsson et al 1984:1401, Racette et al 2006:673,677, Ricciardi et al 2009:2,5–8, WHO 2011:1). A Seca 201 measuring tape was used.

WC was measured after a participant removed his outer clothing (Lee and Nieman 2013:186). The participant stood upright with feet close together, arms at the side, body weight evenly distributed with the abdominal muscles relaxed (Lee and Nieman 2013:186, WHO 2011:21). The measurement was taken around the midpoint between the top of the iliac crest and the lower margin of the last palpable rib in the mid axillary line (Lean et al 1995:159, WHO

2011:20) within 0.1 cm or 1 mm (Lee and Nieman 2013:186, Lean et al 1995:159). The measurement was taken at the end of a normal expiration, with a non-stretch tape, wrapped snugly around the waist, without constricting the waist, at a level parallel to the floor (Lee and Nieman 2013:186, NHANES 2007:3–16, WHO 2011:20). Each measurement was repeated twice. If the measurements differed with more than 0.1 cm (Lee and Nieman 2013:186), a third measurement was taken and the average of the three measurements used. A WC of more than 94 cm in males is considered as an indication for increased risk for metabolic complications, while a circumference of more than 102 cm is considered as an even higher risk and an independent risk factor for metabolic complications and chronic disease in Caucasians (Alberti et al 2009:1642, Hammond and Litchford 2012:169, WHO 2000:11). The values of <102 cm and ≥102 cm was used for categorising purposes.

3.5.5.6 Waist-to-Height Ratio

Waist-to-height ratio (WHtR) is a simple measure of the ratio of WC and standing height that reflects abdominal adiposity. WHtR has been suggested as superior to BMI in predicting central obesity and associated health risks (Ashwell and Hsieh 2005:303–305) in a systematic review and meta-analysis (Ashwell et al 2012:275,279). The recommended cut-off value of 0.5 is suitable for males across all ethnic groups (Ashwell et al 2012:284, Ashwell and Hsieh 2005:303–305).

3.5.5.7 Hip Circumference

Hip circumference is a measurement of the widest portion of the buttocks which is used to determine the waist-to-hip ratio (WHO 2011:5,20). Hip circumference was measured while the participant wore minimal clothing and stood erect, arms at the side, feet together with weight evenly distributed (WHO 2011:20,21, Gibson 2005:281). Hip circumference was measured around the widest portion of the buttocks, with non-stretch tape held in a horizontal plane (WHO 2011:5,20). The tape did not indent the soft tissue (WHO 2011:20, Gibson 2005:281). Each measurement was repeated twice and if measurements were within 0.1 cm of one another, the average was calculated.

3.5.5.8 Waist-to-Hip Ratio

Waist and hip circumference were used to calculate the waist-to-hip ratio (WHR). A ratio of more than one in males indicates an increased risk for lifestyle diseases (Lee and Nieman 2013:185, Hammond and Litchford, 2012:470).

3.5.5.9 Body Adiposity Index

Body adiposity index (BAI) is a calculation to estimate percentage body adiposity using hip circumference and standing height in a formula, BAI = (hip circumference) / ((height)$^{1.5}$) – 18). The BAI can be used to indicate percentage body fat for adult men of different ethnicities (Bergman et al 2011:1083) and fat percentage ranges for men are indicated in Table 3.3. BAI was categorised for association purposes as low and normal body fat, and overweight/ obesity.

Table 3.3 Fat percentage ranges for men (Ricciardi et al 2009:4–5)

Low body fat	10-15%
Average body fat	16-18%
Above average body fat	19-20%
Overweight	21-25%
Obese	≥26%

3.5.6 Semen parameters

In this study semen analysis were done to describe semen volume, sperm concentration, motility and morphology, as well as pH and fatty acid composition of semen as an indication of potential male fertility (Pacey 2012:740,741,744, WHO 2010:7–9). WHO reference values were used (WHO 2010:224) as standard for assessing semen quality and semen analysis were done by the Department of Obstetrics and Gynaecology and the Department of Microbial, Biochemical and Food Biotechnology, University of the Free State.

Semen samples were collected in a private room. The samples were transported to a near-by laboratory within 30 minutes of collection. It is recommended that samples be collected after a minimum of two days and a maximum of seven days of sexual abstinence (WHO 2010:10) and that the number of days of sexual abstinence between visits be as constant as possible

(WHO 2010:10) and was noted. With an increase in duration of abstinence sperm content or numbers or concentration and semen volume increase (De Jonge et al 2004:57, Karavolos et al 2013:5, Sherwood 2013:761,794). Sperm motility tends to fall with abstinence of more than 5 days, therefore semen for analysis should be collected within three days after the last ejaculation (Karavolos et al 2013:5). Abstinence normally does not influence pH, viability, morphology, total or grade A motility of sperm, or sperm DNA fragmentation (De Jonge et al 2004:57). At baseline of this study, two samples, produced three days apart, were analysed. This was repeated ninety days after nutrient supplementation or the use of placebo started.

3.5.6.1 Semen volume

Semen volume was determined by a graduated pipette. As indicated in Table 3.4, the lower reference limit for semen volume is 1.5 ml (5th centile, 95% confidence interval (CI) 1.4–1.7) (WHO 2010:16,224).

3.5.6.2 Sperm concentration

Sperm concentration is an indication of the number of sperm per volume (ml) of fluid diluting the sperm (WHO 2010:33). The WHO sets the lower reference limit (Table 3.4) for sperm concentration at 15×10^6 spermatozoa per ml (5th centile, 95% CI 12–16 × 106) (WHO 2010:44,224). The sperm concentration was multiplied by the volume of the whole ejaculate to calculate the total number of sperm (WHO 2010:33). However it is not the total number of sperm that is important, but it is the ability of the sperm to function effectively that is essential (Aitken and Baker 2006:69, Ng et al 2004:1811). The lower reference limit for total sperm number is 39×10^6 sperm per ejaculate (5th centile, 95% CI 33–46 × 106) as indicated in Table 3.4 (WHO 2010:33,224).

3.5.6.3 Sperm motility

Sperm motility was assessed as soon as possible after liquefaction of the sample, but within 30 – 60 minutes following ejaculation, as motility is affected by the time the sample is exposed to dehydration, pH or changes in temperature (WHO 2010:21).

The motility of each sperm was graded as follows:

- Progressive motility (PR): spermatozoa moving actively, either linearly or in a large circle, regardless of speed;
- Non-progressive motility (NP): all other patterns of motility with an absence of progression, e.g. swimming in small circles, the flagellar force hardly displacing the head, or when only a flagellar beat can be observed; and
- Immotility (IM): no movement (WHO 2010:22).

Total motility was expressed as a percentage of PR plus NP (WHO 2010:224). The lower reference limit is 40 (38–42)% (WHO 2010:224).

3.5.6.4 Sperm morphology

Sperm vary significantly among individuals (WHO 2010:69) which makes assessing morphology a challenge. Semen samples was prepared as a smear on a slide and assessed under a microscope as described by the WHO (2010:62–67). The WHO standard procedures help to define the morphology of a potentially fertilizing sperm cell, and the appearance of abnormal morphology (WHO 2010:69). The lower reference limit for normal forms is 4% as indicated in Table 3.4 (5th centile, 95% CI 3.0–4.0) (WHO 2010:100,224).

Table 3.4 Lower reference limits (5th centiles and their 95% confidence intervals) for semen characteristics (WHO 2010:224)

Parameter	Lower reference limit
Semen volume(ml)	1.5(1.4–1.7)
Total sperm number (10^6 per ejaculate)	39(33–46)
Sperm concentration (10^6 per ml)	15 (12–16)
Total motility (PR + NP, %)	40 (38–42)
Progressive motility (PR, %)	32 (31–34)
Vitality (live sperm, %)	58 (55–63)
Sperm morphology (normal forms, %)	4 (3.0–4.0)
Other consensus threshold values	
pH	≥7.2
Peroxidase-positive leukocytes (10^6per ml)	<1.0
MAR test (motile sperm with bound particles, %)	<50
Immunobead test (motile sperm with bound beads, %)	<50
Seminal zinc(µmol/ejaculate)	≥2.4
Seminal fructose (µmol/ejaculate)	≥13
Seminal neutral glucosidase (mU/ejaculate)	≥20

3.5.6.5 pH

The different accessory gland secretions, mainly alkaline seminal vesicular secretion and acidic prostatic secretion is responsible for the pH value of a sample. The pH was measured after liquefaction within 30 - 60 of ejaculation AS the pH value is influenced by the loss of CO2 in the sample (WHO 2010:16).

3.5.6.6 Fatty acid composition

The method of Folch et al (1957:497–500) was used to quantitatively extract total lipids from semen samples (± 500 μL). Chloroform and methanol was used in a ratio of 2:1 (Folch et al 1957:497–500). A concentration of 0.001% butylated hydroxytoluenen (an antioxidant) was added to the chloroform:methanol mixture (Folch et al 1957:497–500). The fat extracts under vacuum was dried in a rotary evaporator (Folch et al 1957:497–500). The extracts were then dried overnight in a vacuum oven at 50°C (Folch et al 1957:497–500). Phosphorus pentoxide was used as moisture adsorbent (Folch et al 1957:497–500). The extracted fat was stored in a polytop (glass vial, with push-in top) under a blanket of nitrogen and frozen at –20°C until further analysis was done (Folch et al 1957:497–500).

Approximately 10 mg of total lipid obtained from the method used by Folch et al (1957:497-500) was transferred into a Teflon-lined screw-top test tube by means of a disposable glass pasteur pipette. Fatty acids were transesterified to form methyl esters using 0.5 N NaOH in methanol and 14% boron trifluoride in methanol (Park and Goins 1994:1262–1263). Fatty acid methyl esters were quantified using a Varian GX 3400 flame ionization GC, with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm ID, 0.2 μm film thickness) (Park and Goins 1994:1262–1263). Column temperature was 40–230°C (hold 2 minutes; 4°C/minute; hold 10 minutes). Fatty acid methyl esters in hexane (1 μL) were injected into the column using a Varian 8200 CX Autosampler with a split ratio of 100:1 (Park and Goins 1994:1262–1263). The injection port and detector were both maintained at 250°C (Park and Goins 1994:1262–1263). Hydrogen, at 45 psi, functioned as the carrier gas, while nitrogen was employed as the makeup gas. Varian Star Chromatography Software recorded the chromatograms (Park and Goins 1994:1262–1263). Fatty acid methyl ester samples were identified by comparing the relative retention times of FAME peaks from samples with those of standards obtained from SIGMA (189-19). Fatty acids were reported as the relative percentage of each individual fatty acid as a percentage of the total of all fatty acids present in the sample. Fatty acid combinations and ratios were calculated by using the fatty acid data.

The Practice Committee of the American Society for Reproductive Medicine (2015:e19) emphasised that semen parameters within reference ranges do not equate with the minimum values needed for conception. The Committee also stated that men with semen parameters outside reference ranges may still be fertile and men with semen parameters within reference ranges may still be sterile.

3.5.7 Validity and Reliability

Validity is defined as the degree to which instruments accomplish the purpose for which they are being used, thus that the instrument accurately measures the concept being questioned (Leedy and Ormrod, 2010:28).

Reliability refers to a measuring instrument's capability to replicate the results each time the instrument is used and to produce consistent results that do not change unless there are discrepancies in the variable being measured (Leedy and Ormrod, 2010:29).

3.5.7.1 Environmental and Lifestyle Factors

Validity was ensured by measuring physical activity with the short, self-administered version of the International Physical Activity Questionnaire (IPAQ). The questions have been designed for use in various countries and have been validated and used in South Africa (Craig et al 2003:1388).

Reliability was ensured with the questionnaire being compiled in simple, clear, easily understood English, Afrikaans and Sesotho. The short self-administered version of IPAQ is also perceived as less repetitive than longer versions of the IPAQ (Craig et al 2003:1388).

3.5.7.2 Anthropometry

Validity of measurements was ensured by using the same scale, measuring tape and stadiometer to measure all participants. The scale was calibrated with a known weight before each session. The scale was moved to zero point before each weight measurement.

To ensure reliability of anthropometric measures, measurement procedures were pre-tested in the pilot study and the researcher followed standard procedures (Aswathappa et al

2013:29, Ben-Noun and Laor 2006:15, Ben-Noun et al 2001:470, Gibson 2005:247,253, Hingorjo et al 2012:37, Lee and Nieman 2013:186, NHANES 2004, WHO 2005:15).

3.5.7.3 Dietary Intake

Questions to determine vegetables and fruit, fibre, fatty food, meat and processed meat, as well as alcohol intakes were based on published recommendations.

Reliability was ensured with the questionnaire being compiled in simple, clear, easily understood English, Afrikaans and Sesotho. All queries were resolved by the researcher.

3.5.7.4 Semen Analysis

The validity of results was ensured by the Department of Obstetrics and Gynaecology, in keeping with the laboratory practices and techniques standardised according to the WHO criteria. The semen analysis was done by an accredited laboratory.

Reliability was ensured by using semen analysis, a standard diagnostic procedure routinely performed at the Department of Obstetrics and Gynaecology. Standard operating procedures were in place for maintenance and calibration of equipment. The tests were performed and interpreted by the same qualified and trained health care professionals.

3.5.7.5 Nutrient Supplement

Validity was ensured by only including nutrients that are directly related to the aims and objectives of the study and based on an in-depth literature review. Compliance was optimised by encouraging participants to take the supplements and placebos daily and they were asked to return the empty containers of the supplements and placebos.

Reliability in terms of compliance was ensured by explaining the importance and significance of the study and the need for compliance in the information document.

Reliability was further sought by reassuring participants that all results were kept strictly confidential. Making use of a placebo controlled study design increased reliability of study results.

3.6 Ethical Considerations

Ethical approval (ECUFS NR 08/2014) was obtained from the Ethics Committee from the Faculty of Health Sciences, University of the Free State before onset of the study. Written informed consent was obtained from all participants and participants were provided with an information document where procedures were explained in simple and understandable language of their choice. Participants were informed that the results of this study will be published.

Participants received R400-00 remuneration to cover their transport and refreshment expenses to the study venue and could withdraw at any time without discrimination or any of their rights being affected. All information and test results from participants were handled confidentially and participants were given a reference number that was used on data sheets to protect their identity. Participants received feed-back on their results if requested.

Results from this study will assist the Departments of Nutrition and Dietetics and Obstetrics and Gynaecology to improve treatment of males with poor semen parameters. Participants in the placebo group received the same nutrient supplementation as the supplementation group after completion of the study.

3.7 Statistical analysis

Data were captured in duplicate by the researcher on Microsoft Excel (2010) for Windows7 spread sheets and checked electronically by a biostatistician to verify accuracy. Data analysis for this study was done using SAS/STAT software, version 9.3 for Windows (Copyright 2010 SAS Institute Inc). Descriptive statistics include frequencies and percentages for categorical data. For continuous data means and standard deviations or medians and percentages were calculated. Chi-squared tests or Fisher exact tests and two tailed Pearson or Spearman's correlations, as well as analysis of variance were used to determine associations between variables, nutrient intakes and semen parameters. Multiple regression analysis was used to describe the relationship between variables. Statistical significance was set at a p-value of 0.05 or less. Statistical analysis was performed by the Department of Biostatistics, Faculty of Health Sciences, University of the Free State.

3.8 Limitations of this study

Obtaining sponsorship for the nutrient supplements took almost a year. Numerous e-mails were sent to finalise the protocol and contracts for this study.

The main limitation of this study was the sensitivity of obtaining semen samples from participants for the semen analysis that have been performed. This has severely limited the number of volunteers willing to participate. The researcher posted electronic advertisements in the Faculty of Health Sciences, sent advertisements to all staff within the faculty, and heads and primes of all male residences, did personal recruitment at the National Defence Force and in classes within the faculty. Of the 56 volunteers recruited only 50 participants provided all four semen samples. Follow-up phone calls, text messages and e-mail messages were used to try and locate the 6 volunteers.

It was planned to determine ROS during the study. ROS analysis was done during the pilot study, but during the base-line semen analysis the time period between obtaining semen samples and ROS analysis, was too long. This time frame impacted on the ROS results. It was decided at a research meeting not to continue with ROS analysis.

3.9 Conclusion

In this chapter, the selection of the study sample, procedures followed as well as methods and techniques used to conduct this study are described. Ethical considerations, limitations to the study and a description of the statistical methods used are included. The methodology described in this chapter was selected and followed according to the objectives stated in chapter 1 to reach the aim of this study.

Chapter 4 EFFECT OF AGE, ENVIRONMENTAL-, LIFESTYLE, ANTHROPOMETRIC- AND DIETARY FACTORS ON SEMEN PARAMETERS.

This chapter is presented in an article format, with the purpose to report on the findings from data collected according to the first and fifth objective set for this study, namely to describe age, environmental-, lifestyle-, anthropometric- and dietary factors in the study sample that may impact baseline semen parameters. This article also presents data on associations and correlations between age, environmental-, lifestyle-, anthropometric- and dietary factors and semen parameters, according to the last objective of this study. The article will be submitted to the South African Journal of Clinical Nutrition (SAJCN) and is prepared according to the authors instructions (Addendum F). For the purpose of this thesis, references will however be done according to the requirements of the Department of Nutrition and Dietetics, University of the Free State.

4.1 Abstract

Objective: The aim of the study was to describe the effect of age, environmental, lifestyle and dietary factors on semen parameters.

Design: A cross-sectional descriptive study was conducted.

Setting: Participants were recruited in the Bloemfontein area and data collected at the Faculty of Health Sciences, University of the Free State, Bloemfontein, South Africa.

Subjects: Fifty apparently healthy males between 18 and 45 years, responding to advertisements volunteered to participate.

Outcome measures: Age, environmental, lifestyle and dietary data were collected by means of a structured questionnaire and anthropometric measures and semen parameters determined, using standard techniques.

Results: In this young sample (median age of 24 years), 60% were classified as overweight/ obese according to BMI. Eighteen percent did not meet the lower reference limit for sperm concentration, 8% for total sperm motility, 2% for percentage sperm with normal morphology and 14% for semen volume. Reported vegetable and fruit intake was inadequate with only 10

(20%) reporting to consume fruit and 20 (40%) to consuming vegetables on a daily basis. Mean alcohol intake was 10.1 units per week and an intake of equal and more than 5 units per week was associated with lower sperm concentration. The use of electronic devices connected to Wi-Fi for four or more hours per day was associated with lower sperm motility. In this study we failed to find an association between sperm parameters and anthropometric indices, position where cellular phones are carried, the use of hot baths or tight underwear, stress, smoking, drug use or activity levels.

Conclusion:

A high incidence of overweight/ obesity and low vegetable and fruit consumption in this young sample, although not associated with sperm parameters, are areas of concern. The effect of alcohol and electronic devices on sperm parameters should be taken in to account when counselling couples about fertility.

4.2 Introduction

The health and lifestyle of parents influence the health of their children, and although maternal health is recognized as especially significant (Black et al 2008:243, 2013:427, Ferguson-Smith and Patti 2011:115, Levy et al 2005:182), the paternal diet may also play an important role in pregnancy health and embryotic development of the offspring (Binder et al 2012:e52304). Paternal health also influence lifelong health of children, even before intrauterine development takes place (Wu and Suzuki 2006:201). Animal and human studies suggest a link between paternal diet, metabolic health, body weight and semen parameters (Bakos et al 2010:402,408,409, Ferguson-Smith and Patti 2011:115,116). Sperm parameters and semen quality have therefore been labelled as a potential biomarker of overall male health to provide an indication of general health (Jensen et al 2009:559). Various factors including age, environmental-, lifestyle-, and psychological factors have been described to influence the general health and fertility of males (Begum et al 2009:18, Braga et al 2012:53,56,57,58, Campagne 2013:214,220, Homan et al 2007:209). Some of these factors, such as age, are irreversible, while others are modifiable and include smoking, alcohol consumption, caffeine intake, recreational drug use, psychological stress, excessive exercise,

body weight and dietary intake (Braga et al 2012:53,56,57,58, Campagne 2013:214,220, Homan et al 2007:209). A short overview of these factors are given in the following section.

Reproductive function progressively deteriorates as an individual ages (Stewart and Kim 2011:498,499) with age having a significant effect on all the basic sperm parameters (Cooke and Nelson 2011:167, Stewart and Kim 2011:498, Zitzmann 2013:617,625).

Lifestyle factors on the other hand are described as behaviours and circumstances that are modifiable which may impact general health and fertility potential (Campagne 2013:214, Homan et al 2007:209,219).

Temperature is one of the lifestyle factors that may have an effect on semen parameters and is modifiable to a large extend. Optimal spermatogenesis requires a temperature of approximately 34°C (Agarwal et al 2008b:550, Ivell 2007:Online). A work environment, where individuals are sedentary for long periods of time (Jung and Schuppe 2007:203,205, Thonneau et al 1998:2124) contributes to an increase in scrotal temperature (Ivell 2007:Online, Sharpe 2010:1697,1703). Other factors that may also cause an increase in scrotal temperature include hot baths (Dohle et al 2005:709), the use of saunas (Dohle et al 2005:709, Sharpe 2010:1703) and wearing thermal or tight-fitting underwear (Dohle et al 2005:709, Jung and Schuppe 2007:203,205, Povey et al 2012:2799). Any factor or situation that has an influence on the normal cooling of the testes or scrotum may therefore have a negative effect on spermatogenesis (Sharpe 2010:1704).

Modern technology may also have an effect on semen parameters. The use of wireless internet-connected laptops (Avendaño et al 2012:39,41,44), cellular phones (Argarwal et al 2009:1318,1324), especially smartphones (Agarwal et al 2011:449) should be investigated as modern technology is widely used on a daily basis.

A Western lifestyle, comprising of a more sedentary lifestyle with a high energy intake and a resultant increased incidence of obesity may also play a role in decreased semen parameters (Agarwal et al 2011:432,446, Sharma et al 2013:75, Sharpe 2010:1697). Regular exercise is recommended as part of a healthy lifestyle (Haskell et al 2007:1081) and contributes to general health and wellbeing (Botha et al 2013:S18, Homan et al 2007:213). In South Africa

levels of activity are generally not adequate to improve general health status (Botha et al 2013:S18).

Alcohol negatively impacts on semen parameters (Practice Committee of the American Society for Reproductive Medicine 2015:e19), especially on sperm concentration, motility (Braga et al 2012:53,56,58), and semen volume (Li et al 2011:116,119,120). Alcohol dependency may further impact negatively on spermatogenesis, sperm count and morphology (Gaur et al 2010:35, Muthusami and Chinnaswamy 2005:919,922). A moderate intake of more than five units of alcohol per week impacts negatively on semen quality; and as alcohol intake increases to more than 25 units per week the association becomes more prominent (Jensen et al 2014:Online). Povey et al (2012:2799) on the other hand reported no significant association between alcohol consumption and low-motile sperm concentration in young men if their wives/ partners were unable to conceive in the previous 12 months. It is however recommended that young men should not consume alcohol regularly (Jensen et al 2014:Online) and that individuals trying to conceive should abstain from alcohol (Anderson et al 2010:10).

Gaur et al (2010:38) label smoking as a health risk for both active and passive smokers, that affects sperm count, motility and/or morphology (Braga et al 2012:53,56,58, Campagne 2013:215,220, Gaur et al 2010:38,39, Li et al 2011:116,119,120). The effect that smoking has on the decline in semen parameters, including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) damage appears to be directly related to the number of cigarettes smoked (Gaur et al 2010:39,40, Selit et al 2013:35). Some researchers have failed to find an association between smoking (sometimes combined with alcohol intake) and semen parameters (De Jong et al 2014:112), low-motile sperm concentration (Povey et al 2012:2799) and male factor sub-fertility (Wong et al 2003:53).

Marijuana is considered to be the most used recreational drug (Battista et al 2008:82). Recreational drugs are more often used during the reproductive years (Fronczak et al 2012:525) and have shown a negative effect on spermatogenesis and sperm parameters (Fronczak et al 2012:525,526, Safarinejad et al 2013:18,21) with individuals sometimes using more than one drug (Anderson et al 2010:11, Fronczak et al 2012:525, Safarinejad et al 2013:23). Povey et al (2012:2799,2803) however found no significant association between

recreational drug use and low-motile sperm concentration. Underreporting of recreational drug use (Anderson et al 2010:11); and the fact that studies are only retrospective (Fronczak et al 2012:525), limit the evidence available on the true effect of drugs on reproductive health.

Stress can potentially inhibit spermatogenesis (Hall and Burt 2012:434,438) and influence semen quality, including sperm density and progressive motility (Li et al 2011:116,120,121). Research has however, not yet provided clear answers on the association between psychological stress and male fertility (Hall and Burt 2012:434,438, Li et al 2011:116,120,121).

According to Campagne (2013:221) there are no commonly appropriate 'safe' and 'unsafe' thresholds for lifestyle, environmental and psychological factors that may influence semen parameters and suggests that assessment and recommendations should be individualised.

A larger body of data on the effect of body weight and adiposity on semen quality are available. Being underweight has a significant negative impact on semen parameters (Jensen et al 2004:863, Luque et al 2015:Online, Qin et al 2007:827). On the other hand various factors may contribute to the negative effect of obesity on sperm production and infertility (Hammoud et al 2008a:900,902). It has been reported that the effect of overweight/ obesity on semen quality is insignificant (Chavarro et al 2010:2222,2225–2227,2230, Duits et al 2010:1356–1358, Teerds et al 2011:667) and some authors report no significant correlation between BMI and semen parameters (Aggerholm et al 2008:619, Eskandar et al 2012:1,2, MacDonald et al 2010:293), or low-motile sperm concentration (Povey et al 2012:2799).

Other authors disagree and reported that overweight and obese men tend to have a higher risk for poor semen parameters (Kay and Barratt 2009:237, Martini et al 2010:1739,1741,1742, Stewart et al 2009:1561), sexual dysfunction and subfertility (Du Plessis et al 2010:153,159, Hammoud et al 2008b:2222).

A healthy and varied diet may be crucial for good overall and reproductive health (Sharma et al 2013:66,67). Wong et al (2003:53) identified a low intake of vegetables and fruits as a risk factor for male factor sub-fertility. A positive link has been described between antioxidant rich vegetables and fruit intake (Braga et al 2012:53,55,56, Mendiola et al 2010:1128,1130,1131, Mendiola et al 2009:812,814, Mínguez-Alarcón et al 2012:2810–2811), a prudent dietary pattern (fish, chicken, vegetables, fruit, legumes and whole grains) (Gaskins

et al 2012:2899), a high cereal (carbohydrate) and fibre intake (Braga et al 2012:53,55,56, Mendiola et al 2010:1128,1130,1131), as well as a higher intake of omega-3 fatty acids (Attaman et al 2012, Olsen and Ramlau-Hansen 2012:511) and certain sperm parameters.

Total fat, saturated fat (Attaman et al 2012:1,5,6,8, Jensen et al 2013:411,415,417, Olsen and Ramlau-Hansen 2012:511), trans fatty intake (Chavarro et al 2014:429,431–434,436–438) and full fat dairy products, mainly cheese (Afeiche et al 2013:2265,2267–2270,2273) have been negatively related to certain sperm parameters. A lower intake of fats and protein may therefore have a positive effect on semen quality (Mendiola et al 2010:1128,1130,1131).

It is realistic to accept that the general health benefits obtained by healthy living, including moderate exercise and a balanced diet, may similarly apply to fertility (Homan et al 2007:214). As lifestyle practices are mostly modifiable and small lifestyle changes could have a positive effect on spermatogenesis (Sharpe 2010:1704), lifestyle changes should be recommended to couples trying to conceive (Homan et al 2007:214,220).

This study formed part of a larger study, with the aim to determine the impact of micro-nutrient supplementation on semen parameters. This report will however focus on age, environmental, lifestyle, anthropometric and dietary factors of the study sample at baseline that may influence semen parameters.

4.3 Methods

4.3.1 Study design, sample size and ethical considerations

Approval to perform this study was obtained from the Health Research Ethics Committee of the Faculty of Health Sciences, University of the Free State (UFS) (ECUFS nr 08/2014). For this particular sub-study a cross-sectional descriptive design was followed. The study sample consisted of 50 apparently healthy men between the ages of 18 and 45 years, who responded to advertisements on the campus of the UFS and the National Defence Force. Written informed consent was obtained and participants were informed that participation is voluntary and that they could withdraw from the study at any point.

4.3.2 Data collection

Data collection took place at the Faculty of Health Sciences, University of the Free State. Anthropometric measurements included body weight and height that were used to calculate body mass index (BMI). A platform electronic Soehnle Professional scale and Seca stadiometer were used for weight and height measurements. Standard techniques were used to determine weight to the nearest 100 g and height to the nearest 0.1 cm (Lee and Nieman 2013:168,170). Participants removed their shoes and wore minimal clothing before measurements were taken twice. If weight deviated with more than 100 g or height with more than 0.1 cm, a third measurement was taken and the average calculated. A BMI lower than 18.5 kg/m^2 defined underweight, between 18.5 kg/m^2 and 24.9 kg/m^2 normal weight, 25 kg/m^2 to 29.9 kg/m^2 overweight and 30 kg/m^2 or more obesity (WHO 2006:Online).

Participants completed a self-administered questionnaire to report on age, environmental-, lifestyle- and dietary factors that could influence semen parameters. Environmental factors included work environment, use of modern technology, use of saunas and hot baths, as well as wearing tight-fitting underwear/clothes. Lifestyle factors referred to physical activity, alcohol consumption, smoking, recreational drug use, supplement use and perception of stress. Physical activity was determined using the validated last seven day, self-administered short International Physical Activity Questionnaires (IPAQ) (Craig et al 2003:1381,1388) and activity level was categorised as low, moderate and high. Dietary factors referred to usual intake of specified foods which included vegetables and fruits, starchy foods, dairy products, protein-rich foods, high-fat foods, and high-salt foods as well as beverages. Frequency of intake was reported to categorise intake for data analysis and comparisons.

For semen analysis, two semen samples were collected and the average used to provide a representative reflection of sperm parameters. Participants were requested to abstain from sexual intercourse or masturbation for three days before the first sample; and the second sample was collected three days later. Samples were collected in a private room and transported to a near-by laboratory within 30 minutes of collection. Semen analysis was done by the Department of Obstetrics and Gynaecology, UFS and included semen volume, sperm concentration, quantitative and qualitative motility, sperm morphology and pH. The World Health Organisation (WHO) reference values were used to interpret these parameters (WHO

2010:224) and the cut-off values were used to categorize variables into groups when investigating associations.

4.3.3 Statistical analysis

Data were captured in duplicate by the researcher on Microsoft Excel (2010) for Windows7 spread sheets and checked electronically by a biostatistician to verify accuracy. Data analysis was performed using SAS/STAT software, version 12.4 of the SAS system for Windows (© 2010 SAS Institute Inc.). Statistical analysis was performed by the Department of Biostatistics, Faculty of Health Sciences, UFS. Descriptive statistics were used to describe the sample and included frequencies and percentages for categorical data. For continuous data means and standard deviations or medians and percentages were calculated. Chi-squared tests or Fisher exact tests were used to determine associations between variables and two tailed Pearson's or Spearman's correlations, as well as analysis of variance were used to describe correlations. Statistical significance was set at a p-value of 0.05 or less.

4.4 Results

The median age of the sample was 24 years (ranging from 18 to 43 years). Weight ranged between 58.7kg and 123.8 kg and according to BMI classification 40% (n=20) had a normal weight, 34% (n=17) were overweight and 26% (n=13) obese.

Table 4.1 summarises the results obtained from the semen analysis of participants.

Table 4.1 Median and mean semen parameters of participants

Variable			Minimum	Lower quartile	Median	Upper quartile	Maximum	Mean	±SD	WHO reference limit	Lower than reference limit		Higher than reference limit	
	n	%									n	%	n	%
Semen volume (ml)	50	100	0.7	1.8	3.0	3.8	7.0	2.8	1.3	1.5 (1.4-1.7)	7	14	43	86
Sperm concentration (10^6 per ml)	50	100	2.0	22.5	33.0	63.5	105.5	42.0	29.2	15 (12-16)	9	18	41	82
Sperm total motility (%)	50	100	25.0	53.0	62.8	70.0	82.5	60.1	13.8	40 (38-42)	4	8	46	92
Progressive motility	50	100	10.0	39.0	47.0	54.0	62.5	44.4	12.7					
Non-progressive motility	50	100	20.0	23.5	27.5	35.0	65.0	31.1	10.7					
Immotility	50	100	10.0	20.0	23.5	25.0	52.5	24.0	8.3					
Sperm morphology (normal forms %)	48	96	3.0	9.0	12.0	13.5	19.5	11.2	3.6	4 (3.0-4.0)	1	2.1	47	97.9
pH	50	100	7.3	8.0	8.0	8.5	9.0	8.2	0.3	≥7.2	0	0	50	100

*Evaluated according to the WHO criteria in WHO laboratory manual for the Examination and processing of human semen. 2010. 5th Edition, Switzerland, 2010:224.

Semen volume ranged between 0.7 to 7.0 ml with 14% producing an inadequate semen volume (Table 4.1). Median sperm concentration was 33 x 10^6 per ml, ranging between 2.0 to 105.5 x 10^6 per ml, median total sperm motility was 62.8% ranging between 25.0% and 82.5% and sperm morphology varied between 3.0 to 19.5% sperm with normal forms. Eighteen percent of the sample did not meet the lower WHO reference values for sperm concentration, 8% not for total sperm motility and 2% did not meet the minimum recommended percentage sperm with normal morphology. The semen pH for all the men met the recommended criteria of ≥7.2.

A description of environmental factors that could affect semen parameters are reported in Table 4.2.

Table 4.2 Environmental factors that could have an effect on semen parameters

Variable / Question	Category	n	%
Work environment – Number of sitting hours	0 - ≤8 hours	40	80
	>8 hours	9	18
Do you work with a laptop on your lap?	Yes	16	32
	No	34	68
How many hours a day do you use electronic devices connected to Wi-Fi (laptops, tablets, smartphones)?	None	8	16
	1-3 hours	14	28
	4-8 hours	15	30
	>8 hours	13	26
Where do you carry your cell phone?	Belt	1	2
	Hip pocket	37	74
	Shirt	8	16
	Other	4	8
Do you use a sauna?	Yes	3	6
	No	47	94
Do you take a hot bath?	Yes	30	60
	No	20	40
Do you wear tight fitting underwear or trousers (cycling shorts or tight fitting jeans)?	Yes	19	38
	No	31	62

Most men (68%) did not use laptops computers, but more than half spent more than four hours per day using electronic devices connected to Wi-Fi on a daily basis. More than three quarters (76%) carried cellular phones in their hip pockets or on their belt. Only three men (6%) used a sauna, but 60% (n=30) used hot baths. Tight-fitting underwear or trousers were worn by 38% (n=19) of participants.

Table 4.3 reports on lifestyle factors of this study sample and includes activity level, alcohol consumption, smoking, recreational drug use, use of nutritional supplements and perception of stress.

Table 4.3 Lifestyle factors of participants

Variable / Question	Category	n	%
Activity* (n=49)	Low	7	14
	Moderate	16	32
	High	27	54
Weekly alcohol consumption	Yes	39	78
	No	11	22
Do you currently smoke of have you smoked cigarettes in the last 3 months? (n=50)	Yes	19	38
	No	31	62
How many cigarettes do you smoke per day (n=19)?	0.5-9	7	14
	10-20	11	22
	30	1	2
How many packs do you smoke per week? (n=19)	<1	4	8
	1-5	11	22
	6-10	3	6
	>10	1	2
How many years have you been smoking? (n=19)	≤1	4	8
	2-5	8	16
	6-10	2	4
	11-20	4	8
	>20	1	2
Are you regularly exposed to secondary/passive smoke at home or work? (n=50)	Yes	29	58
	No	21	42
Have you used any recreational drugs in the past three months? (n=50)	Yes	10	20
	No	40	80
Do you sometimes use nutritional supplements? (n=49)	Yes	17	34
	No	32	64
Stress perception (n=50)	High	30	60
	Low	20	40

*Evaluated according to the IPAQ (Craig et al 2003:1381,1388,1391–1395)

Most men (92%) reported that their activity level represented usual level of activity and reported a high level of activity (54%, n=27), with 14% (n=7) indicating low activity levels.

More than a third (38%; n=19) of the men smoked and more than half (58%) indicated exposure to secondary or passive smoking (n=29) at home or at work. Twenty percent (n=10)

of participants indicated that they had used recreational drugs during the last three months. Self-reported perception of stress was high with 60% of participants indicating that they experience high levels of stress on a daily basis.

Table 4.4 summarises dietary aspects that could have an influence on sperm parameters. These factors include intake of fruit and vegetables, fibre-rich starchy foods, white or brown bread / rolls milk and -products, protein-rich foods, high-fat foods and high-salt foods.

Table 4.4 Frequency of dietary intake of specific foods

	Type of food	None or less than 1 a week		Once per week		2-3 times per week		4-6 times per week		1 x per day		2 x per day		3 x day		>3 x per day	
		N	%	N	%	n	%	n	%	N	%	n	%	n	%	n	%
	Fruit	9	18	9	18	16	32	6		5		4		1			
	Vegetables			3	6	13	26	14		14		4		2			
	Milk / milk products			1		9	18	8		3		8		12		9	
Protein-rich foods	Beef	5	10	18		20	40	5		2							
	Chicken	2	4	13		25	50	8		1							
	Pork	16	36	21		12	24									1	
	Liver	37	74	8		3	6	1				1					
	Seafood (including fish)	25	50	19		4	8			1		1					
High-fat foods	Bacon	21	42	18		9	18	1						1			
	Russians/salami/Vienna's	23	46	16		8	16	3									
	Biltong with fat	31	62	10		6		1		2							
	Cheese or cheese spread, regular	7	14	6		21		13		2		1					
	Nuts (all nuts, including peanuts)	18	36	18		9		3		1				1			
High-salt foods	Vegetable/meat spread	31	62	6		8		3		1							
	Popcorn/chips	23	46	14		9		1				1		2			
	Gravy	21	42	17		10		1		1							
	Chutney	10	20	10		15		9		4		2					
	Soup	26	52														

Intake of vegetables and fruits was inadequate, with only 10 (20%) reporting intake of fruit and 20 (40%) intake of vegetables on a daily basis. Participants were also asked to list vegetables and fruit consumed most often. Bananas, apples, pumpkin, green beans, carrots, potatoes, oranges, spinach, broccoli and tomatoes were listed as the most frequently consumed vegetables and fruit. Almost two thirds (n=32) of participants used milk and milk products on a daily basis, mostly full cream products 76% (n=38).

Protein-rich foods, high-fat foods (including processed meats) and high-salt foods were used on weekly and sometimes daily basis. Beef and chicken were commonly consumed with only 10% and 4% of participants respectively consuming these meats never or less than once per week. The cooking method used most at home when preparing food was frying or stir-frying in fat, oil or butter (48%, n=24) compared to grilling (2%, n=11) or steaming / boiling (30%, n=15). Forty percent of participants added salt to their food before tasting it.

Table 4.5 provides an outline of the frequency of specific beverages that participants consumed. The beverages listed included water, fruit and sweetened juice, softs drink (sugar sweetened and artificially sweetened), sports and caffeine containing energy drinks, tea or coffee with or without sugar, as well as alcoholic drinks.

Table 4.5 Frequency of beverage intake

	None or less than once a week		Once per week		2-3 times per week		4-6 times per week		Once per day		2 x per day		3 x per day		>3 times per day	
	n	%	n	%	N	%	n	n	N	%	n	%	n	%	n	%
Water			1	2	3	6	5	10	7	14	10	20	6	12	18	36
100% fruit juice	16	32	16	32	10	20	4	8	4	8						
Sweetened juice	18	36	11	22	14	28	5	10	1	2					1	2
Soft drink, sugar sweetened	13	26	13	26	14	28	5	10	2	4	2	4			1	2
Soft drink, artificially sweetened	32	64	6	12	5		4	8	2	4	1	2				
Sports drinks	27	54	10	20	6	12	3	6	1	2	2	4				
Energy drinks (caffeine containing)	34	68	6	12	6	12	4	8								
Tea/coffee with sugar	8	16	4	8	7	14	5	10	7	14	8	16	5	10	6	12
Tea/coffee without sugar	36	72	4	8	4	8	1	2	3	6			1	2	1	2
Hard liquor (25 ml)	28	56	12	24	5	10	4	8	1	2						
Beer/ales/wine coolers (340 ml)	21	42	14	28	11	22	4	8								
Wine (120 ml)	32	64	11	22	4	8	2	4	1	2						

According to Table 4.5 sugar sweetened beverages were consumed frequently by most of the participants. Sports drinks were used less frequently (n=27, 54%) and the majority (68%) of participants did not use caffeine containing energy drinks. Coffee and tea with sugar were consumed by most participants. Most participants did not consume hard liquor (56%), beer/ales/wine coolers (42%) or wine (64%) at all or less than once a week. Only one participant reported using hard liquor and/or wine on a daily basis. The mean number of estimated alcohol units consumed was 10.1 per week.

The number of days that participants did not consume any alcohol ranged between three and seven days per week, with 42 (84%) consuming alcohol on two or less days per week. Eleven participants (22%) did not usually consume any alcohol.

Table 4.6 reports on associations between age, anthropometric measures, environmental-, lifestyle- and dietary factors and sperm concentration, -motility and -morphology.

Table 4.6 Associations between age, anthropometric measures, environmental-, lifestyle- and dietary factors and sperm parameters

	Sperm concentration			Sperm motility			Sperm morphology		
Factor	**<15X10^6/ml (n)**	**≥15X10^6/ml (n)**	***p*-value**	**<40% (n)**	**≥ 40% (n)**	***p*-value**	**<4% (n)**	**≥4% (n)**	***p*-value**
Anthropometric factors									
Body Mass Index (kg/m^2)									
≤24.9 (n)	3	17	0.72	3	17	0.29	1	18	0.40
≥25 (n)	6	24		1	29		0	29	
Body Adiposity Index (%)									
Low and normal body fat (n)	7	32	1.00	3	21	0.56	1	21	0.48
Overweight/ obese (n)	2	9		1	25		0	25	
Neck circumference (cm)									
<38 (n)	3	15	1.00	3	15	0.13	1	16	0.35
≥38 (n)	6	26		1	31		0	31	
Waist circumference (cm)									
<102 (n)	7	33	1.00	4	36	0.57	1	37	1.00
≥102 (n)	2	8		0	10		0	10	
Waist-to-height ratio									
<0.5 (n)	4	26	1.00	4	25	0.13	1	26	1.00
≥0.5 (n)	3	17		0	21		0	21	
Environmental factors									
Laptop on lap									
Yes (n)	2	14	0.19	1	15	1.00	0	16	1.00
No (n)	7	27		3	31		1	31	
Electronic devices connected to Wi-Fi									
0-3 hours (n)	6	31	0.68	1	36	0.05*	0	35	0.27
≥ 4 hours (n)	3	10		3	10		1	12	
Cellular phone									
Pocket or Hip (n)	6	32	0.67	3	35	1.00	1	35	1.00
Other (n)	3	9		1	11		0	12	
Tight underwear /trousers									
Yes (n)	2	14	0.69	2	17	1.00	1	18	0.39
No (n)	7	27		2	29		0	29	

	Sperm concentration			Sperm motility			Sperm morphology		
	<15X10^6/ml (n)	**≥15X10^6/ml (n)**	***p*-value**	**<40% (n)**	**≥ 40% (n)**	***p*-value**	**<4% (n)**	**≥4% (n)**	***p*-value**
Hot bath									
Yes (n)	4	26	0.45	3	27	0.64	1	28	1.00
No (n)	5	15		1	19		0	19	
Work stress									
Low (n)	3	17	0.72	1	19	0.64	0	20	1.00
High (n)	6	24		3	27		1	27	
Lifestyle factors									
Activity									
Low/ moderate (n)	4	19	1.00	2	21	1.00	1	21	0.46
High(n)	5	22		2	25		0	26	
Alcohol									
<5 units / week (n)	1	22	0.03*	0	23	0.12	0	22	1.00
≥5 units / week (n)	8	19		4	23		1	25	
Smoking									
Yes (n)	4	15	0.72	2	17	0.63	1	17	0.36
No (n)	5	26		2	29		0	30	
Recreational drugs									
Yes (n)	2	8	1.00	1	9	1.00	1	9	0.21
No (n)	7	33		3	37		0	38	
Dietary factors									
Fruit and vegetables									
<2 per day (n)	9	39	1.00	4	44	1.00	1	45	2
≥2 per day (n)	0	2		0	2		0	2	
Milk consumption									
<500ml/day (n)	7	28	0.71	3	32	1.00	1	34	1.00
≥500ml / day (n)	2	13		1	14		0	13	
Fish consumption									
<2 / week (n)	8	36	1.00	4	40	1.00	1	41	1.00
≥2 / week (n)	1	5		0	6		0	6	
Take aways									
≤1 per week	5	27	0.70	1	31	0.13	0	30	0.38
>1 per week	14	140		3	15		1	17	

When grouped together and compared with the WHO lower reference limits for sperm concentration, - motility and –morphology, no statistically significant association were found for any of the anthropometric-, environmental-, lifestyle- or dietary factors, except for an alcohol intake of more than five units per week that were associated with lower sperm concentration and the use of electronic devices for four or more hours a day that were associated with lower sperm motility.

4.5 Discussion

A relationship between aging and sperm concentration and motility has been described in overweight men (Eskandar et al 2012:1–4) and between aging and total sperm motility, progressive motility, normal sperm morphology and sperm concentration (Tsao et al 2015:Online). In this younger study sample however, no correlation was found between age and semen parameters, probably because age related changes are only expected as the individual ages (Dunson et al 2004:51; Stewart and Kim 2011:498,499).

Although approximately 60% of participants presented with an overweight/ obese BMI classification, expected higher lean muscle mass in this younger and physically active population (reported elsewhere) could also have contributed to their higher BMI (Kay and Barratt 2009:239). This percentage of overweight/ obesity is similar to that reported in Turkey where 55.2% of healthy male volunteers in a study on neck circumference and obesity (Saka et al 2014:572) and 68.4% of men that were partner attending an infertility clinic in the United States of America (USA) (Biekniek et al 2015:e67). Sperm concentration has been linked to overweight according to BMI classification (Aggerholm et al 2008:619,623, Jensen et al 2004:863,866). A number of researchers reported a negative association between increase in BMI and semen volume, sperm concentration/ count and sperm motility/ low progressively motile sperm count/ rapid motility and percentage cells with normal morphology was observed in obese men (Biekniek et al 2015:e67, Hammoud et al 2008b:2222,2224, Hofny et al 2010:581,582, Kort et al 2006:450,451, Martini et al 2010:1739,1741,1742, Paasch et al 2010:2898,2899, Sermondade et al 2012:2,9, Shayeb et al 2011:717,720,722, Tsao et al 2015:Online).

Other researchers however, failed to find a link between overweight/ obesity and basic sperm parameters (Chavarro et al 2010:2222,2225,2227, Duits et al 2010:1356,1357,1359, Egwurugwu et al 2011:29,30, Eskandar et al 2012:1–4, MacDonald et al 2010:293,306,307, Povey et al 2012:2799). When categorizing anthropometric measures and semen parameters according to acknowledged cut-off values in the current study, none of the anthropometric measures showed an association with semen concentration, –motility or morphology.

The mean semen volume of 2.8 ml (±1.3) in this study was similar to the mean of 2.96 ml (±1.54) reported for a comparable age group (20-40 years) of sperm donors (Van Waeleghem et al 1996:327). In this study the median sperm concentration (33 x 10^6 per ml) was lower than the 41 x 10^6 per ml median reported in Denmark (Andersen et al 2000:366), 66.8 x 10^6 per ml mean reported for a comparable age group of sperm donors (Van Waeleghem et al 1996:327) and 56 x 10^6 per ml and 42 x 10^6 per ml median reported for fertile and infertile men in the USA (Guzick et al 2001:1389). The median of 62.8% for motile sperm was higher than 55% reported in the USA for both fertile and infertile males (Guzick et al 2001:1389). The median of 13.5% sperm with normal morphology was observed in this study, was slightly lower than the 14% reported for the fertile group in the USA (Guzick et al 2001:1389). The pH of all semen samples were within the WHO reference limits.

When the semen quality of groups of men were compared, sedentary work and obesity was reported to negatively affect semen quality (Magnusdottir et al 2005:208,214). The number of sitting hours per day is positively linked to daytime scrotal temperatures which affects semen quality (Jung and Schuppe 2007:203,205,212). Although scrotal temperature was not measured, 18% of men in this study indicated that they spend more than eight hours per day sitting, which did correlate with the lower cut-off values for sperm -morphology.

Working with a laptop computer near the testes, may contribute to an increase in scrotal temperature which may negatively influence sperm quality (Jung and Schuppe 2007:203,205,212). Most men (68%) in this study did not use laptops computers, but more than half of participants spent more than four hours per day using electronic devices connected to Wi-Fi on a daily basis. A study by Avendaño et al (2012:39,41,44) showed that when sperm are exposed *in vitro* to a wireless internet-connected laptop for four hours, motility are negatively influenced by a non-thermal effect. The low levels of radiation

produced by the computer may therefore also influence semen parameters. This effect on motility was also observed in the current study, where a significant association between using electronic devices connected to Wi-Fi for four hours or more per day and a lower sperm motility was found.

More than three quarters (76%) of participants carried cellular phones in their hip pockets or on their belt. The position where a cellular phone is worn on the body may reduce sperm motility, count, viability and morphology (Adams et al 2014:106,111, Agarwal et al 2009:1318, 2011:442, Agarwal et al 2008b:552, Jurewicz et al 2009:305, Kilgallon and Simmons 2005:254). In this study, no statistically significant association between where the cellular phone is carried and normal or abnormal sperm parameters were shown. It is recommended that the use of wireless internet-connected phones and devices should be investigated (Agarwal et al 2009:1318,1324; Agarwal et al 2011:449), as the daily use of this technology is on the increase.

Although very few participants in this study used a sauna, other researchers reported that spermatogenesis in males with normal semen parameters were significantly but reversibly impaired after exposure to two sauna sessions of 15 minutes per week for three months (Garolla et al 2013:877,884). Exposure to saunas should only have a small effect, as the scrotum is not immersed in water and therefore intrascrotal temperature can still be regulated (Sharpe 2010:1703). More than half of participants in this study however took hot baths, which may contribute to an increase in scrotal temperature (Dohle et al 2005:709) No significant association was however found between the use of hot baths and below reference limits for sperm parameters.

Almost 40% of participants wore tight fitting underwear or trouser, which may also contribute to an elevation in scrotal temperature and consequently poor semen quality (Dohle et al 2005:709). However the current study could not find an association between wearing tight fitting clothing and poor sperm parameters.

A high level of activity was reported by more than 50% of participants, which compares to 50.2% of males (19 years and older) in the South African Youth Risk Behaviour Survey of 2008, that indicates that they participated in sufficient vigorous activity (Reddy et al 2010:117). The

high activity levels reported however did not show an association with poor sperm parameters.

It is recommended that young men should not drink alcohol regularly (Jensen et al 2014:Online) and that individuals trying to conceive should abstain from alcohol (Anderson et al 2010:10) Sperm concentration, -motility (Braga et al 2012:53,56,58) and semen volume (Li et al 2011:116,119,120) have been shown to be negatively influenced by alcohol intake. In this study, alcohol was consumed by more than a third of participants and a statistically significant association was found between participants that consumed five or more units of alcohol per week and below reference values for sperm concentration. Participants consumed a mean of 10.1 units of alcohol per week, exceeding the five units per week, which has been reported to negatively influence semen quality (Jensen et al 2014:Online).

According to Li et al (2011:116) smoking is a risk factor for lower semen quality. Gaur et al (2010:38) label smoking as a lifestyle risk for both active and passive smokers that affects sperm count, motility and/or morphology (Braga et al 2012:53,56,58, Campagne 2013:215,220, Gaur et al 2010:38,39, Li et al 2011:116,119,120). The effect that smoking has on sperm quality, appears to be directly related to the number of cigarettes smoked (Gaur et al 2010:39,40, Selit et al 2013:35). Some researchers however failed to find an association between smoking (sometimes combined with alcohol intake) and semen parameters (De Jong et al 2014:112), low-motile sperm concentration (Povey et al 2012:2799) and male factor sub-fertility (Wong et al 2003:53). In this study, the majority of participants who smoked, smoked between ten to twenty cigarettes per day, but smoking was not associated with low sperm concentration, -motility or -morphology.

The relative high reported use of recreational drugs in this sample, who are in their reproductive years, has the potential to affect semen parameters (Fronczak et al 2012:525,526, Safarinejad et al 2013:18,21) Povey et al (2012:2799,2803) however report no significant association between recreational drug use and low-motile sperm concentration. Similarly this study also failed to find an association between recreational drug use and sperm parameters.

Stress alone can potentially reduce spermatogenesis (Hall and Burt 2012:434,438) and sperm quality, including sperm progressive motility and an increase in the number of abnormal sperm (Li et al 2011:116,120,121). Investigations have not yet provided clear answers related to the association between psychological stress and male fertility (Hall and Burt 2012:434,438, Li et al 2011:116,120,121). Although high levels of stress were reported, this study found no association between high levels of stress and sperm parameters

Almost a third of the participants indicated that they consumed fruit only two to three times per week. Although participants indicated that more of them eat vegetables on a daily basis when compared to fruit, they may not meet the recommended fruit and vegetable intake and may be at risk for sub-fertility (Wong et al 2003:49,51). In this study no significant association was found.

Milk and milk products are good sources of calcium, but the possible link between full-fat dairy, specifically cheese and percent progressively motile sperm should be considered if a pregnancy is planned (Afeiche et al 2013:2265). As full-cream dairy, protein rich foods of animal origin and processed foods are sources of saturated fats, the high intake of these fats may impact on sperm concentration (Attaman et al 2012:1,7,8). In the current study, no association was found between a full cream milk intake and sperm parameters.

Gaskins et al (2012:2899) stated that a prudent dietary pattern, rich in fruits, vegetables, chicken, fish and whole grains may be an economical and safe way to improve the percentage progressively motile sperm.

4.6 Conclusion

In this study, the use of electronic devices connected to Wi-Fi and alcohol intake were the only two factors that were associated with sperm parameters. A high incidence of overweight/ obesity and low fruit and vegetable consumption stood out as areas of concern, although not associated with sperm parameters.

The main limitation of this study was the sensitive nature of the tests that have been performed, which severely limited the number of volunteers willing to participate and kept their appointments. The researcher used various methods to recruit volunteers, including

electronic advertisements and personal recruitment and reminded participants of their appointments by means of text messages. As dietary intake was self-reported, it is difficult to define associations between intake and other parameters. For future studies, it is recommended that a larger sample be included if more resources are available and that other geographic areas in South Africa be included.

This study provided valuable information about the effect of alcohol and use of electronic devices on sperm parameters in a South African setting. Results from this study can be used when advising males about reproductive health, in order to optimise sperm parameters, which could influence the health of future generations.

4.7 Acknowledgements

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4.8 References

Adams J a., Galloway TS, Mondal D, Esteves SC and Mathews F (2014) Effect of mobile telephones on sperm quality: A systematic review and meta-analysis. *Environment International*. Elsevier B.V. 70: 106–112. Available at: http://dx.doi.org/10.1016/j.envint.2014.04.015.

Afeiche M, Williams PL, Mendiola J, Gaskins AJ, Jørgensen N, Swan SH and Chavarro JE (2013) Dairy food intake in relation to semen quality and reproductive hormone levels among physically active young men. *Human reproduction (Oxford, England)* 28(8): 2265–75. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3712661&tool=pmcentrez&rendertype=abstract.

Agarwal A, Desai NR, Makker K, Varghese A, Mouradi R, Sabanegh E and Sharma R (2009) Effects of radiofrequency electromagnetic waves (RF-EMW) from cellular phones on human ejaculated semen: an in vitro pilot study. *Fertility and Sterility*. Elsevier Ltd 92(4): 1318–1325. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0015028208033566.

Agarwal A, Desai NR, Ruffoli R and Carpi A (2008b) Lifestyle and testicular dysfunction: a brief

update. *Biomedicine & pharmacotherapy = Biomédecine & pharmacothérapie* 62(8): 550–3. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18771892.

Agarwal A, Singh A, Hamada A and Kesari K (2011) Cell phones and male infertility: A review of recent innovations in technology and consequences. *International Braz J Urol* 37(4): 432–454.

Aggerholm AS, Thulstrup AM, Toft G, Ramlau-Hansen CH and Bonde JP (2008) Is overweight a risk factor for reduced semen quality and altered serum sex hormone profile? *Fertility and sterility* 90(3): 619–26. Available at: http://www.sciencedirect.com/science/article/pii/S0015028207015282.

Andersen AG, Jensen TK, Carlsen E, Jørgensen N, Andersson AM, Krarup T, Keiding N and Skakkebaek NE (2000) High frequency of sub-optimal semen quality in an unselected population of young men. *Human reproduction (Oxford, England)* 15(2): 366–72. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10655308.

Anderson K, Nisenblat V and Norman R (2010) Lifestyle factors in people seeking infertility treatment - A review. *The Australian & New Zealand journal of obstetrics & gynaecology* 50(1): 8–20.

Attaman JA, Toth TL, Furtado J, Campos H, Hauser R and Chavarro JE (2012) Dietary fat and semen quality among men attending a fertility clinic. *Human reproduction (Oxford, England)* 27(5): 1466–74. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3329193&tool=pmcentrez&rendertype=abstract.

Avendaño C, Mata A, Sanchez Sarmiento CA and Doncel GF (2012) Use of laptop computers connected to internet through Wi-Fi decreases human sperm motility and increases sperm DNA fragmentation. *Fertility and sterility* 97(1): 39–45.e2. Available at: http://www.sciencedirect.com/science/article/pii/S0015028211026781.

Bakos HW, Mitchell M, Setchell BP and Lane M (2010) The effect of paternal diet-induced obesity on sperm function and fertilization in a mouse model. *International journal of andrology* 34(5 Pt 1): 402–10. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20649934.

Battista N, Pasquariello N, Di Tommaso M and Maccarrone M (2008) Interplay between endocannabinoids, steroids and cytokines in the control of human reproduction. *Journal of neuroendocrinology* 20 Suppl 1(12): 82–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18426505.

Begum H, Moniruddin ABM and Nahar K (2009) REVIEW ARTICLES ENVIRONMENTAL AND NUTRITIONAL ASPECT IN. 16–19.

Biekniek J, Kashanian J, Deibert C, Grober E, Brannigan R, Sandlow J and Jarvi K (2015) DDeleterious effects of male obesity on reproductive parameters among a loarge multi-institutional cohort. *Fertility and Sterility*. Elsevier Ltd 104(3): e67. Available at: http://dx.doi.org/10.1016/j.fertnstert.2015.07.203.

Binder NK, Hannan NJ and Gardner DK (2012) Paternal Diet-Induced Obesity Retards Early Mouse Embryo Development, Mitochondrial Activity and Pregnancy Health. *PLoS ONE* 7(12): e52304. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3531483&tool=pmcentrez&rendertype=abstract.

Black RE, Allen LH, Bhutta Z a., Caulfield LE, de Onis M, Ezzati M, Mathers C and Rivera J (2008) Maternal and child undernutrition: global and regional exposures and health consequences. *The Lancet* 371(9608): 243–260.

Black RE, Victora CG, Walker SP, Bhutta Z a., Christian P, De Onis M, Ezzati M, Grantham-Mcgregor S, Katz J, Martorell R and Uauy R (2013) Maternal and child undernutrition and overweight in low-income and middle-income countries. *The Lancet* 382(9890): 427–451.

Botha C, Wright H, Moss S and Kolbe-Alexander T (2013) 'Be active!'Revisiting the South African food-based dietary guideline for activity. *Food-Based Dietary Guidelines for South Africa* 26(3): 18–27.

Braga DP de AF, Halpern G, Figueira R de CS, Setti AS, Iaconelli A and Borges E (2012) Food intake and social habits in male patients and its relationship to intracytoplasmic sperm injection outcomes. *Fertility and sterility* 97(1): 53–9. Available at: http://www.sciencedirect.com/science/article/pii/S001502821102677X.

Campagne DM (2013) Can Male Fertility Be Improved Prior to Assisted Reproduction through The Control of Uncommonly Considered Factors? *International journal of fertility & sterility* 6(4): 214–223. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3850314&tool=pmcentrez&rendertype=abstract.

Chavarro JE, Mínguez-Alarcón L, Mendiola J, Cutillas-Tolín A, López-Espín JJ and Torres-Cantero AM

(2014) Trans fatty acid intake is inversely related to total sperm count in young healthy men. *Human reproduction (Oxford, England)* 29(3): 429–40. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24419496.

Chavarro JE, Toth TL, Wright DL, Meeker JD and Hauser R (2010) Body mass index in relation to semen quality, sperm DNA integrity, and serum reproductive hormone levels among men attending an infertility clinic. *Fertility and sterility*. Elsevier Ltd 93(7): 2222–31. Available at: http://www.sciencedirect.com/science/article/pii/S0015028209001502.

Cooke L and Nelson SM (2011) Reproductive ageing and fertility in an ageing population. *The Obstetrician & Gynaecologist* 13(3): 161–168. Available at: http://doi.wiley.com/10.1576/toag.13.3.161.27668.

Craig C, Marshall A, Sjostrom M, Bauman A, Booth M, Ainsworth B, Pratt M, Ekelund U, Yngve A, Sallis J and Oja P (2003) International Physical Activity Questionnaire: 12 Country Reliability and Validity. *Medicine & Science in Sports & Exercise* 35(8): 3508–1381. Available at: http://addssa.com/intranets/idrd/2011_10_activos_y_saludables/03_recursos/study_material/gestores/Articulos Ingles/Vigilancia y Evaluacion/1Q. International PAQ.pdf.

Dohle GR, Colpi GM, Hargreave TB, Papp GK, Jungwirth a. and Weidner W (2005) EAU guidelines on male infertility. *European Urology* 48: 703–711.

Duits FH, Van Wely M, Van Der Veen F and Gianotten J (2010) Healthy overweight male partners of subfertile couples should not worry about their semen quality. *Fertility and Sterility*. Elsevier Ltd 94(4): 1356–1359. Available at: http://dx.doi.org/10.1016/j.fertnstert.2009.05.075.

Dunson DB, Baird DD and Colombo B (2004) Increased infertility with age in men and women. *Obstetrics and gynecology* 103(1): 51–56.

Egwurugwu JN, Nwafor a, Chike CPR, Ufearo CS, Uchefuna RC, Iwuji SC, Okwara JE and Alagwu E a (2011) The relationship between body mass index, semen and sex hormones in adult male. *Nigerian journal of physiological sciences : official publication of the Physiological Society of Nigeria* 26(1): 29–34. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22314983.

Eskandar M, Al-Asmari M, Babu Chaduvula S, Al-Shahrani M, Al-Sunaidi M, Almushait M, Donia O and Al-Fifi S (2012) Impact of Male Obesity on Semen Quality and Serum Sex Hormones. *Advances in Urology* 2012: 1–4.

Ferguson-Smith AC and Patti ME (2011) You are what your dad ate. *Cell Metabolism*. Elsevier Inc. 13(2): 115–117. Available at: http://dx.doi.org/10.1016/j.cmet.2011.01.011.

Fronczak CM, Kim ED and Barqawi AB (2012) The insults of illicit drug use on male fertility. *Journal of andrology* 33(4): 515–28. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21799144.

Garolla A, Torino M, Sartini B, Cosci I, Patassini C, Carraro U and Foresta C (2013) Seminal and molecular evidence that sauna exposure affects human spermatogenesis. *Human Reproduction* 28(4): 877–85. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23411620.

Gaskins AJ, Colaci DS, Mendiola J, Swan SH and Chavarro JE (2012a) Dietary patterns and semen quality in young men. *Human reproduction* 27(10): 2899–907. Available at: http://humrep.oxfordjournals.org/content/27/10/2899.short.

Gaskins AJ, Colaci DS, Mendiola J, Swan SH and Chavarro JE (2012b) Dietary patterns and semen quality in young men. *Human reproduction (Oxford, England)* 27(10): 2899–907. Available at: http://humrep.oxfordjournals.org/content/27/10/2899.short.

Gaur DS, Talekar MS and Pathak VP (2010) Alcohol intake and cigarette smoking: impact of two major lifestyle factors on male fertility. *Indian journal of pathology & microbiology* 53(1): 35–40. Available at: http://www.ijpmonline.org/article.asp?issn=0377-4929;year=2010;volume=53;issue=1;spage=35;epage=40;aulast=Gaur.

Gibson RS. 2005. Anthropometric Assessment. *Principles of nutritional assessment*. 2nd edition. New York: Oxford University Press:6-283.

Guzick DS, Overstreet JW, Factor-Litvak P, Brazil CK, Nakajima ST, Coutifaris C, Carson SA, Cisneros P, Steinkampf MP, Hill JA, Xu D and Vogel DL (2001) Sperm morphology, motility, and concentration in fertile and infertile men. *The New England journal of medicine* 345(19): 1388–1393.

Hall E and Burt VK (2012) Male fertility: psychiatric considerations. *Fertility and sterility*. Elsevier Inc. 97(2): 434–9. Available at: http://www.sciencedirect.com/science/article/pii/S0015028211028032.

Hammoud AO, Gibson M, Peterson CM, Meikle a. W and Carrell DT (2008a) Impact of male obesity on infertility: a critical review of the current literature. *Fertility and Sterility* 90(4): 897–904.

Hammoud AO, Wilde N, Gibson M, Parks A, Carrell DT and Meikle AW (2008b) Male obesity and alteration in sperm parameters. *Fertility and sterility*. Elsevier Ltd 90(6): 2222–5. Available at: http://www.sciencedirect.com/science/article/pii/S0015028207037752.

Haskell WL, Lee IM, Pate RR, Powell KE, Blair SN, Franklin B a., MacEra C a., Heath GW, Thompson PD and Bauman A (2007) Physical activity and public health: Updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. *Medicine and Science in Sports and Exercise* 39(8): 1423–1434.

Hofny ERM, Ali ME, Abdel-Hafez HZ, Kamal EE-D, Mohamed EE, Abd El-Azeem HG and Mostafa T (2010) Semen parameters and hormonal profile in obese fertile and infertile males. *Fertility and sterility*. Elsevier Ltd 94(2): 581–4. Available at: http://www.sciencedirect.com/science/article/pii/S0015028209007559.

Homan GF, Davies M and Norman R (2007) The impact of lifestyle factors on reproductive performance in the general population and those undergoing infertility treatment: A review. *Human Reproduction Update* 13(3): 209–223.

Ivell R (2007) Lifestyle impact and the biology of the human scrotum. *Reproductive biology and endocrinology : RB&E* 5: 15.

Jensen T, Andersson A, Jørgensen N, Andersen A, Carlsen E, Petersen J and Skakkebaek N (2004) Body mass index in relation to semen quality and reproductive hormones among 1,558 Danish men. *Fertility and sterility* 82(4): 863–70. Available at: http://www.sciencedirect.com/science/article/pii/S0015028204022241.

Jensen T, Heitmann B, Jensen M, Halldorsson T, Andersson A, Skakkebrek N, Joensen U, Lauritsen M, Christiansen P, Dalgård C, Lassen T and JØrgensen N (2013) High dietary intake of saturated fat is associated with reduced semen quality among 701 young Danish men from the general population. *American Journal of Clinical Nutrition* 97.

Jensen T, Jacobsen R, Christensen K, Nielsen N and Bostofte E (2009) Good semen quality and life expectancy: A cohort study of 43,277 men. *American Journal of Epidemiology* 170(5): 559–565.

Jensen TK, Gottschau M, Madsen JOB, Andersson A-M, Lassen TH, Skakkebaek NE, Swan SH, Priskorn L, Juul A and Jorgensen N (2014) Habitual alcohol consumption associated with reduced semen quality and changes in reproductive hormones; a cross-sectional study among 1221 young Danish men. *BMJ Open* 4(9): e005462–e005462. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4185337&tool=pmcentrez&rendertype=abstract.

De Jong a. ME, Menkveld R, Lens JW, Nienhuis SE and Rhemrev JPT (2014) Effect of alcohol intake

and cigarette smoking on sperm parameters and pregnancy. *Andrologia* 46: 112–117.

Jung A and Schuppe H (2007) Influence of genital heat stress on semen quality in humans. *Andrologia* 39(6): 203–215.

Jurewicz J, Hanke W, Radwan M and Bonde JP (2009) Environmental factors and semen quality. *International journal of occupational medicine and environmental health* 22(4): 305–29. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20053623.

Kay VJ and Barratt CLR (2009) Male obesity: impact on fertility. *The British Journal of Diabetes & Vascular Disease* 9(5): 237–241. Available at: http://dvd.sagepub.com/cgi/doi/10.1177/1474651409343132.

Kefer JC, Agarwal A and Sabanegh E (2009) Role of antioxidants in the treatment of male infertility. 449–457.

Kilgallon SJ and Simmons LW (2005) Image content influences men's semen quality. *Biology letters* 1(3): 253–255.

Kleinhaus K, Perrin M, Friedlander Y, Paltiel O, Malaspina D and Harlap S (2006) Paternal age and spontaneous abortion. *Obstetrics and gynecology* 108(2): 369–377.

Kort HI, Massey JB, Elsner CW, Mitchell-Leef D, Shapiro DB, Witt M a and Roudebush WE (2006) Impact of body mass index values on sperm quantity and quality. *Journal of andrology* 27(3): 450–2. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16339454.

Lee RD and Nieman DC. (2013) *Nutritional Assessment*, 6th edition, New York: McGraw-Hill:168,170.

Levy A, Fraser D, Katz M, Mazor M and Sheiner E (2005) Maternal anemia during pregnancy is an independent risk factor for low birthweight and preterm delivery. *European Journal of Obstetrics Gynecology and Reproductive Biology* 122(2): 182–186.

Li Y, Lin H, Li Y and Cao J (2011) Association between socio-psycho-behavioral factors and male semen quality: systematic review and meta-analyses. *Fertility and sterility*. Elsevier Ltd 95(1): 116–23. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20674912.

MacDonald a a, Herbison GP, Showell M and Farquhar CM (2010) The impact of body mass index on semen parameters and reproductive hormones in human males: a systematic review with meta-analysis. *Human reproduction update* 16(3): 293–311. Available at:

http://www.ncbi.nlm.nih.gov/pubmed/19889752.

Magnusdottir E V, Thorsteinsson T, Thorsteinsdottir S, Heimisdottir M and Olafsdottir K (2005) Persistent organochlorines, sedentary occupation, obesity and human male subfertility. *Human reproduction (Oxford, England)* 20(1): 208–15. Available at: http://humrep.oxfordjournals.org/content/20/1/208.short.

Martini AC, Tissera A, Estofán D, Molina RI, Mangeaud A, de Cuneo MF and Ruiz RD (2010) Overweight and seminal quality: a study of 794 patients. *Fertility and sterility* 94(5): 1739–43. Available at: http://www.sciencedirect.com/science/article/pii/S0015028209040461.

Mendiola J, Torres-Cantero AM and Agarwal A (2009) Lifestyle factors and male infertility: an evidence-based review. *Arch Med Sci* 5(1A): S3–12.

Mendiola J, Torres-Cantero AM, Vioque J, Moreno-Grau JM, Ten J, Roca M, Moreno-Grau S and Bernabeu R (2010) A low intake of antioxidant nutrients is associated with poor semen quality in patients attending fertility clinics. *Fertility and Sterility* 93(4): 1128–1133.

Mínguez-Alarcón L, Mendiola J, López-Espín JJ, Sarabia-Cos L, Vivero-Salmerón G, Vioque J, Navarrete-Muñoz EM and Torres-Cantero AM (2012) Dietary intake of antioxidant nutrients is associated with semen quality in young university students. *Human reproduction (Oxford, England)* 27(9): 2807–14. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22752607.

Muthusami KR and Chinnaswamy P (2005) Effect of chronic alcoholism on male fertility hormones and semen quality. *Fertility and sterility* 84(4): 919–24. Available at: http://www.sciencedirect.com/science/article/pii/S0015028205012513.

Olsen J and Høst Ramlau-Hansen C (2012) Dietary fats may impact semen quantity and quality. *Asian Journal of Andrology* 14: 511–512.

Paasch U, Grunewald S, Kratzsch J and Glander HJ (2010) Obesity and age affect male fertility potential. *Fertility and Sterility*. Elsevier Ltd 94(7): 2898–2901. Available at: http://dx.doi.org/10.1016/j.fertnstert.2010.06.047.

Du Plessis SS, Cabler S, McAlister D a, Sabanegh E and Agarwal A (2010) The effect of obesity on sperm disorders and male infertility. *Nature reviews. Urology* 7(3): 153–161. Available at: http://dx.doi.org/10.1038/nrurol.2010.6.

Povey a. C, Clyma J a., McNamee R, Moore HD, Baillie H, Pacey a. a. and Cherry NM (2012)

Modifiable and non-modifiable risk factors for poor semen quality: A case-referent study. *Human Reproduction* 27(9): 2799–2806.

Practice Committee of the American Society for Reproductive Medicine (2015) Diagnostic evaluation of the infertile male: a committee opinion. *Fertility and Sterility*. American Society for Reproductive Medicine 103(3): e18–e25. Available at: http://linkinghub.elsevier.com/retrieve/pii/S001502821402528X.

Qin D-D, Yuan W, Zhou W-J, Cui Y-Q, Wu J-Q and Gao E-S (2007) Do reproductive hormones explain the association between body mass index and semen quality? *Asian journal of andrology* 9(6): 827–34. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17968470.

Reddy SP, James S, Sewpaul R, Koopman F, Funani N, Sifunda S, Josie J, Masuka P, Kambaran N and Omardien R (2010) *Umthente Uhlaba Usamila-The South African Youth Risk Behaviour Survey 2008*. *Cape Town: South African Medical Research Council*.

Safarinejad MR, Asgari SA, Farshi A, Ghaedi G, Kolahi AA, Iravani S and Khoshdel AR (2013) The effects of opiate consumption on serum reproductive hormone levels, sperm parameters, seminal plasma antioxidant capacity and sperm DNA integrity. *Reproductive toxicology (Elmsford, N.Y.)*. Elsevier Inc. 36: 18–23. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23207164.

Saka M, Türker P, Ercan A, Kiziltan G and Baş M (2014) Is neck circumference measurement an indicator for abdominal obesity? A pilot study on Turkish Adults. *African health sciences* 14(3): 570–575. Available at: http://www.scopus.com/inward/record.url?eid=2-s2.0-84906924509&partnerID=tZOtx3y1.

Selit I, Basha M, Maraee a, El-Naby SH, Nazeef N, El-Mehrath R and Mostafa T (2013) Sperm DNA and RNA abnormalities in fertile and oligoasthenoteratozoospermic smokers. *Andrologia* 45(1): 35–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22564026.

Sermondade N, Faure C, Fezeu L, Shayeb a. G, Bonde JP, Jensen TK, Van Wely M, Cao J, Martini a. C, Eskandar M, Chavarro JE, Koloszar S, Twigt JM, Ramlau-Hansen CH, Borges E, Lotti F, Steegers-Theunissen RPM, Zorn B, Polotsky a. J, La Vignera S, Eskenazi B, Tremellen K, Magnusdottir E V., Fejes I, Hercberg S, Lévy R and Czernichow S (2012) BMI in relation to sperm count: An updated systematic review and collaborative meta-analysis. *Human Reproduction Update* 19(3): 221–231.

Sharma R, Biedenharn KR, Fedor JM and Agarwal A (2013) Lifestyle factors and reproductive health: taking control of your fertility. *Reproductive biology and endocrinology : RB&E*. Reproductive Biology

and Endocrinology 11(1): 66. Available at: http://www.rbej.com/content/11/1/66.

Sharpe RM (2010) Environmental/lifestyle effects on spermatogenesis. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 365(1546): 1697–1712.

Shayeb a. G, Harrild K, Mathers E and Bhattacharya S (2011) An exploration of the association between male body mass index and semen quality. *Reproductive BioMedicine Online* 23(6): 717–723. Available at: http://dx.doi.org/10.1016/j.rbmo.2011.07.018.

Stewart AF and Kim ED (2011) Fertility concerns for the aging male. *Urology*. Elsevier Inc. 78(3): 496–9. Available at: http://www.sciencedirect.com/science/article/pii/S0090429511006200.

Stewart TM, Liu DY, Garrett C, Jørgensen N, Brown EH and Baker HWG (2009) Associations between andrological measures, hormones and semen quality in fertile Australian men: inverse relationship between obesity and sperm output. *Human reproduction (Oxford, England)* 24(7): 1561–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19351657.

Teerds KJ, de Rooij DG and Keijer J (2011) Functional relationship between obesity and male reproduction: from humans to animal models. *Human reproduction update* 17(5): 667–83. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21546379.

Thonneau P, Bujan L, Multigner L and Mieusset R (1998) Occupational heat exposure and male fertility: a review. *Human reproduction (Oxford, England)* 13(8): 2122–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9756281.

Tsao C-W, Liu C-Y, Chou Y-C, Cha T-L, Chen S-C and Hsu C-Y (2015) Exploration of the Association between Obesity and Semen Quality in a 7630 Male Population. *PloS one* 10(3): e0119458. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4379020&tool=pmcentrez&rendertype=abstract.

Van Waeleghem K, De Clercq N, Vermeulen L, Schoonjans F and Comhaire F (1996) Deterioration of sperm quality in young healthy Belgian men. *Human reproduction (Oxford, England)* 11(2): 325–9.

WHO (2010) Examination and processing of human semen. *Online* Fifth edit(10): 286. Available at: http://whqlibdoc.who.int/publications/2010/9789241547789_eng.pdf.

World Health Organization (WHO). 2006. BMI classification: Global Database on Body Mass Index. Available at: http://apps.who.int/bmi/index.jsp?introPage=intro_3.html. (Accessed: 24 November

2014).

Wong WY, Zielhuis GA, Thomas CMG, Merkus HMWM and Steegers-Theunissen RPM (2003) New evidence of the influence of exogenous and endogenous factors on sperm count in man. *European journal of obstetrics, gynecology, and reproductive biology* 110(1): 49–54. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12932871.

Wu Q and Suzuki M (2006) Parental obesity and overweight affect the body-fat accumulation in the offspring: The possible effect of a high-fat diet through epigenetic inheritance. *Obesity Reviews* 7(2): 201–208.

Zitzmann M (2013) Effects of age on male fertility. *Best practice & research. Clinical endocrinology & metabolism*. Elsevier Ltd 27(4): 617–28. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24054934.

Chapter 5 ANTHROPOMETRIC MEASUREMENTS, PHYSICAL ACTIVITY AND SEMEN PARAMETERS OF HEALTHY MALE VOLUNTEERS

Data in this chapter are presented in article format, to report on the findings according to the second objective set for this study. The objective was to measure anthropometric indices of a group of healthy male at baseline and after three months. This article also includes data on correlations between activity levels, anthropometric measures and semen parameters. This article will be submitted to the journal, South African Family Practice; and is prepared according to the author's instructions (Addendum G), although referencing will be done according to the requirements of the Department of Nutrition and Dietetics, University of the Free State.

5.1 Abstract

Background: High body adiposity and inactivity are associated with lower semen parameters. This study describes anthropometric status and activity level amongst young, healthy men, in relation to semen parameters.

Methods: Fifty apparently healthy young volunteers were included and age, anthropometric measurements (weight, height, neck-, waist- and hip circumference), activity levels and semen parameters (volume, sperm concentration, morphology, motility and pH) were recorded.

Results: Median age was 24 years, with 60% of participants presenting with an overweight/ obese BMI and 46% with a neck circumference of ≥38cm. At baseline 18% and after three months 14% of participants had lower sperm concentrations than the reference values. At baseline and after three months, four participants (8%) did not meet the lower reference limits for sperm motility. No statistically significant correlations were found between anthropometric measures and semen parameters at baseline. Fifty-four percent of participants reported high activity levels and the median time spent sitting was 330.0 minutes/week. Eighty percent spent less than eight hours per day sitting during the week. The time spent sitting during the week, did not correlate significantly with sperm concentration (r=0.04, p= 0.76) or -motility (-0.17, p=0.24), but showed a weak negative correlation with sperm morphology (-0.29, p=0.05).

Conclusion: In this sample of healthy, young males the high incidence of overweight/ obesity and larger neck circumference was concerning in terms of long term metabolic health. Anthropometric measures and activity did not however, influence semen parameters, other than a weak negative correlation between time sitting and sperm morphology.

Key words: Anthropometric measures, Physical activity, Semen parameters

5.2 Introduction

Globally the prevalence of obesity is on the increase. In 2014, 1.9 billion people aged 18 years and older were overweight, and of these 600 million were obese (WHO 2015:Online, Ghanayem et al 2010:96). Overweight and obese men tend to have a higher risk for poor semen parameters (Eisenberg et al 2014:193,198–199, 2015:493,494, Kay and Barratt 2009:237, Magnusdottir et al 2005:208, Martini et al 2010:1739,1741,1742, Sermondade et al 2012:2), sexual dysfunction and subfertility (Du Plessis et al 2010:159).

With the global increase in the prevalence of overweight and obesity, an increase in infertility has also reported (Petraglia et al 2013:S4). The increase in infertility is debated by others, with a systematic review showing that fertility remained consistent between 1990 and 2010 (Mascarenhas et al 2012:Online). Certain parts of Africa, including Southern Africa, however report high levels of infertility, compared to North and East Africa (Mascarenhas et al 2012:Online). It remains a challenge to estimate the total global prevalence of infertility (Inhorn and Patrizio 2015:Online) and although sperm count and other sperm parameters are widely investigated, not enough scientific evidence exists to confirm a reduction in these parameters (Cocuzza and Esteves 2014:1,6).

Despite limited scientific evidence, it is concerning that semen parameters in a group of young males (18 years of age), subjected to compulsory medical examinations for military service in Denmark, already displayed sub-optimal semen quality (Andersen et al 2000:366,368,371). A decline in conception rates amongst women born in the same country was linked to the possible influence of this declining reproductive health in men (Jensen et al 2008:81) with a concern that these trends are already reported in younger populations (Jensen et al 2008:81).

In various cultures, women are frequently considered to be responsible for the couple's infertility (Ombelet et al 2008:605), but it is estimated that seven percent of men in the general population is affected by infertility (Krausz 2011:271). Sperm parameters do not only provide an indication of fertility potential, but also provide an indication of the general health of males (Jensen et al 2009:559). Both animal and human studies further suggest a link between paternal diet, metabolic health, body weight and semen parameters (Bakos et al 2010:402,408,409, Ferguson-Smith and Patti 2011:115,116). For example, a link has been described between high pre-conception body mass index (BMI) in males and the semen quality of their offspring (Ramlau-Hansen et al 2007:2758,2762), possibly due to a decrease in Sertoli cell numbers and sperm count (Sharpe 2010:1697, Winters et al 2006:560). A link has also been described between physical activity and semen quality has also been described in a younger group of males (18 to 22 years) (Gaskins et al 2013:Online).

Various practical and affordable methods can be used to provide an indication of anthropometric nutritional status in individuals, including body mass index (BMI), body adiposity index (BAI), waist-to-height ratio (WHtR), waist-to-hip ratio (WHR) and neck circumference.

Weight and height are used to calculate BMI, by dividing a participant's weight in kilogram by the square of the height in meters (kg/m^2) (WHO 2014:Online, Lee and Nieman 2013:181, WHO 2000:8-9, WHO 1995:329,364). BAI iss calculated, using hip circumference and standing height, to estimate percentage body fat. BAI can be used to indicate percentage body fat for adult men of different ethnicities (Bergman et al 2011:1083).

Waist and hip circumferences are used to calculate the waist-to-hip ratio WHR. WHtR is a simple measure of the ratio of waist circumference to standing height, which reflects abdominal adiposity. WHtR has been proposed as superior to BMI in predicting central obesity and associated health risks (Ashwell et al 2012:275,279, Ashwell and Hsieh 2005:303–305).

Neck circumference is a simple screening tool that can be used as an indicator of upper body fat distribution to help identify overweight and obesity (Adamu et al 2013:82, Ben-Noun et al 2001:470, Preis et al 2010:3701, Saka et al 2014:570). The measurement is easy to use, non-

invasive, not time consuming, cost-effective and correlates well with other standard anthropometric parameters (Adamu et al 2013:82, Aswathappa et al 2013:28,31, Ben-Noun et al 2001:470). Neck circumference of 35 cm or more, in men with metabolic syndrome, may predict erectile dysfunction (Akin et al 2014:963,965,969).

The aim of this study was to describe anthropometric measures and activity levels of young healthy males before and after micronutrient intervention and compare these with semen parameters with the aim to find a simple screening tool that could identify the risk for poor semen parameters in resource poor settings.

5.3 Methods

5.3.1 Study design, sample size and ethical considerations

This descriptive study formed part of a larger intervention study, examining the effect of micronutrient supplementation on sperm parameters. The study sample consisted of 50 apparently healthy men between the ages of 18 and 45 years. Advertising to recruit volunteers was done on the campus of the University of the Free State (UFS) and at the National Defence Force in Bloemfontein, South Africa. Written informed consent was obtained from each participant and participants were informed that they could withdraw from the study at any time. The Ethics Committee of the Faculty of Health Sciences, UFS approved the protocol of this study (ECUFS number 08/2014).

5.3.2 Data collection

Data collected included age, anthropometric measurements, activity levels and semen parameters.

In this study anthropometric measures included weight and height, as well as neck circumference (NC), waist circumference (WC) and hip circumference (HC). All anthropometric measurements were taken by the researcher in a private area (WHO 2005:3-3-4).

A platform electronic Soehnle Professional scale and Seca stadiometer were used for weight and height measurements. Two measurements were taken in direct succession, and if weight

differed with more than 100g or height with more than 0.1cm (Lee and Nieman 2013:170,186) a third measurement was taken and the average calculated. Before each weighing session a known weight was used to calibrate the scale. Standard measuring techniques were used (Gibson 2005:247,253, Lee and Nieman 2013:168,170; NHANES 2007:3–3,3–7 – 3–9). A BMI lower than 18.5 kg/m^2 reflected underweight, between 18.5 kg/m^2 and 24.9 kg/m^2 normal weight, 25 kg/$m^2$to 29.9 kg/m^2 overweight and 30 kg/m^2 or more obesity (WHO 2006:Online).

Neck, waist and hip measurements were taken with a non-stretch, Seca 201 tape measure. If two measurements taken in succession differed with more than 0.1cm a third measurement was taken and the average calculated. NC was measured in the midway of the neck between the mid cervical spine and mid anterior neck, to the nearest 0.1cm (Aswathappa et al 2013:29, Ben-Noun and Laor 2006:15, Ben-Noun et al 2001:473). Participants stood upright, head erect with the shoulders not hunched in a relaxed position, and looked straight ahead towards the researcher (Aswathappa et al 2013:29, Ben-Noun and Laor 2006:15, Ben-Noun et al 2001:473). The measuring tape was placed just below the laryngeal prominence (Aswathappa et al 2013:29, Ben-Noun et al 2001:473, Hingorjo et al 2012:37) parallel with the floor, with the tape in front at the same height as the tape at the back of the neck (Hingorjo et al 2012:37). The trapezius muscles were not included in the measurement of the neck circumference (Aswathappa et al 2013:29). A cut-off value of equal or more than 38 cm was used to identify overweight (Yang et al 2010:2465,2467).

WC was measured with the participant standing upright, feet close together, arms at the side, body weight evenly distributed with the abdominal muscles relaxed (Lee and Nieman 2013:186, WHO 2011:21). The measurement was taken around the midpoint, between the top of the iliac crest and lower margin of the last palpable rib in the mid axillary line within 0.1cm (Lean et al 1995:159, Lee and Nieman 2013:186, WHO 2005:20). Measurements were taken at the end of a normal expiration, with the tape wrapped snugly around the waist, without constricting the waist, at a level parallel to the floor (Lee and Nieman 2013:186,NHANES 2007:3–16, WHO 2011:20). A WC of more than 94cm in males is considered as an indication for increased risk for metabolic complications, while a circumference of more than 102cm is considered as a higher risk and an independent risk factor for metabolic complications and chronic disease (Alberti et al 2009:1642, Hammond and Litchford 2012:169, WHO 2000:11).

Hip circumference was measured around the widest portion of the buttocks, with the tape in a horizontal plane while the participant stood upright, arms at the side, feet together, with weight evenly distributed (Gibson 2005:281, WHO 2011:20,21). The tape did not indent the soft tissue (Gibson 2005:281,WHO 2011:20).

A global cut-off value of 0.5 is used to interpret WHtR for all genders and ethnic groups (Ashwell et al 2012:284, Ashwell and Hsieh 2005:303–305). A WHtR ratio of more than one indicates an increased risk for lifestyle diseases (Hammond and Litchford 2012:470, Lee and Nieman 2013:185).

BAI was calculated to estimate percentage body adiposity, using hip circumference and standing height in a formula, BAI = (hip circumference) / ((height)$^{1.5}$) – 18) (Bergman et al 2011:1083), with the same formulae and cut-off values used for adult men of different ethnicities (Bergman et al 2011:1083). The body fat percentage for males are interpreted as low when between 10-15%, average when between 16-18%, above average when between 19-20%, overweight when between 21-25% and obese when equal or above 26% (Ricciardi et al 2009:4–5).

Physical activity was determined using the International Physical Activity Questionnaire (IPAQ) (Craig et al 2003:1381,1388). Activity was categorised as low, moderate and high, using level and duration of activity according to metabolic equivalents of tasks (METs), as reported on the self-reported questionnaire. One MET is defined as the amount of energy required at rest and is equal to 3.5 ml O_2 per kg body weight x min (Jetté et al 1990:555).

Low activity referred to the activity level of participants who do not meet the criteria for moderate or high physical activity levels. Activity levels were only compared to anthropometric measures at baseline.

Moderate activity referred to three or more days of vigorous-intensity activity of at least20 minutes per day; or five or more days of moderate-intensity activity and/or walking of at least 30 minutes per day; or five or more days of any combination of walking, moderate-intensity or vigorous intensity activities achieving a minimum total physical activity of at least 600 Metabolic Equivalent of Task (MET)-minutes/week. Individuals meeting at least one of the

above criteria were defined as accumulating a minimum level of activity and therefore were classified as 'moderate'.

High activity levels referred to vigorous-intensity activity on at least 3 days achieving a minimum total physical activity of at least 1500 MET-minutes/week; or seven days of any combination of walking, moderate-intensity or vigorous-intensity activities achieving a minimum total physical activity of at least 3000 MET-minutes/week.

Results were evaluated according to current recommendations for adults for activity which is 150 minutes (2 hours and 30 minutes) per week of moderate-intensity, or 75 minutes (1 hour and 15 minutes) per week of vigorous-intensity aerobic physical activity (Lambert et al 2001:S12, USDA 2010:18).

Two semen samples were collected. The first sample was collected after abstaining from sexual intercourse or masturbation for three days. The second sample was collected three days later. Samples were collected in a private room and transported to a near-by laboratory within 30 minutes of collection. Semen analysis was done by the Department of Obstetrics and Gynaecology, UFS and included semen volume, sperm concentration, morphology, quantitative /qualitative motility and pH. The World Health Organisation (WHO) reference values were used to interpret semen and sperm parameters (WHO 2010:224). Qualitative motility of sperm was described as progressive motility (PR), non-progressively motility (NP) and immotility (IM). Progressive motility (PR) is considered active linear or slightly curved forward movement of the sperm, non-progressive motility (NP) is defined when there is no forward movement or circular forward movement, while immotility (IM) describes no visible movement. Average values of the two samples provided were used. The same procedure for collection of semen samples and analysis were repeated after three months.

Participants were randomized into two groups, using randomization tables, one group receiving nutrient supplements (vitamin-mineral and omega-3) and the other receiving a placebo. The vitamin-mineral supplement used was Centrum® Adult and the omega-3 supplement was Centrum® My Nutrients™ Omega-3 (Pfizer South Africa (Pty) Ltd). The placebo consisted of glucose and fructose. Participants were instructed to take the vitamin mineral supplement twice daily and one Omega-3 mini-gel in the morning and two Omega-3

mini-gels in the evening. The placebo group were also instructed to take the placebo twice daily.

5.3.3 Statistical analysis

Data were captured in duplicate on Microsoft Excel (2010) for Windows7 spread sheets and checked electronically by a biostatistician to verify accuracy. SAS/STAT software, version 12.4 of the SAS system for Windows (© 2010 SAS Institute Inc.) was used to analyse data. Analysis was performed by the Department of Biostatistics, Faculty of Health Sciences, UFS. Descriptive statistics were used to describe the sample and included frequencies and percentages for categorical data. For continuous data means and standard deviations or medians and percentages were calculated. Chi-squared tests or Fisher exact tests and two tailed Pearson's or Spearman's correlations, as well as analysis of variance were used to determine associations between variables. Statistical significance was set at a p-value of 0.05 or less.

5.4 Results

Results from this study are presented as a description of the anthropometric status, semen parameters and activity levels and also report on correlations and associations between these variables. The study sample included healthy young men with a median age of 24 years, ranging between 18 and 43 years. Table 5.1 summarises the anthropometric measurements of the study sample.

Table 5.1 Median and mean anthropometric measures at baseline and after three months

Variable	N	%	Minimum	Lower quartile	Median	Upper quartile	Maximum	Mean	±SD	Lower 95% CL for Mean	Upper 95% CL for Mean
Baseline											
Weight (kg)	50	100	58.7	76.4	83.4	100.1	123.8	87.6	16.8	83.0	92.4
Height (cm)	50	100	155.5	174.2	180.0	185.5	200.0	179.4	8.2	177.1	182.0
Body Mass Index (kg/m^2)	50	100	19.5	23.6	26.9	30.7	42.5	27.2	5.1	26.0	29.0
Neck circumference (cm)	50	100	34.3	37.2	38.7	40.7	44.4	39.0	2.3	38.3	39.6
Waist circumference (cm)	50	100	71.0	80.0	86.5	99.0	119.6	90.6	13.9	87.0	95.0
Hip circumference (cm)	50	100	91.5	98.9	105.0	109.5	125.0	105.5	8.8	103.0	108.0
Waist-to-hip ratio	50	100	0.7	0.8	0.8	0.9	1.0	0.9	0.1	0.8	0.9
Waist-to-height ratio	50	100	0.4	0.4	0.5	0.6	0.7	0.5	0.1	0.5	0.5
Body Adiposity Index (%)	50	100	17.0	23.1	25.1	28.3	38.7	26.0	4.4	25.0	27.2
After three months											
Weight (kg)	50	100	58.6	77.0	83.4	98.0	118.4	87.5	16.1	83.0	92.1
Height (cm)	50	100	155.6	174.3	180.0	185.5	200.0	179.4	8.2	177.1	182.0
Body Mass Index (kg/m^2)	50	100	19.5	23.8	27.0	30.5	40.1	27.2	5.0	25.8	28.7
Neck circumference (cm)	50	100	33.5	37.6	38.4	40.9	45.3	38.9	2.3	38.2	39.6
Waist circumference (cm)	50	100	68.25	80.0	87.3	99.6	118.5	90.1	13.1	86.4	93.9
Hip circumference (cm)	50	100	87.5	101.0	104.8	109.5	123.8	105.2	8.0	102.9	107.5
Waist-to-hip ratio	50	100	0.7	0.8	0.8	0.9	1.0	0.9	0.1	0.8	0.9
Waist-to-height ratio	50	100	0.4	0.5	0.5	0.5	0.7	0.5	0.1	0.5	0.5
Body Adiposity Index (%)	50	100	17.9	22.8	25.3	28.5	38.8	25.9	4.4	26.7	27.2

Median weight for this group at baseline and after three months were the same (83.4 kg) (Table 5.1). At baseline body weight ranged between 58.7 and 123.8 kg and after three months between 58.6 and 118.4kg. Median height was 180.0cm ranging from 155.5 to 200.0cm. BMI at baseline for the group ranged between 19.5 and 42.5kg/m^2, and after three months between 19.5 and 40.1kg/m^2 with the BMI categories indicated in Table 2.1. Median neck circumference at baseline was 38.7cm, ranging between 34.3 and 44.4cm and after three months between 33.5 and 45.3cm. 46% of participants had a neck circumference of 38 cm or more. Median waist circumference (Table 5.1) was 86.5 cm at baseline varying between 71.0 and 119.6cm and after three months 87.3 cm, varying between 68.3 and 118.5cm. BAI at baseline ranged between 17.0 and 38.7% and after three months 17.9 to 38.8%. Median WHR at baseline and after three months was the same (0.8). Table 5.2 reports on the incidence of overweight and obesity in the study sample according to BMI and BAI.

Table 5.2 Overweight and obesity according to BMI and BAI at baseline and after three months

Category	Baseline		After three months	
BMI				
	n	**%**	**n**	**%**
Normal	20	40	21	42
Overweight	17	34	15	30
Obese Class 1	9	18	10	20
Obese Class 2	3	6	3	6
Obese Class 3	1	2	1	2
BAI				
Low (14-20%)	3	6	4	8
Average (21-25%)	21	42	20	40
Above average (26-29%)	15	30	15	30
Overweight (30-35%)	9	18	9	18
Obese (>36%)	2	4	2	4

Forty percent of participants were classified in the normal BMI category at baseline and 42 percent after three months. When applying the BAI formulae, 78% of participants fell in the low to above average category at baseline and after three months (Table 5.2). According to BMI 60% (n=30) was overweight/obese at baseline and according to BAI 22% (n=11). After three months 58% (n=29) were classified as overweight/obese according to BMI, while overweight/obesity according to BAI classification remained the same (22%). In Table 5.3 the results from the semen analysis of participants are reported.

Table 5.3 Semen parameters at baseline and after three months

Variable			Minimum	Lower quartile	Median	Upper quartile	Maximum	Mean	±SD	WHO reference limit	Lower than reference limit		Higher than reference limit	
	n	%									n	%	n	%
Baseline														
Semen volume (ml)	50	100	0.7	1.8	3.0	3.8	7.0	2.8	1.3	1.5 (1.4-1.7)	7	14	43	86
Sperm concentration (10^6 per ml)	50	100	2.0	22.5	33.0	63.5	105.5	42.0	29.2	15 (12-16)	9	18	41	82
Sperm total motility (%)	50	100	25.0	53.0	62.8	70.0	82.5	60.1	13.8	40 (38-42)	4	8	46	92
Progressive motility	50	100	10.0	39.0	47.0	54.0	62.5	44.4	12.7					
Non-progressive motility	50	100	20.0	23.5	27.5	35.0	65.0	31.1	10.7					
Immotility	50	100	10.0	20.0	23.5	25.0	52.5	24.0	8.3					
Sperm morphology (normal forms %)	48	96	3.0	9.0	12.0	13.5	19.5	11.2	3.6	4 (3.0-4.0)	1	2.1	47	97.9
pH	50	100	7.3	8.0	8.0	8.5	9.0	8.2	0.3	≥7.2	0	0	50	100
After three months														
Semen volume (ml)	50	100	0.8	2.3	2.8	3.5	6.5	2.9	1.1	1.5 (1.4-1.7)	5	10	45	90
Sperm concentration (10^6 per ml)	50	100	1.0	21.0	30.5	52.5	120.5	40.6	29.2	15 (12-16)	7	14	43	86
Sperm motility (%)	50	100	15.0	47.5	60.0	65.0	75.0	55.4	13.9	40 (38-42)	4	8	46	92
Progressive motility	49	98	12.5	36.5	45.0	51.5	57.5	41.6	11.4					
Non-progressive motility	49	98	22.0	25.0	32.5	35.0	50.0	32.1	8.2					
Immotility	49	98	15.0	20.0	25.0	30.0	55.0	25.8	6.9					
Sperm morphology (normal forms %)	44	88	1.0	10.0	12.0	13.3	18.0	11.6	3.5	4 (3.0-4.0)	1	2.3	43	97.7
pH	49	98	7.3	8.0	8.0	8.3	9.3	8.1	0.3	≥7.2	0	0	49	98

*Evaluated according to the WHO criteria in WHO laboratory manual for the Examination and processing of human semen. 2010. 5th Edition, Switzerland, 2010:224.

At baseline and after three months 98% of the participants provided a complete semen sample (table 5.3). Semen volume at baseline ranged between 0.7 to 7.0 ml with seven (14%) participants producing an insufficient semen volume. After three months semen volume ranged between 0.8 and 6.5 ml. No significant change in means (p=0.43) were found for semen volume. Median sperm concentration at baseline was 33 x 10^6 per ml, with a range between 2.0 to 105.5 x 10^6 per ml. The median sperm concentration after three months was 30.5 x 10^6 per ml ranging from 1.0 to 120.5 x 10^6 per ml. Eighteen percent of participants had a lower sperm concentration at baseline than the reference values; and after three months, 14% did not meet the lower reference limit. Semen concentration did not change significantly (p=0.74) over the three month period. The median for total sperm motility was 62.8% at baseline and 60% after three months. Total sperm motility varied between 25.0% and 82.5% and 15.0 and 75% respectively. Progressive motility at baseline ranged between 10.0 to 62.5% and 12.5 to 57.5% after three months. Median non-progressive motility at baseline and after three months were the same (35%). At baseline and after three months, four participants (8%) did not meet the lower reference limit of the WHO for sperm motility. No change in sperm motility were found between baseline and after three months (p=0.56) At baseline sperm morphology (n=48) varied between 3.0 to 19.5% sperm with normal forms, while ranging from 1.0 to 18.0% after three months (n=44). The number of participants with less sperm with normal forms than specified by the WHO remained the same and a significant improvement were found over the three month period (p=0.05). Semen pH for all the participants met the reference pH of ≥7.2 and did not change significantly (p=0.14) after three months.

Table 5.4 reports on the activity level of participants at baseline according to the IPAQ, using intensity and duration of activity according to metabolic equivalent of task (METs)

Table 5.4 Activity level of participants at baseline

Activity level	n	%
Low	7	14
Moderate	16	32
High	27	54

Fifty-four percent of participants (Table 5.4) had a high self-reported activity level. Seventy two percent of participants reported that they are active every day of the week, with an

additional 16% being active at least 5 days per week. In this study sample, the median time spent sitting per day during the week was 330.0 minutes (n=49). Eighty percent of participants (n=40) indicated that spent less than eight hours per day sitting during the week.

Table 5.5 shows the correlation between anthropometric measures and semen parameters at baseline.

Table 5.5 Correlation between baseline anthropometric measures and semen parameters

Variables	Sperm concentration			Sperm motility			Sperm morphology		
	n	r	*p*-value	n	r	*p*-value	n	r	*p*-value
BMI	50	-0.04	0.80	50	0.11	0.46	48	0.20	0.17
BAI	50	-0.002	0.99	50	0.16	0.27	48	0.22	0.14
Neck circumference	50	-0.05	0.74	50	0.08	0.60	48	0.41	0.78
Waist circumference	50	-0.06	0.67	50	0.08	0.56	48	0.16	0.29
WHtR	50	-0.05	0.75	50	0.12	0.40	48	0.17	0.24

No statistically significant correlations were found between anthropometric measures and semen parameters (Table 5.5).

Table 5.6 reports on the influence of reported physical activity levels on semen parameters.

Table 5.6 Effect of physical activity on baseline semen parameters

Variable	n	Degrees of freedom (df)	Sum of squares	F value	*p*-value	R-square
Semen volume	49	2	0.2	0.06	0.94	0.003
Semen concentration	49	2	444.4	0.25	0.78	0.010
Semen motility	49	2	9.9	0.02	0.98	0.0001
Progressive motility (PR)	49	2	19.4	0.06	0.95	0.002
Non-progressive motility (NP)	49	2	147.3	0.78	0.47	0.032
Immotility (IM)	49	2	119.9	0.71	0.5	0.030
pH	49	2	0.003	0.01	0.99	0.004

In this sample, time spent sitting during the week, did not correlate significantly with sperm concentration (r=0.04, p=0.76) or sperm motility (-0.17, p=0.24). However, the total number

of minutes spent sitting during the week showed a weak negative correlation with sperm morphology (-0.29, p=0.05).

5.5 Discussion

In this young sample, with a median age of 24 years, most men met the reference limit for semen parameters as set by the WHO and except for morphology, semen parameters remained the same over a period of three months.

More than half of participants were classified as being overweight /obese according to BMI. However when applying calculated BAI, only less than a quarter were categorised as overweight /obese. This discrepancy in estimating body adiposity by using the two different calculations could partly be explained by the influence of muscle mass on BMI (Kay and Barratt 2009:239). Muscle mass could have influenced the interpretation of BMI in this study sample of young active men, reporting mostly moderate or high activity levels (86%). Median anthropometric measures did not change significantly over the three month period. The BMI reported in this study agrees with the incidence of overweight/ obesity reported by other researchers of between 55.2% amongst volunteers in Turkey (Saka et al 2014:572) and 68.4% in men attending an infertility clinic in the USA (Biekniek et al 2015:e67).

Although only a small number of participants did not meet the lower reference limits specified by the WHO, further investigation might be warranted, especially when considering that this sample was relatively young and apparently healthy.

In the literature, overweight /obesity in men is associated with changes in spermatogenesis and sexual dysfunction (Hammoud 2006:624, Hammoud et al 2008a:897, Kay and Barratt 2009:237,240) and a higher BMI has been associated with lower sperm volume (Eisenberg et al 2014:193,196,197), concentration (Braga et al 2012:53; Aggerholm et al 2008:619,623, Jensen et al 2004:863,866, Kasturi et al 2008:257; Hammoud et al 2008b:2222,2224; Kort et al 2006:450,451), motility (Braga et al 2012:53; Kasturi et al 2008:257; Sermondade et al 2012:2,9; Kort et al 2006:450,451) and morphology (Biekniek et al 2015:e67, Tsao et al 2015:Online; Sermondade et al 2012:2,9). In this study however no statistically significant correlation was found between any of the anthropometric measures and semen parameters. As found in this sample, other researchers also failed to find a link between overweight /

obesity and specific sperm parameters (Campbell et al 2015:593,600, Chavarro et al 2010:2222,2225,2227, Duits et al 2010:1356,1357,1359, Egwurugwu et al 2011:29,30, Eisenberg et al 2014:193,196,197, Eskandar et al 2012:1–4, MacDonald et al 2010:293,306,307, Povey et al 2012:2799, Zhang et al 2014:1861,1863).

Neck circumference is related to body weight, BMI, waist and hip circumference and waist-to-hip ratio (Saka et al 2014:570,572,573) and can be used as an indicator for upper body fat distribution to identify overweight and obesity (Adamu et al 2013:82, Ben-Noun et al 2001:470, Preis et al 2010:3701, Saka et al 2014:570). In this sample, almost half of participants had a neck circumference of 38cm or more, reflecting overweight (Yang et al 2010:2465,2467). Ben-Noun et al (2001:470,477) recommend that men with a neck circumference of 37 cm or more need further evaluation of body composition. The majority of men in this study will therefore need further evaluation in terms of overweight, as the lower quartile median neck circumference was 37.2cm.

With an increase in waist circumference, a decline in semen volume and sperm count has been reported (Eisenberg et al 2014:193,196,197, 2015:493–494). In this study median waist circumference of this sample fell in the healthy category (Alberti et al 2009:1642, Hammond and Litchford 2012:169, WHO 2000:11). Although Fejes et al (2005:155,157) describe a correlation between waist and hip circumference and sperm count, total motility and number of progressive motile sperm in infertile couples, no correlation between waist circumference or hip circumference and semen parameters were found in this healthy population. At baseline and after three months, WHR and WHtR were classified in the normal category and did not indicate an increased risk for lifestyle diseases (Lee and Nieman 2013:185, Hammond and Litchford 2012:470).

The majority of participants indicated that they are moderately to highly active and did not spend more than four hours per day sitting during the week. The level of activity did not have a statistically significant effect on any of the semen parameters and only the amount of time spent sitting during the week showed a weak negative correlation with sperm morphology. Gaskins et al (2013:Online) however reported that higher moderate-to-vigorous activity levels with less time spent watching TV, significantly influenced sperm concentration. Vaamonde et al (2012:3267,3270) also described the positive effect of physical activity on semen

parameters compared to inactive men. If a male is seated, air does not circulate effectively around the scrotum resulting in less effective cooling (Sharpe 2010:1703). The duration of sitting during a work day is positively linked to daytime scrotal temperatures which may have a negative effect on semen quality (Jung and Schuppe 2007:203,205,212, Magnusdottir et al 2005:205). Although activity levels did not significantly influence semen parameters in this study, it has been documented that different training modalities might have an effect on semen parameters, especially as intensity and volume of exercise increase (Vaamonde et al 2009:1941,1943).

5.6 Conclusion

This study provided novel information on semen parameters in relation to anthropometric measures and activity in a South African setting. From these results it seems that anthropometric measures and activity do not influence semen parameters in healthy, young males, except for a weak negative correlation between time spent sitting and sperm morphology. The high incidence of overweight and obesity amongst participants is however concerning in terms of long term metabolic health.

5.7 Limitations

The main limitation of this study was the sensitivity of obtaining semen samples and limited resources, which limited the number of participants volunteering to participate in the study and honouring their appointments. Compliance of some participants in terms of honouring their appointments was also a limitation. In order to overcome the limitations, a variety of methods were used to recruit volunteers, including electronic advertisements and personal recruitment and participants were reminded of their appointments by means of text messages.

The use of indirect methods to describe body adiposity is also acknowledged as a limitation and future studies amongst healthy active males could consider the use of direct methods to determine body adiposity like dual X-ray absorptiometry, if resources allow.

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5.9 References

Adamu L, Asuku A, Taura M, Tela I, Datti S and Imam A (2013) Neck circumference: An upcoming tool of adiposity indices. *Nigerian Journal of Basic Clinical Sciences* 10(2): 82–85.

Aggerholm AS, Thulstrup AM, Toft G, Ramlau-Hansen CH and Bonde JP (2008) Is overweight a risk factor for reduced semen quality and altered serum sex hormone profile? *Fertility and sterility* 90(3): 619–26. Available at: http://www.sciencedirect.com/science/article/pii/S0015028207015282.

Akin Y, Gulmez H, Bozkurt A, Nuhoglu B and Usta MF (2014) Usage of neck circumference as novel indicator of erectile dysfunction: a pilot study in Turkish population. *Andrologia* 1–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24147908.

Alberti KGMM, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato K a., Fruchart JC, James WPT, Loria CM and Smith SC (2009) Harmonizing the metabolic syndrome: A joint interim statement of the international diabetes federation task force on epidemiology and prevention; National heart, lung, and blood institute; American heart association; World heart federation; International . *Circulation* 120(16): 1640–1645.

Andersen AG, Jensen TK, Carlsen E, Jørgensen N, Andersson AM, Krarup T, Keiding N and Skakkebaek NE (2000) High frequency of sub-optimal semen quality in an unselected population of young men. *Human reproduction (Oxford, England)* 15(2): 366–72. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10655308.

Ashwell M, Gunn P and Gibson S (2012) Waist-to-height ratio is a better screening tool than waist circumference and BMI for adult cardiometabolic risk factors: systematic review and meta-analysis. *Obesity reviews : an official journal of the International Association for the Study of Obesity* 13(3): 275–86. Available at:

http://www.ncbi.nlm.nih.gov/pubmed/22106927.

Ashwell M and Hsieh SD (2005) Six reasons why the waist-to-height ratio is a rapid and effective global indicator for health risks of obesity and how its use could simplify the international public health message on obesity. *International journal of food sciences and nutrition* 56(5): 303–307.

Aswathappa J, Garg S, Kutty K and Shankar V (2013) Neck circumference as an anthropometric measure of obesity in diabetics. *North American Journal of Medical Sciences* 5(1): 28–31.

Bakos HW, Mitchell M, Setchell BP and Lane M (2010) The effect of paternal diet-induced obesity on sperm function and fertilization in a mouse model. *International journal of andrology* 34(5 Pt 1): 402–10. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20649934.

Ben-Noun LL and Laor A (2006) Relationship between changes in neck circumference and cardiovascular risk factors. *Experimental and clinical cardiology* 11(1): 14–20. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2274839&tool=pmcentrez&rendertype=abstract.

Ben-Noun LL, Sohar E and Laor A (2001) Neck Circumference as a Simple Screening Measure for Identifying Overweight and Obese Patients. *Obesity Research* 9(8): 470–477. Available at: http://doi.wiley.com/10.1038/oby.2001.61.

Bergman RN, Stefanovski D, Buchanan TA, Sumner AE, Reynolds JC, Sebring NG, Xiang AH and Watanabe RM (2011) A Better Index of Body Adiposity. *Obesity*. Nature Publishing Group 19(5): 1083–1089. Available at: http://dx.doi.org/10.1038/oby.2011.38/nature06264.

Biekniek J, Kashanian J, Deibert C, Grober E, Brannigan R, Sandlow J and Jarvi K (2015) DDeleterious effects of male obesity on reproductive parameters among a loarge multi-institutional cohort. *Fertility and Sterility*. Elsevier Ltd 104(3): e67. Available at: http://dx.doi.org/10.1016/j.fertnstert.2015.07.203.

Campbell JM, Lane M, Owens JA and Bakos HW (2015) Paternal obesity negatively affects

male fertility and assisted reproduction outcomes: a systematic review and meta-analysis. *Reproductive BioMedicine Online*. Reproductive Healthcare Ltd. 31(5): 593–604. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1472648315003776.

Chavarro JE, Toth TL, Wright DL, Meeker JD and Hauser R (2010) Body mass index in relation to semen quality, sperm DNA integrity, and serum reproductive hormone levels among men attending an infertility clinic. *Fertility and sterility*. Elsevier Ltd 93(7): 2222–31. Available at: http://www.sciencedirect.com/science/article/pii/S0015028209001502.

Cocuzza M and Esteves SC (2014) Shedding light on the controversy surrounding the temporal decline in human sperm counts: a systematic review. *TheScientificWorldJournal* 2014: 365691. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3929517&tool=pmcentrez&rendertype=abstract.

Craig C, Marshall A, Sjostrom M, Bauman A, Booth M, Ainsworth B, Pratt M, Ekelund U, Yngve A, Sallis J and Oja P (2003) International Physical Activity Questionnaire: 12 Country Reliability and Validity. *Medicine & Science in Sports & Exercise* 35(8): 3508–1381. Available at: http://addssa.com/intranets/idrd/2011_10_activos_y_saludables/03_recursos/study_material/gestores/Articulos Ingles/Vigilancia y Evaluacion/1Q. International PAQ.pdf.

Duits FH, Van Wely M, Van Der Veen F and Gianotten J (2010) Healthy overweight male partners of subfertile couples should not worry about their semen quality. *Fertility and Sterility*. Elsevier Ltd 94(4): 1356–1359. Available at: http://dx.doi.org/10.1016/j.fertnstert.2009.05.075.

Egwurugwu JN, Nwafor a, Chike CPR, Ufearo CS, Uchefuna RC, Iwuji SC, Okwara JE and Alagwu E a (2011) The relationship between body mass index, semen and sex hormones in adult male. *Nigerian journal of physiological sciences : official publication of the Physiological Society of Nigeria* 26(1): 29–34. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22314983.

Eisenberg ML, Kim S, Chen Z, Sundaram R, Schisterman EF and Buck Louis GM (2014) The

relationship between male BMI and waist circumference on semen quality: data from the LIFE study. *Human reproduction (Oxford, England)* 29(2): 193–200. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3896223&tool=pmcentrez&rendertype=abstract.

Eisenberg ML, Kim S, Chen Z, Sundaram R, Schisterman EF and Buck Louis GM (2015) The relationship between male BMI and waist circumference on semen quality: data from the LIFE study. *Human reproduction (Oxford, England)* 29(2): 193–200. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3896223&tool=pmcentrez&rendertype=abstract.

Eskandar M, Al-Asmari M, Babu Chaduvula S, Al-Shahrani M, Al-Sunaidi M, Almushait M, Donia O and Al-Fifi S (2012) Impact of Male Obesity on Semen Quality and Serum Sex Hormones. *Advances in Urology* 2012: 1–4.

Fejes I, Koloszar S, Szöllösi J, Zavaczki Z and Pal A (2005) Is semen quality affected by male body fat distribution ? *Andrologia* 37: 155–159.

Ferguson-Smith AC and Patti ME (2011) You are what your dad ate. *Cell Metabolism*. Elsevier Inc. 13(2): 115–117. Available at: http://dx.doi.org/10.1016/j.cmet.2011.01.011.

Gaskins AJ, Mendiola J, Afeiche M, Jørgensen N, Swan SH and Chavarro JE (2013) Physical activity and television watching in relation to semen quality in young men. *British Journal of Sports Medicine* 1–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23380634.

Ghanayem BI, Bai R, Kissling GE, Travlos G and Hoffler U (2010) Diet-induced obesity in male mice is associated with reduced fertility and potentiation of acrylamide-induced reproductive toxicity. *Biology of reproduction* 82(1): 96–104. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2802115&tool=pmcentrez&rendertype=abstract.

Gibson RS (2005) Anthropometric Assessment. *Principles of nutritional assessment*. 2nd edition. New York: Oxford University Press:6-283.

Hammond KA and Litchford MD (2012) 'Nutrition Assessment: Clinical: Inflammation,

Physical, and Functional Assessments',in Mahan KL, Escott-Stump S and Raymond J.L., *Krause's Food and the Nutrition Care Process*,13th edition, Philadelphia: W.B Saunders Company: 169,470.

Hammoud AO (2006) Obesity and Male Reproductive Potential. *Journal of Andrology* 27(5): 619–626.

Hammoud AO, Gibson M, Peterson CM, Meikle a. W and Carrell DT (2008a) Impact of male obesity on infertility: a critical review of the current literature. *Fertility and Sterility* 90(4): 897–904.

Hammoud AO, Wilde N, Gibson M, Parks A, Carrell DT and Meikle AW (2008b) Male obesity and alteration in sperm parameters. *Fertility and sterility*. Elsevier Ltd 90(6): 2222–5. Available at: http://www.sciencedirect.com/science/article/pii/S0015028207037752.

Hingorjo MR, Qureshi MA and Mehdi A (2012) Neck circumference as a useful marker of obesity: a comparison with body mass index and waist circumference. *JPMA. The Journal of the Pakistan Medical Association* 62(1): 36–40. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22352099.

Inhorn MC and Patrizio P (2015) Infertility around the globe: new thinking on gender, reproductive technologies and global movements in the 21st century. *Human Reproduction Update* 21(4): 411–426. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25801630.

Jensen T, Andersson A, Jørgensen N, Andersen A, Carlsen E, Petersen J and Skakkebaek N (2004) Body mass index in relation to semen quality and reproductive hormones among 1,558 Danish men. *Fertility and sterility* 82(4): 863–70. Available at: http://www.sciencedirect.com/science/article/pii/S0015028204022241.

Jensen TK, Jacobsen R, Christensen K, Nielsen NC and Bostofte E (2009) Good semen quality and life expectancy: A cohort study of 43,277 men. *American Journal of Epidemiology* 170(5): 559–565.

Jensen TK, Sobotka T, Hansen M a, Pedersen AT, Lutz W and Skakkebaek NE (2008) Declining trends in conception rates in recent birth cohorts of native Danish women: a possible role of

deteriorating male reproductive health. *International journal of andrology* 31(2): 81–92. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2440321&tool=pmcentrez&rendertype=abstract.

Jetté M, Sidney K and Blümchen G (1990) Metabolic equivalents (METS) in exercise testing, exercise prescription, and evaluation of functional capacity. *Clinical Cardiology* 13(8): 555–565.

Jung a. and Schuppe HC (2007) Influence of genital heat stress on semen quality in humans. *Andrologia* 39(6): 203–215.

Kasturi SS, Tannir J and Brannigan RE (2008) The metabolic syndrome and male infertility. *Journal of andrology* 29(3): 251–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18222914.

Kay VJ and Barratt CLR (2009) Male obesity: impact on fertility. *The British Journal of Diabetes & Vascular Disease* 9(5): 237–241. Available at: http://dvd.sagepub.com/cgi/doi/10.1177/1474651409343132.

Krausz C (2011) Male infertility: pathogenesis and clinical diagnosis. *Best practice & research. Clinical endocrinology & metabolism*. Elsevier Ltd 25(2): 271–85. Available at: http://www.sciencedirect.com/science/article/pii/S1521690X10000916.

Lambert E, Bohlmann T and Kolbe-Alexander T (2001) 'Be active' - Physical activity for health in South Africa. *South African Journal of Clinical Nutrition* 14(3): S12–S16.

Lean ME, Han TS and Morrison CE (1995) Waist circumference as a measure for indicating need for weight management. *BMJ (Clinical research ed.)* 311(6998): 158–161.

Lee RD and Nieman DC. (2013) *Nutritional Assessment*, 6th edition, New York: McGraw-Hill:168,170,185,186.

MacDonald a a, Herbison GP, Showell M and Farquhar CM (2010) The impact of body mass index on semen parameters and reproductive hormones in human males: a systematic review with meta-analysis. *Human reproduction update* 16(3): 293–311. Available at:

http://www.ncbi.nlm.nih.gov/pubmed/19889752.

Magnusdottir E V, Thorsteinsson T, Thorsteinsdottir S, Heimisdottir M and Olafsdottir K (2005) Persistent organochlorines, sedentary occupation, obesity and human male subfertility. *Human reproduction (Oxford, England)* 20(1): 208–15. Available at: http://humrep.oxfordjournals.org/content/20/1/208.short.

Martini AC, Tissera A, Estofán D, Molina RI, Mangeaud A, de Cuneo MF and Ruiz RD (2010) Overweight and seminal quality: a study of 794 patients. *Fertility and sterility* 94(5): 1739–43. Available at: http://www.sciencedirect.com/science/article/pii/S0015028209040461.

Mascarenhas MN, Flaxman SR, Boerma T, Vanderpoel S and Stevens G a. (2012) National, Regional, and Global Trends in Infertility Prevalence Since 1990: A Systematic Analysis of 277 Health Surveys. *PLoS Medicine* 9(12): 1–12.

NHANES (2007) National Health and Nutrition Examinatory Survey. Anthropometry Procedures Manual. 1–1–5–16. Available at: http://www.cdc.gov/nchs/data/nhanes/nhanes_07_08/manual_an.pdf.

Ombelet W, Cooke I, Dyer S, Serour G and Devroey P (2008) Infertility and the provision of infertility medical services in developing countries. *Human reproduction update* 14(6): 605–21. Available at: http://humupd.oxfordjournals.org/content/14/6/605.

Petraglia F, Serour GI and Chapron C (2013) The changing prevalence of infertility. *International Journal of Gynecology and Obstetrics*. International Federation of Gynecology and Obstetrics 123(SUPPL. 2): S4–S8. Available at: http://dx.doi.org/10.1016/j.ijgo.2013.09.005.

Du Plessis SS, Cabler S, McAlister D a, Sabanegh E and Agarwal A (2010) The effect of obesity on sperm disorders and male infertility. *Nature reviews. Urology* 7(3): 153–161. Available at: http://dx.doi.org/10.1038/nrurol.2010.6.

Povey a. C, Clyma J a., McNamee R, Moore HD, Baillie H, Pacey a. a. and Cherry NM (2012) Modifiable and non-modifiable risk factors for poor semen quality: A case-referent study. *Human Reproduction* 27(9): 2799–2806.

Preis SR, Massaro JM, Hoffmann U, D'Agostino RB, Levy D, Robins SJ, Meigs JB, Vasan RS, O'Donnell CJ and Fox CS (2010) Neck circumference as a novel measure of cardiometabolic risk: the Framingham Heart study. *The Journal of clinical endocrinology and metabolism* 95(8): 3701–10. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2913042&tool=pmcentrez&rendertype=abstract.

Ramlau-Hansen CH, Nohr EA, Thulstrup AM, Bonde JP, Storgaard L and Olsen J (2007) Is maternal obesity related to semen quality in the male offspring? A pilot study. *Human reproduction (Oxford, England)* 22(10): 2758–2762. Available at: http://www.scopus.com/inward/record.url?eid=2-s2.0-34548745728&partnerID=tZOtx3y1.

Ricciardi R, Metter EJ, Cavanaugh EW, Ghambaryan A and Talbot L a (2009) Predicting cardiovascular risk using measures of regional and total body fat. *Applied nursing research : ANR*. Elsevier Inc. 22(1): 2–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19171289.

Saka M, Türker P, Ercan A, Kiziltan G and Baş M (2014) Is neck circumference measurement an indicator for abdominal obesity? A pilot study on Turkish Adults. *African health sciences* 14(3): 570–575. Available at: http://www.scopus.com/inward/record.url?eid=2-s2.0-84906924509&partnerID=tZOtx3y1.

Sermondade N, Faure C, Fezeu L, Shayeb a. G, Bonde JP, Jensen TK, Van Wely M, Cao J, Martini a. C, Eskandar M, Chavarro JE, Koloszar S, Twigt JM, Ramlau-Hansen CH, Borges E, Lotti F, Steegers-Theunissen RPM, Zorn B, Polotsky a. J, La Vignera S, Eskenazi B, Tremellen K, Magnusdottir E V., Fejes I, Hercberg S, Lévy R and Czernichow S (2012) BMI in relation to sperm count: An updated systematic review and collaborative meta-analysis. *Human Reproduction Update* 19(3): 221–231.

Sharpe RM (2010) Environmental/lifestyle effects on spermatogenesis. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 365(1546): 1697–1712.

Tsao C-W, Liu C-Y, Chou Y-C, Cha T-L, Chen S-C and Hsu C-Y (2015) Exploration of the Association between Obesity and Semen Quality in a 7630 Male Population. *PloS one* 10(3):

e0119458. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4379020&tool=pmcentrez&rendertype=abstract.

USDA (2010) *Dietary guidelines for Americans*. . Available at: http://www.health.gov/dietaryguidelines/dga2010/DietaryGuidelines2010.pdf.

Vaamonde D, Da Silva-Grigoletto ME, Garcia-Manso JM, Barrera N and Vaamonde-Lemos R (2012) Physically active men show better semen parameters and hormone values than sedentary men. *European Journal of Applied Physiology* 112(9): 3267–3273. Available at: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=medl&AN=22234399\nhttp://openurl.ac.uk/athens:_edu//lfp/LinkFinderPlus/Display?sid=OVID:Ovid+MEDLINE%28R%29&id=pmid:22234399&id=10.1007%2Fs00421-011-2304-6&issn=1439-6319&isbn=&volume=11.

Vaamonde D, Da Silva-Grigoletto ME, García-Manso JM, Vaamonde-Lemos R, Swanson RJ and Oehninger SC (2009) Response of semen parameters to three training modalities. *Fertility and sterility* 92(6): 1941–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19013565 (accessed 02/12/15).

WHO (2000) Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organization Technical Report Series*, i–xii, 1–253. Available at: http://libdoc.who.int/trs/WHO_TRS_894.pdf\nhttp://www.ncbi.nlm.nih.gov/pubmed/11234459.

WHO (2010) Examination and processing of human semen. *Online* Fifth edit(10): 286. Available at: http://whqlibdoc.who.int/publications/2010/9789241547789_eng.pdf.

WHO (2011) Waist Circumference and Waist-Hip Ratio Report of a WHO Expert Consultation. , 8–11.

World Health Organization (WHO). 2006. BMI classification: Global Database on Body Mass Index. Available at: http://apps.who.int/bmi/index.jsp?introPage=intro_3.html. (Accessed: 24 November 2014).

World Health Organization (WHO). 2015. Obesity and overweight. WHO Fact Sheet N°311.

Available at: http://www.who.int/mediacentre/factsheets/fs311/en/. (Accessed: 22 June 2015).

Winters SJ, Wang C, Abdelrahaman E, Hadeed V, Dyky MA and Brufsky A (2006) Inhibin-B levels in healthy young adult men and prepubertal boys: is obesity the cause for the contemporary decline in sperm count because of fewer Sertoli cells? *Journal of andrology* 27(4): 560–564. Available at: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=16582411&retmode=ref&cmd=prlinks.

Yang GR, Yuan S, Fu H, Wan G, Zhu L, Bu X, Zhang J, Du X, Li Y, Ji Y, Gu X and Li Y (2010) Neck Circumference Positively Related With Central Obesity, Overweight, and Metabolic Syndrome in Chinese Subjects With Type 2 Diabetes: Beijing Community Diabetes Study 4. *Diabetes Care* 33(11): 2465–2467.

Zhang E, Zhang H, Zang Z, Chen J and Zhang B (2014) Association of Body Mass Index with Semen Quality and Sexual Hormone Levels among Men in Intrauterine Insemination. (July): 1861–1865.

Chapter 6 EFFECT OF NUTRIENT SUPPLEMENTATION ON ANTHROPOMETRY AND SEMEN PARAMETERS IN HEALTHY MALES

Data in this chapter are presented in an article format, reporting on the findings according to the third to fourth objective set for this study. The main focus was to compare semen parameters from baseline to three months after nutrient supplementation. This article is prepared according to the author's guidelines (Addendum H) of the South African Journal of Obstetrics and Gynaecology. Referencing as required by the Department of Nutrition and Dietetics, University of the Free State is used.

6.1 Abstract

Background: Nutrition play an important role in the development of the reproductive system, as well as in spermatogenesis and sperm maturation. Antioxidants protect sperm from oxidative damage and several nutrients and antioxidants have been implicated in male fertility.

Methods: An intervention study on a sample of 50 apparently healthy volunteers, between the ages of 18 and 45 years was approved by the Health Research Ethics Committee from the Faculty of Health Sciences, University of the Free State. Data collected included age, various anthropometric measurements and semen parameters. Participants were randomized in two groups, one receiving micro-nutrient and omega-3 supplements and the other a placebo over a period of three months.

Results: Mean age was 24 years. No statistically significant difference in means of anthropometric measurements between the two groups and between baseline and post-intervention was observed. After the intervention period, the only statistical significant difference in means was an improvement in the mean number of sperm with normal forms in the supplement group when compared to the placebo group (p=0.05).

Conclusions: Supplementation with multi-vitamin-mineral and omega-3 supplements did not significantly influence anthropometric measures in a young group of apparently healthy men. However, after three months of supplementation, sperm morphology differed significantly

between the placebo and supplement group, suggesting a possible role for nutrient supplementation to improve sperm morphology.

Keywords: Nutrient supplementation, anthropometric measures, sperm parameters

6.2 Introduction

Various nutrients are involved in the development of the reproductive system, spermatogenesis and sperm maturation (Cheah and Yang 2011:182). Antioxidants protect sperm from oxidative damage (Agarwal et al 2008c:2, Cheah and Yang 2011:182, Sikka 2004:5) and several nutrients and antioxidants have been proven of value to improve male fertility (Agarwal and Sekhon 2010:217, Begum et al 2009:16).

Vitamin C supports normal functioning of the male reproductive system, genetic integrity of sperm (Begum et al 2009:17, Cheah and Yang 2011:186) and protects cells from oxidative stress (Cheah and Yang 2011:196, Gallagher 2012:88). According to Mínguez-Alarcón et al. (2012:2807,2813), the present recommendations for vitamin C intake may not be sufficient to benefit semen quality. Folate, Vitamin B_{12} and zinc are involved in the synthesis of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) (Begum et al 2009:17, Gallagher 2012:86, Wong et al 2000:435).

Vitamin A is needed for spermatogenesis (Gallagher 2012:60), while vitamin E is involved in the functioning of reproductive system (Gallagher 2012:113) and protects cell membranes from oxidative degradation from reactive oxygen species (ROS) (Gallagher 2012:70,72, Suleiman et al 1996:530). Vitamin E can also restore incomplete acrosomal membranes which are needed for successful fertilization (Cheah and Yang 2011:194).

Vitamin E and selenium may reinforce each other in their antioxidant function to reduce oxidative damage (Gallagher 2012:120). Selenium is an ultra-trace element, which acts as a potent antioxidant and is essential to human health (Beckett and Arthur 2005:455, Gallagher 2012:117,120, Rayman 2012:1256,1257).

Zinc, a trace mineral, is indispensable for normal functioning of the male reproductive system, including spermatogenesis, androgen production, proliferative activity of germ cells and capacitation of sperm (Begum et al 2009:17, Tikkiwal et al 1987:30,33, Wong et al 2000:435).

An inadequate intake of zinc may impede testicle development and may contribute to termination of spermatogenesis (Cheah and Yang 2011:183). Zinc deficiency has been linked to decreased spermatogenesis and impaired male fertility in sub-fertile males (Wong et al 2000:435) and may be linked to decreased sperm count, progressive motility and number of normal sperm in oligospermic males (Tikkiwal et al 1987:30,32).

Zinc and selenium are needed for morphological integrity of sperm, particularly midpiece formation (Cheah and Yang 2011:194). If midpiece formation is abnormal, the connection of the sperm head and tail to this piece will be affected, which in turn will negatively impact on fertilization (Cheah and Yang 2011:194).

6.3 Objectives

The main objective of this study was to investigate the effect of micronutrient and omega-3 supplementation on semen parameters by comparing semen parameters before and after nutrient supplementation.

6.4 Methods

6.4.1 Research design, sample size and ethical considerations

An intervention study to examine the effect of micronutrient and omega-3 supplementation on anthropometric measures and sperm parameters, was conducted on a sample of 50 apparently healthy men, between the ages of 18 and 45 years. Volunteers were recruited by means of advertisements and personal canvassing on the campus of the University of the Free State (UFS) and the South African National Defence Force. The study protocol was approved by the Health Research Ethics Committee from the Faculty of Health Sciences, (UFS) (ECUFS nr 08/2014). Written informed consent was obtained from all participants, who were informed that participation was voluntary.

6.4.2 Data collection

Age, anthropometric measurements and semen parameters were measured before and after intervention. Anthropometric measures included weight and height to calculate body mass index (BMI), as well as neck-, waist- and hip circumference to calculate waist-to-hip ratio

(WHR) and waist-to-height ratio (WHrR). All anthropometric measurements were taken by the researcher in a private area (WHO 2005:3–3–4).

A platform electronic Soehnle Professional scale was used for weight measurements and a Seca stadiometer for height measurements. The stadiometer could measure a height to a maximum of two meters. Participants were weighed and measured without outer clothing and shoes (Gibson 2005:247,253, Lee and Nieman 2013:168,186, NHANES 2007:3–3,3–7 – 3–9). Two measurements were taken in direct succession, and if weight measures differed with more than 100g or height more than 0.1cm (Lee and Nieman 2013:168,170) a third measurement was taken and the average calculated. Before each weighing session, a known weight was used to calibrate the scale. Standard measuring techniques were used (Gibson 2005:247,253, Lee and Nieman 2013:168,170; NHANES 2007:3–3,3–7 – 3–9).

A BMI lower than 18.5 kg/m^2 indicated underweight, between 18.5 kg/m^2 and 24.9 kg/m^2 normal weight, 25 kg/$m^2$to 29.9 kg/m^2 overweight and 30 kg/m^2 or more obesity (WHO 2006:Online).

All neck, waist and hip measurements were taken with a non-stretch, Seca 201 tape measure. If two measurements taken in succession differed with more than 0.1cm a third measurement was taken and the average calculated. Neck circumference was measured in the midway of the neck between the mid cervical spine and mid anterior neck, to the nearest 0.1cm (Aswathappa et al 2013:29, Ben-Noun and Laor 2006:15, Ben-Noun et al 2001:473). Participants stood upright, head erect with the shoulders not hunched in a relaxed position, and looked straight ahead towards the researcher (Aswathappa et al 2013:29, Ben-Noun and Laor 2006:15, Ben-Noun et al 2001:473). The measuring tape was placed just below the laryngeal prominence (Aswathappa et al 2013:29, Ben-Noun et al 2001:473, Hingorjo et al 2012:37) parallel with the floor, with the tape in front at the same height as the tape at the back of the neck (Hingorjo et al 2012:37). The trapezius muscles were not included in the measurement of the neck circumference (Aswathappa et al 2013:29). A neck circumference of more and equal to 38 cm indicates overweight.

Waist circumference was measured, with the participant standing upright, feet close together, arms at the side, body weight evenly distributed with the abdominal muscles

relaxed (Lee and Nieman 2013:186, WHO 2011:21). The measurement was taken around the midpoint, between the top of the iliac crest and lower margin of the last palpable rib in the mid axillary line within 0.1cm (Lean et al 1995:159, Lee and Nieman 2013:186, WHO 2005:20). The measurement was taken at the end of a normal expiration, with the tape wrapped snugly around the waist, without constricting the waist, at a level parallel to the floor (Lee and Nieman 2013:186,NHANES 2007:3–16, WHO 2011:20).

A waist circumference of equal and more than 102cm is considered as a high risk and an independent risk factor for metabolic complications and chronic disease (Alberti et al 2009:1642, Hammond and Litchford 2012:169, WHO 2000:11).

Hip circumference was measured around the widest portion of the buttocks, with the tape in a horizontal plane while the participant stood upright, arms at the side, feet together, with weight evenly distributed (Gibson 2005:281, WHO 2011:20,21). The tape did not indent the soft tissue (Gibson 2005:281,WHO 2011:20).

To evaluate WHR, a ratio of more than one were used as cut-off value to indicate risk for lifestyle diseases (Hammond and Litchford 2012:470, Lee and Nieman 2013:185). For WHtR, the universal cut-off value of 0.5 for males of different ethnic groups were applied (Ashwell et al 2012:284, Ashwell and Hsieh 2005:303–305).

Two semen samples were collected, three days apart. Participants were requested to ejaculate and then abstain from ejaculation for three days before providing the first sample and again before the second sample. Samples were obtained in a private room and transported to a near-by laboratory within 30 minutes of collection. Semen analysis was done by the Department of Obstetrics and Gynaecology, UFS and included semen volume, sperm concentration, quantitative and qualitative motility, sperm -morphology, as well as pH. Semen and sperm parameters were assessed according to the World Health Organisation (WHO) reference values (WHO 2010:224). Mean values of each of the two samples were used and reported to ensure validity.

Participants were randomized into two groups, using randomization tables, one group receiving nutrient supplements (vitamin-mineral and omega-3) and the other receiving a placebo. The vitamin-mineral supplement used was Centrum® Adult and the omega-3

supplement was Centrum® My Nutrients™ Omega-3 (Pfizer South Africa (Pty) Ltd). The placebo consisted of glucose and fructose. Participants were instructed to take the vitamin mineral supplement twice daily and one Omega-3 mini-gel in the morning and two Omega-3 mini-gels in the evening. The placebo group were also instructed to take the placebo twice daily. The multi-vitamin mineral supplement provided the following nutrients per day: 7 000 IU vitamin A, 120 mg vitamin C, 2 000 IU vitamin D, 60 IU vitamin E, 50 µg vitamin K, 3 mg thiamin, 3.4 mg riboflavin, 40 mg niacin, 4 mg vitamin B_6, 800 µg folic acid, 12 µg vitamin B_{12}, 60 µg biotin, 20 mg panthothenic acid, 400 mg calcium, 36 mg iron, 40 mg phosphorus, 300 µg iodine, 100 mg magnesium, 22 mg zinc, 110 µg selenium, 1 mg copper, 4.6 mg manganese, 70 µg chromium, 90 µg molybdenum, 144 mg chloride, 160 mg potassium, 10 µg nickel, 4 mg silicon, 20 µg tin and 20 µg vanadium. The omega-3 supplement provided 660 mg docosahexaenoic acid (EPA) and 330 mg docosahexaenoic acid (DHA) per day. Supplements or placebos were taken for a period of 90 days, to allow for the period of 72-74 days that sperm takes to develop; and an added 12 days for final maturation of sperm (Iammarrone et al 2003:214–216, Wong et al 2000:440, 2002:492). Compliance was encouraged by requesting participants to return empty containers after the intervention period. Participants were also encouraged to maintain their habitual lifestyle during the intervention period.

6.4.3 Statistical analysis

Data were captured on two separate Microsoft Excel (2010) for Windows7 spread sheets and checked electronically. Data were statistically analysed using SAS/STAT software, version 12.4 of the SAS system for Windows (© 2010 SAS Institute Inc.). Descriptive statistics were used to describe the sample and included frequencies and percentages. For continuous data means and standard deviations or medians with ranges and confidence intervals were calculated, as appropriate. Chi-square and Fisher's exact tests as appropriate were used to test for significance of associations and two tailed Pearson's or Spearman's correlations to determine associations between variables. Analysis of variance were used to test for differences in means. Statistical significance was set at a p-value of 0.05 or less.

6.5 Results

The median age of the sample was 24, ranging between 18 and 43 years. Anthropometric measures for the supplementation and control group before and after intervention are indicated in Table 6.1.

Table 6.1 Mean anthropometric measures at baseline and after three months of nutrient supplementation

	Baseline							Post-intervention							
	Placebo group			Supplement group			Difference in means between placebo and supplement groups (A)	Placebo group			Supplement group			Difference in means between placebo and supplement groups (B)	Difference in means (C = B-A)
	N	%	Mean ±SD	n	%	Mean ±SD	*p*-value	n	%	Mean ±SD	n	%	Mean ±SD	*p*-value	*p*-value
Weight (kg)	23	46	83.9 16.5	27	54	90.8 16.7	0.15	23	46	84.2 16.0	27	54	90.4 16.0	0.18	0.34
Height (cm)	23	46	178.3 8.1	27	54	180.3 8.3	0.40	23	46	178.3 8.0	27	54	180.3 8.3	0.40	0.72
BMI (kg/m^2)	23	46	26.3 4.7	27	54	28.0 5.5	0.24	23	46	23.4 4.5	27	54	28.0 5.4	0.28	0.49
Neck circumference (cm)	23	46	38.6 2.3	27	54	39.2 2.3	0.38	23	46	38.7 2.2	27	54	39.0 2.4	0.66	0.23
Waist circumference (cm)	23	46	88.6 12.7	27	54	92.4 14.9	0.35	23	46	88.5 12.4	27	54	91.5 13.8	0.42	0.45
Hip circumference (cm)	23	46	103.3 7.8	27	54	107.3 9.3	0.12	23	46	103.5 7.8	27	54	106.6 8.2	0.18	0.32
Waist-to-hip ratio	23	46	0.9 0.1	27	54	0.9 0.1	0.92	23	46	0.9 0.1	27	54	0.9 0.1	0.93	0.98
Waist-to-height ratio	23	46	0.5 0.1	27	54	0.5 0.1	0.49	23	46	0.5 0.1	27	54	0.5 0.1	0.58	0.50

Mean weight for the placebo group (Table 6.1) at baseline was 83.9±16.5 kg and post-intervention 84.2±16.0 kg and mean height was 178.3±8.1 cm. Mean BMI at baseline was 26.3±4.7 kg/m^2 and remained approximately the same at 26.4±4.5 kg/m^2 post-intervention. Neck circumference for the placebo group at baseline was 38.6±2.3 cm and post-intervention 38.7±2.2 cm. Mean waist circumference for this group varied very little between baseline (88.6±12.7 cm) and post-intervention (88.5±12.4 cm). The small variation was also observed in hip circumference of the placebo group at baseline (103.3±7.8 cm) and post-intervention (103.5±7.8 cm). In the placebo group mean WHR (0.9±0.1) and WHtR (0.5±0.1) remained the same at baseline and after three months.

In the supplement group (Table 6.1) the mean weight at baseline (90.8±16.7 kg) differed very slightly from the post-intervention weight (90.4±15.9 kg). Mean height was 180.3±8.3 cm. Mean BMI for the supplement group at baseline (28.0±5.5 kg/m^2) was about the same as post-intervention (28.0±5.4 kg/m^2). Neck circumference for the group at baseline (39.2±2.3 cm) was similar to the post-intervention circumference (39.0±2.5 cm). Mean waist circumference for the supplement group was 92.4±14.9 cm at baseline and 91.5±13.8 cm post-intervention, while hip circumference from baseline to post-intervention varied between 107.3±9.3 cm and 106.6±8.2 cm. In this group mean WHR (0.9±0.1) and WHtR (0.5±0.1) also remained the same. No statistically significant differences in anthropometric measures were observed between the placebo and supplement group at baseline and after intervention, as well when the difference in means was compared.

The results of semen analysis before and after nutrient supplementation are presented in Table 6.2.

Table 6.2 Mean semen parameters at baseline and after three months of nutrient supplementation

	Baseline							Post-intervention							
Variable	**Placebo group)**			**Supplement group**			**Difference in means between placebo and supplement groups (A)**	**Placebo group**			**Supplement group**			**Difference in means between placebo and supplement groups (B)**	**Comparison of difference in means (C = B-A)**
	n	**%**	**Mean (±SD)**	**n**	**%**	**Mean (±SD)**	***p*-value**	**n**	**%**	**Mean (±SD)**	**n**	**%**	**Mean (±SD)**	***p*-value**	***p*-value**
Semen volume (ml)	23	46	2.7 (1.0))	27	54	3.0 (1.5)	0.42	23	46	2.9 (0.8)	27	54	3.0 (1.3)	0.71	0.43
Sperm concentration (10^6 per ml)	23	46	41.1 (30.5)	27	54	42.8 (28.6)	0.84	23	46	38.5 (29.7)	27	54	42.5 (29.2)	0.63	0.74
Sperm total motility (%)	23	46	58.4 (15.7)	27	54	61.5 (12.1)	0.43	23	46	55.1 (14.6)	27	54	55.7 (13.5)	0.87	0.56
Progressive motility (PR) (%)	23	46	43.4 (14.7)	27	54	45.1 (11.4)	0.63	22	44	41.2 (11.9)	27	54	42.1 (11.2)	0.78	0.82
Non-progressive motility (NP) (%)	23	46	29.2 (7.4)	27	54	31.9 (11.3)	0.33	22	44	32.9 (8.5)	27	54	31.5 (8.0)	0.55	0.14
Immotility (IM) (%)	23	46	27.3 (10.0)	27	54	22.2 (7.7)	0.05*	22	44	25.7 (8.5)	27	54	25.7 (5.3)	0.96	0.11
Sperm morphology (normal forms %)	22	44	11.4 (4.0)	26	52	11.0 (3.2)	0.68	22	44	10.9 (4.3)	24	48	12.5 (2.6)	0.13	0.05*
pH	23	46	8.2 (0.3)	27	54	8.2 (0.4)	0.47	22	44	8.2 (0.3)	27	54	8.1 (0.3)	0.18	0.14

*p=≤0.05

Evaluated according to the WHO criteria in WHO laboratory manual for the Examination and processing of human semen. 2010. 5th Edition, Switzerland, 2010:224.

As indicated in Table 6.2, the two groups were comparable with regard to semen parameters, with the exception of a difference in the percentage of immotile sperm (p=0.05), between the placebo group and supplement group at baseline. After intervention, no significant difference in means for any of the sperm parameters were found between the placebo and supplement groups. When comparing the means of the two groups from baseline to post-intervention, a statistical significant improvement was noted in the mean number of sperm with normal forms when compared to the placebo group (p=0.05).

6.6 Discussion

From baseline to post intervention, no statistically significant changes were observed for body weight, BMI, neck circumference, waist circumference, hip circumference, WHR or WHtR. As participants were requested to maintain their habitual lifestyle and eating habits; and because a multi-vitamin mineral supplement and an omega-3 supplement, with a very small energy contribution were used, no changes in mean anthropometric measurements due to the intervention were expected.

Vitamin- and minerals have shown to improve semen parameters in sub-fertile and infertile males. Vitamin C supplementation in infertile men may increase sperm count and sperm motility, and may improve sperm morphology and thus be used to improve semen parameters (Akmal et al, 2006:440). Vitamin C and vitamin E supplementation showed a significant reduction in sperm DNA damage in males attending an infertility clinic (Greco et al 2005a:349, Greco et al 2005b:2590). This reduction in sperm DNA damage was also seen with vitamin C, vitamin E, β-carotene, zinc and selenium supplementation, but an unforeseen negative result, an increase in sperm decondensation, was reported (Ménézo et al 2007:418,419). A combination of vitamin C, vitamin E and carnitine is used in the treatment of male infertility (Agarwal and Sekhon 2010:217). Sperm motility significantly improved and sperm malondialdehyde concentration was reduced with an intake of vitamin E and selenium compared to a placebo in a wide age range (Keskes-Ammar et al 2003:83,86). A significant reduction in MDA concentration was also found after supplementation with vitamin E on its own in asthenospermic males (Suleiman et al 1996:530,533). Miller et al (2005:37) however stated that the daily intake of vitamin E should not exceed 400 IU/day, as a meta-analysis has shown that higher dosages have been linked to all-cause mortality.

Folic acid combined with zinc sulphate in subfertile males significantly increased sperm concentration, but also caused a small increase in percentage abnormal sperm, despite normal blood folic acid or zinc levels (Wong et al 2002:491,496). Selenium and/ or N-acetyl-cysteine supplementation improved semen parameters significantly in males with idiopathic oligo-asthenoteratospermia (Safarinejad and Safarinejad 2009:741,745).

Vitamins have also been used as supplement in healthy males. Vitamin C intake of more than the RDA in healthy males is linked to sperm number and concentration (Eskenazi et al 2005:1006,1010), therefore the present recommendations of vitamin C intake may not be sufficient to be of benefit to semen quality (Mínguez-Alarcón et al 2012:2807,2813). Folic acid combined with zinc sulphate in healthy males significantly increased sperm concentration, but also caused a small increase (4%) in abnormal sperm, despite normal blood folic acid or zinc levels (Wong et al 2002:491,496). As vitamin A is needed for spermatogenesis (Gallagher 2012:60), an intake of β-carotene in healthy men showed a positive correlation with sperm concentration and progressive motility (Eskenazi et al 2005:1006,1010).

Although supplementation with various micronutrients (individual or combinations of a few nutrients) have shown a positive effect on semen parameters in other studies (Agarwal and Sekhon 2010:217, Begum et al 2009:16), multi-vitamin mineral supplementation within the limits of the RDA and UL in this study failed to show an effect on most semen parameters. A significant mean improvement from baseline to post-intervention however was shown for the percentage of sperm with normal morphology (p=0.05).

6.7 Conclusion

In this study, supplementation with a multi-vitamin mineral and an omega-3 supplement did not have a significant influence on anthropometric measures of a young group of apparently healthy males. After a three month period of nutrient supplementation however, the only variable that changes significantly and only in the supplement group was mean sperm morphology, suggesting that nutrient supplementation might have the potential to improve sperm morphology.

6.8 Limitations

The sensitivity of obtaining semen samples and limited resources were the main limitations for this study, which had a negative effect on the number of participants volunteering to participate in the study and honouring their appointments. Electronic advertisements and personal recruitment were used to recruit volunteers and participants were reminded of their appointments by means of text messages.

6.9 Acknowledgements

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6.10 References

Agarwal A, Makker K and Sharma R (2008c) Clinical relevance of oxidative stress in male factor infertility: An update. *American Journal of Reproductive Immunology* 59(1): 2–11.

Agarwal A and Sekhon LH (2010) The role of antioxidant therapy in the treatment of male infertility. *Human fertility (Cambridge, England)* 13(4): 217–225.

Alberti KGMM, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato K a., Fruchart JC, James WPT, Loria CM and Smith SC (2009) Harmonizing the metabolic syndrome: A joint interim statement of the international diabetes federation task force on epidemiology and prevention; National heart, lung, and blood institute; American heart association; World heart federation; International . *Circulation* 120(16): 1640–1645.

Ashwell M, Gunn P and Gibson S (2012) Waist-to-height ratio is a better screening tool than waist circumference and BMI for adult cardiometabolic risk factors: systematic review and meta-analysis. *Obesity reviews : an official journal of the International Association for the Study of Obesity* 13(3): 275–86. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22106927.

Ashwell M and Hsieh SD (2005) Six reasons why the waist-to-height ratio is a rapid and

effective global indicator for health risks of obesity and how its use could simplify the international public health message on obesity. *International journal of food sciences and nutrition* 56(5): 303–307.

Aswathappa J, Garg S, Kutty K and Shankar V (2013) Neck circumference as an anthropometric measure of obesity in diabetics. *North American Journal of Medical Sciences* 5(1): 28–31.

Beckett GJ and Arthur JR (2005) Selenium and endocrine systems. *Journal of Endocrinology* 184(3): 455–465.

Begum H, Moniruddin ABM and Nahar K (2009) REVIEW ARTICLES ENVIRONMENTAL AND NUTRITIONAL ASPECT IN. 16–19.

Ben-Noun LL and Laor A (2006) Relationship between changes in neck circumference and cardiovascular risk factors. *Experimental and clinical cardiology* 11(1): 14–20. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2274839&tool=pmcentrez&rendertype=abstract.

Ben-Noun LL, Sohar E and Laor A (2001) Neck Circumference as a Simple Screening Measure for Identifying Overweight and Obese Patients. *Obesity Research* 9(8): 470–477. Available at: http://doi.wiley.com/10.1038/oby.2001.61.

Cheah Y and Yang W (2011) Functions of essential nutrition for high quality spermatogenesis. *Advances in Bioscience and Biotechnology* 02(04): 182–197.

Gallagher ML (2012) Intake: The Nutrients and Their Metabolism, In: Mahan KL, Escott-Stump S and Raymond J.L., *Krause's Food and the Nutrition Care Process*,13th edition, Philadelphia: W.B Saunders Company: 462-484.

Gibson RS. (2005) Anthropometric Assessment. *Principles of nutritional assessment*. 2nd edition. New York: Oxford University Press:6-283.

Hammond KA and Litchford MD (2012) 'Nutrition Assessment: Clinical: Inflammation, Physical, and Functional Assessments',in Mahan KL, Escott-Stump S and Raymond J.L., *Krause's Food and the Nutrition Care Process*,13th edition, Philadelphia: W.B Saunders

Company: 169,470.

Hingorjo MR, Qureshi MA and Mehdi A (2012) Neck circumference as a useful marker of obesity: a comparison with body mass index and waist circumference. *JPMA. The Journal of the Pakistan Medical Association* 62(1): 36–40. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22352099.

Iammarrone E, Balet R, Lower a M, Gillott C and Grudzinskas JG (2003) Male infertility. *Best practice & research. Clinical obstetrics & gynaecology* 17(2): 211–229.

Lean ME, Han TS and Morrison CE (1995) Waist circumference as a measure for indicating need for weight management. *BMJ (Clinical research ed.)* 311(6998): 158–161.

Lee RD and Nieman DC. (2013) *Nutritional Assessment*, 6th edition, New York: McGraw-Hill:168,170,185,186.

Mínguez-Alarcón L, Mendiola J, López-Espín JJ, Sarabia-Cos L, Vivero-Salmerón G, Vioque J, Navarrete-Muñoz EM and Torres-Cantero AM (2012) Dietary intake of antioxidant nutrients is associated with semen quality in young university students. *Human reproduction (Oxford, England)* 27(9): 2807–14. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22752607.

NHANES (2007) National Health and Nutrition Examinatory Survey. Anthropometry Procedures Manual. 1–1–5–16. Available at: http://www.cdc.gov/nchs/data/nhanes/nhanes_07_08/manual_an.pdf.

Rayman MP (2012) Selenium and human health. *The Lancet*. Elsevier Ltd 379(9822): 1256–1268. Available at: http://dx.doi.org/10.1016/S0140-6736(11)61452-9.

Sikka SC (2004) Role of oxidative stress and antioxidants in andrology and assisted reproductive technology. *J Androl* 25(1): 5–18. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14662779.

Suleiman SA, Eamin Ali M, Zaki ZMS, El-Malik EM and Nasr MA (1996) Lipid peroxidation and human sperm motility: protective role of vitamin E. *Journal of Andrology* 17(5): 530–537. Available at: http://onlinelibrary.wiley.com/doi/10.1002/j.1939-4640.1996.tb01830.x/abstract.

Tikkiwal M, Ajmera RL and Mathur NK. 1987. Effect of zinc administration on seminal zinc and fertility of oligospermic males. *Indian journal of physiology and pharmacology* 31(1): 30–4. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3666872 (accessed 20/01/16).

WHO (2000) Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organization Technical Report Series*, i–xii, 1–253. Available at: http://libdoc.who.int/trs/WHO_TRS_894.pdf\nhttp://www.ncbi.nlm.nih.gov/pubmed/11234459.

WHO (2005) The WHO STEPwise approach to chronic disease risk factor surveillance. *WHO STEPS Surveillance Manual*.

World Health Organization (WHO). 2006. BMI classification: Global Database on Body Mass Index. Available at: http://apps.who.int/bmi/index.jsp?introPage=intro_3.html. (Accessed: 24 November 2014).

WHO (2010) Examination and processing of human semen. *Online* Fifth edit(10): 286. Available at: http://whqlibdoc.who.int/publications/2010/9789241547789_eng.pdf.

WHO (2011) Waist Circumference and Waist-Hip Ratio Report of a WHO Expert Consultation. , 8–11.

Wong WY, Merkus HMW., Thomas CM., Menkveld R, Zielhuis GA and Steegers-Theunissen RP. (2002) Effects of folic acid and zinc sulfate on male factor subfertility: a double-blind, randomized, placebo-controlled trial. *Fertility and Sterility* 77(3): 491–498. Available at: http://www.sciencedirect.com/science/article/pii/S0015028201032290.

Wong WY, Thomas CM., Merkus JMW., Zielhuis GA and Steegers-Theunissen RP. (2000) Male factor subfertility: possible causes and the impact of nutritional factors. *Fertility and Sterility* 73(3): 435–442. Available at: http://www.sciencedirect.com/science/article/pii/S0015028299005518.

Chapter 7 EFFECT OF OMEGA-3 SUPPLEMENTATION ON SEMEN PARAMETERS OF HEALTHY MALES

According to the third to fourth objective of this study, this article will discuss the effect of omega-3 supplementation on semen parameters and fatty acid composition of semen of participants in this study sample. The article is prepared according to the author's instructions of Andrologia (Addendum I) but referencing is done according to requirements of the Department of Nutrition and Dietetics, University of the Free State.

7.1 Summary

The study investigated the effects of multivitamin mineral and omega-3 supplements on semen parameters and fatty acid composition of semen. Fifty apparently healthy men participated in a 90 day randomized intervention trial with a daily treatment of 660 mg EPA and 330 mg DHA in combination with a vitamin-mineral supplement, or placebo consisting of glucose and fructose. Before and after intervention semen samples were obtained to determine semen volume, sperm concentration, qualitative and quantitative sperm motility, sperm morphology, pH and fatty acid composition. Supplementation did not influence seminal fatty acid composition, but showed a statistically significant improvement in the mean percentage sperm with normal forms (p=0.05). The lack of effect, could possibly be ascribed to the choice of healthy volunteers recruited for inclusion in the study. The slight trends observed in this relatively small study sample, encourages more research in this area. Follow-up studies on omega-3 supplementation should focus on sub-fertile males, using a larger sample size over a longer intervention period and providing a larger dose of omega-3.

7.2 Introduction

Lipids form part of the structure of sperm membranes and are involved in the cell metabolism and functional activity of sperm (Mandal et al 2014:1, Safarinejad et al 2010:46, Tavilani et al 2007:45, Wathes et al 2007:190). Sperm have a high polyunsaturated fatty acid (PUFA) content (Hammadeh et al 2009:87, Ménézo et al 2007:418), especially omega-3 (Docosahexaenoic acid (DHA; 22:6n-3) (Conquer et al 1999:793,798). PUFA's have important antioxidant functions (Showell et al 2012:5) and one of the important functions of PUFA's in the cell membrane is to sustain the properties of the lipid bilayer (Farooqui et al 2000:3). The

fatty acid composition and amount of PUFA's in sperm seem to determine the physiological features, morphology and function of the cells (Lenzi et al 2000:230, Safarinejad et al 2010:103). The lipids in the sperm membrane help to maintain the fluidity and flexibility of sperm, and are thus necessary for successful fertilization (Aksoy et al 2006:75, Safarinejad et al 2010:100,101, Wathes et al 2007:190,197).

Omega-3 and omega-6 fatty acids are subgroups of PUFA's (DeFilippis and Sperling 2006:564,565) and the number indicates the placement of the first double bond from the methyl end of the chain (Gallagher 2012:41). A significant link is described between sperm and serum DHA levels and sperm concentration, -motility, -morphology and antioxidant activity of seminal plasma (Aksoy et al 2006:75,79, Safarinejad et al 2010:104).

Fatty acid composition of sperm may be used as a significant predictor of fertility (Aksoy et al 2006:75,77, Safarinejad et al 2010:100). Infertile males and sub-fertile males have lower concentrations of DHA in sperm, seminal plasma and blood serum (Aksoy et al 2006:75,77, Conquer et al 1999:795,798, Safarinejad et al 2010:100,104). Males with asthenospermia, oligospermia and oligoasthenospermia have significantly higher serum omega-6 : omega-3 ratios (Aksoy et al 2006:75,77, Conquer et al 1999:793,795,798, Safarinejad et al 2010:100,104) and a lower omega-3 index [eicosapentaenoic acid (EPA) + DHA] (Safarinejad et al 2010:100,104) than normospermic males. Lower concentrations of PUFAs and higher concentrations of saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) are found in sperm and seminal plasma of males with asthenospermia, oligospermia and/or oligoasthenospermia compared to normospermic males (Aksoy et al 2006:75,77, Conquer et al 1999:793,798, Tavilani et al 2007:45,48,49). Small variations in fatty acid concentration and composition could have a negative impact on sperm (Tavilani et al 2007:49). It has been suggested that supplementation with omega-3 could improve semen quality in males with oligospermia, asthenospermia (Aksoy et al 2006:75,79) or idiopathic oligoasthenoteratospermia (Safarinejad et al 2010:104).

Due to the outstanding safety profile of omega-3 fatty acid supplements, it has been suggested that it can be used as a nutraceutical to improve semen quality (Safarinejad et al 2010:101). Fish oil supplements seem to be well tolerated with few side-effects other than taste perversion and eructation (Safarinejad et al 2010:45) and are seen as a harmless and

easy way to accomplish adequate omega-3 fatty acid intake (Opperman 2013:10). However, according to Wathes et al (2007:198) PUFA's act as a two-edged sword. PUFA's are vital for optimal cell function, but high levels are potentially detrimental. The high levels of PUFAs needed for fluidity of sperm and eventual fertilization, makes sperm more susceptible to attack by Reactive Oxygen Species (ROS). The amount of PUFA's that should be consumed for optimum fertility therefore remains unclear (Wathes et al 2007:190,198).

The effect of diets high in omega-6 and low in omega-3 fatty acids on semen quality and sperm function needs additional investigation (Safarinejad et al 2010:105). Limited data are available on omega-3 intake through fish consumption in the Free State province, South Africa. However, Tydeman-Edwards (2012:141) reported that the frequency of fish consumption in the Free State does not meet the recommended intake of two times per week, indicating that intake of fatty fish amongst males in the Free State is unlikely to meet recommendations. For this reason, the aim of this study was to determine the effect of omega-3 supplementation on semen parameters.

7.3 Subjects and methods

7.3.1 Study population and ethical considerations

This study formed part of a larger study, examining the impact of micronutrient supplementation on sperm parameters. An intervention study was undertaken in a study sample of 50 apparently healthy men, between the ages of 18 and 45 years. Volunteers were recruited by means of advertisements and personal canvassing on the campus of the University of the Free State (UFS) and the South African National Defence Force. Males between 18 and 45 years that reacted on advertisements were included. Participants were excluded from the study it they had been treated for cancer, had previous testes surgery, and/or used a dietary supplement on a daily basis during the previous three months. The Health Research Ethics Committee from the Faculty of Health Sciences, UFS (ECUFS nr 08/2014) approved the research proposal. Written informed consent was obtained from all participants, who were informed that participation was voluntary and that they could withdraw from the study at any stage.

7.3.2 Data collection

Data collected for this part of the study included age, anthropometric measurements, semen parameters and fatty acid composition of intact semen.

Birth date to calculate age was the only demographic variable obtained. Anthropometric measures included weight and height. All anthropometric measurements were taken by the researcher in a private area (WHO 2005:3–3–4). Participants were measured without outer clothing and shoes (Gibson 2005:247,253, Lee and Nieman 2013:168,186, NHANES 2007:3–3,3–7 – 3–9).

Weight and height measurements were taken using standard measuring techniques (Gibson 2005:247,253, Lee and Nieman 2013:168,170,186, NHANES 2007:3–3,3–7 – 3–9) with a platform electronic Soehnle Professional scale and Seca stadiometer with a maximum height of two meters. All measurements were taken in direct succession, and if two weight measurements differed with more than 100g or two height measurements more than 0.1cm (Lee and Nieman 2013:168,170) a third measurement was taken. The average of the measurements was calculated. Before each weighing session a known weight was used to calibrate the scale.

Weight and height were used to calculate body mass index (BMI) in kg/m^2. A BMI lower than 18.5 kg/m^2 indicated underweight, between 18.5 kg/m^2 and 24.9 kg/m^2 normal weight, 25 $kg/m^2$to 29.9 kg/m^2 overweight and 30 kg/m^2 or more obesity (WHO 2006:Online).

Two semen samples were collected from participants, three days apart. Participants were requested ejaculate and then to abstain from ejaculation for three days before providing the first sample and again before the second sample. Samples were obtained in a private room and transported to a near-by laboratory within 30 minutes of collection. Semen analysis was done by the Department of Obstetrics and Gynaecology, UFS and included semen volume, sperm concentration, quantitative and qualitative motility, sperm morphology, as well as pH. Semen and sperm parameters were assessed according to the World Health Organisation (WHO) reference values (WHO 2010:224). Mean values of each of the two samples were used and reported to improve validity. Fatty acid composition of semen was determined by the

Department of Microbial, Biochemical and Food Biotechnology, UFS, before and after the intervention period.

The method of Folch et al (1957:497–500) was used to quantitatively extract total lipids from semen samples (± 500 μL). Chloroform and methanol were used in a ratio of 2:1 (Folch et al 1957:497–500). A concentration of 0.001% butylated hydroxytoluenen (an antioxidant) was added to the chloroform : methanol mixture (Folch et al 1957:497–500). The fat extracts was dried in a rotary evaporator under vacuum (Folch et al 1957:497–500). The extracts were then dried overnight in a vacuum oven at 50°C (Folch et al 1957:497–500). Phosphorus pentoxide was used as moisture adsorbent (Folch et al 1957:497–500). The extracted fat was stored in a polytop (glass vial, with push-in top) under a blanket of nitrogen and frozen at –20°C until analysis was done (Folch et al 1957:497–500).

Approximately 10 mg of total lipids obtained was transferred into a Teflon-lined screw-top test tube by means of a disposable glass pasteur pipette. Fatty acids were transesterified to form methyl esters using 0.5 N NaOH in methanol and 14% boron trifluoride in methanol (Park and Goins 1994:1262–1263). Fatty acid methyl esters were quantified using a Varian GX 3400 flame ionization GC, with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm ID, 0.2 μm film thickness) (Park and Goins 1994:1262–1263). Column temperature was 40–230°C (hold 2 minutes; 4°C/minute; hold 10 minutes). Fatty acid methyl esters in hexane (1 μl) were injected into the column using a Varian 8200 CX Autosampler with a split ratio of 100:1 (Park and Goins 1994:1262–1263). The injection port and detector were both maintained at 250°C (Park and Goins 1994:1262–1263). Hydrogen, at 45 psi, functioned as the carrier gas, while nitrogen was employed as the makeup gas. Varian Star Chromatography Software recorded the chromatograms (Park and Goins 1994:1262–1263). Fatty acid methyl ester samples were identified by comparing the relative retention times of FAME peaks from samples with those of standards obtained from SIGMA (189-19). Fatty acids were reported as the relative percentage of each individual fatty acid as a percentage of the total of all fatty acids present in the sample. Fatty acid combinations and ratios were calculated by using the fatty acid data.

Using randomization tables, participants were allocated to two groups, one group receiving nutrient supplements (omega-3 and vitamin-mineral) and the other receiving a placebo.

Although participants were randomly assigned to groups, final group sizes differed as a result of participants not providing all the semen samples/ withdrawing from the study. The vitamin-mineral supplement used was Centrum® Adult and the omega-3 supplement was Centrum® My Nutrients™ Omega 3 (Pfizer South Africa (Pty) Ltd). The placebo contained glucose and fructose, without fatty acids. Participants were requested to take the vitamin mineral supplement twice daily, as well as one Omega-3 mini-gel in the morning and two Omega-3 mini-gels in the evening. The total EPA intake was 660 mg and DHA intake was 330 mg per day. The placebo group was instructed to take the placebo twice daily. Supplements or placebos were taken for a period of 90 days, as sperm takes 72-74 days to develop; and an additional 12 days were allowed for final maturation of sperm (Iammarrone et al 2003:214–216, Wong et al 2000:440, 2002:492). Compliance was encouraged by requesting participants to return empty containers after the intervention period. Participants were also encouraged to maintain their habitual lifestyle during the intervention period.

7.3.3 Statistical analysis

Data were captured on two separate Microsoft Excel (2010) for Windows7 spread sheets and checked electronically for verification. Data were statistically analysed using SAS/STAT software, version 12.4 of the SAS system for Windows (© 2010 SAS Institute Inc.). Descriptive statistics were used to describe the sample and included frequencies and percentages. For continuous data means and standard deviations or medians with ranges and confidence intervals were calculated, as appropriate. Chi-square tests and Fisher's exact tests were used to test for significance of associations and two tailed Pearson's or Spearman's correlations were used to determine associations between variables. Student t-tests and analysis of variance were used to test for differences in means. Statistical significance was set at a p-value of <0.05.

7.4 Results

The age of the sample ranged between 18 and 43 years with a median age of 24 years. Median weight at baseline and post-intervention was the same (83.4 kg) and median height was 180.0 cm. BMI for the sample ranged between 19.5 and 42.5kg/m2 at baseline; and between 19.5 and 40.1kg/m^2 after the intervention period. The mean change in BMI was not statistically

significant (p=0.49). According to BMI classification 34% was overweight and 26% obese at baseline compared to 30% being overweight and 28% obese after the intervention period. The fatty acid composition of semen at baseline and after nutrient and omega-3 supplementation is summarized in Table 7.1 and 7.2.

Table 7.1 Fatty acid composition of baseline semen parameters

Fatty acid	Structure	Placebo					Supplement					
		n	Median	Range	Mean	±SD	n	Median	Range	Mean	±SD	Difference in means between placebo and supplement groups (A)
												p-value
Capric	C10:0	21	0.6	0.0-0.7	0.5	0.2	27	0.6	0.0-0.7	0.5	0.1	0.98
Lauric	C12:0	21	1.1	0.4-1.3	1.0	0.2	27	1.0	0.3-1.2	1.0	0.2	0.52
Myristic	C14:0	21	1.2	1.0-2.3	1.3	0.3	27	1.1	1.0-1.5	1.2	0.1	0.16
Myristoleic	C14:1c9	21	1.0	0.0-2.3	1.0	1.0	27	1.7	0.0-2.3	1.4	0.8	0.20
Pentadecylic	C15:0	21	0.5	0.0-0.8	0.5	0.2	27	0.5	0.0-0.8	0.4	0.2	0.60
Palmitic	C16:0	21	57.3	50.6-60.1	56.9	2.2	27	56.8	53.5-61.3	56.9	2.0	0.88
Stearic	C18:0	21	34.6	25.8-37.7	34.0	2.7	27	34.5	31.4-36.4	34.4	1.3	0.48
Elaidic	C18:1t9	21	0.0	0.0-1.0	0.2	0.3	27	0.0	0.0-1.3	0.3	0.5	0.20
Oleic	C18:1c9	21	1.5	0.5-6.8	2.0	1.5	27	1.4	0.5-3.4	1.6	0.8	0.22
Vaccenic	C18:1c7	21	0.1	0.0-0.8	0.2	0.2	27	0.1	0.0-0.5	0.1	0.2	0.34
Linoleic (LA)	C18:2c9,12 (n-6)	21	0.2	0.0-1.9	0.4	0.5	27	0.2	0.0-1.2	0.3	0.3	0.43
Arachidic (AA)	C20:0	21	0.0	0.0-1.2	0.3	0.5	27	0.0	0.0-1.2	0.3	0.5	0.98
γ-Linolenic	C18:3c6,9,12 (n-6)	21	0.2	0.0-0.3	0.2	0.1	27	0.2	0.0-0.3	0.3	0.1	0.82
α-Linolenic (ALA)	C18:3c9,12,15 (n-3)	21	0.0	0.0-04	0.0	0.1	27	0.0	0.0-0.2	0.0	0.0	0.59
Eicosadienoic	C20:2c11,14 (n-6)	21	0.0	0.0-0.1	0.0	0.0	27	0.0	0.0	0.0	0.0	0.11
Behenic	C22:0	21	0.0	0.0-0.5	0.1	0.1	27	0.0	0.0-1.4	0.1	0.3	0.93
Eicosatrienoic (DGLA)	C20:3c8,11,14 (n-6)	21	0.3	0.0-0.7	0.4	0.1	27	0.3	0.0-0.6	0.3	0.1	0.49
Erucic	C22:1c13	21	0.0	0.0-0.6	0.1	0.2	27	0.1	0.0-0.7	0.1	0.2	0.41
Eicosatrienoic (ETE)	C20:3c11,14,17 (n-3)	21	0.2	0.1-0.8	0.2	0.1	27	0.2	0.0-0.3	0.2	0.1	0.24
Arachidonic (ARA)	C20:4c5,8,11,14 (n-6)	21	0.2	0.0-1.2	0.3	0.3	27	0.2	0.0-0.7	0.2	0.2	0.59
Eicosopentaenoic (EPA)	C20:5c5,8,11,14,17 (n-3)	21	0.0	0.0	0.0	0.0	27	0.0	0.0	0.0	0.0	-
Lignoceric	C24:0	21	0.0	0.0-0.3	0.0	0.1	27	0.0	0.0-0.5	0.0	0.1	0.85
Nervonic	C24:1c15	21	0.0	0.0-0.1	0.0	0.0	27	0.0	0.0-0.2	0.0	0.0	0.64
Docosapentaenoic (DPA)	C22:5c7,10,13,16,19 (n-3)	21	0.0	0.0	0.0	0.0	27	0.0	0.0	0.0	0.0	-
Docosahexanoic (DHA)	C22:6c4,7,10,13,16,19 (n-3)	21	0.0	0.0-2.4	0.5	0.7	27	0.3	0.0-3.6	0.5	0.8	0.86
Total Fatty Acid content												
Total SFA	SFA	21	95.1	86.6-98.5	94.6	3.4	27	95.1	89.1-98.6	94.7	2.3	0.87
Total MUFA	MUFA	21	3.0	0.6-8.2	3.4	2.1	27	3.7	0.7-6.6	3.5	1.4	0.92
Total PUFA	PUFA	21	1.3	0.7-5.5	2.0	1.5	27	1.6	0.0-6.0	1.7	1.2	0.65
Total Omega-6 Fatty Acids	Total n-6	21	0.9	0.5-4.0	1.2	0.8	27	1.1	0.0-2.2	1.0	0.5	0.29
Total Omega-3 Fatty Acids	Total n-3	21	0.2	0.1-2.6	0.7	0.8	27	0.5	0.0-3.8	0.7	0.8	0.89
Fatty Acid Ratios												
SFA : PUFA	SFA : PUFA	21	75.5	15.8-138.0	73.0	38.6	26	60.7	14.8-150.4	73.0	38.6	0.93
Omega-6 : Omega-3 fatty acids	n-6 : n-3	21	2.8	0.7-8.5	3.0	2.0	26	2.0	0.4-8.8	2.3	1.7	0.20
AA : DHA		5	0.6	0.0-1.5	0.7	0.6	7	0.5	0.0-3.5	1.2	1.4	0.45

*p≤0.05

Table 7.2 Fatty acid composition of post-intervention semen parameters

		Post-intervention										
		Placebo					Supplement					
Fatty acid	**Structure**	**n**	**Median**	**Range**	**Mean**	**±SD**	**n**	**Median**	**Range**	**Mean**	**±SD**	**Difference in means between placebo and supplement groups (B)**	**Comparison of difference in means C=(B-A)**
												***p*-value**	***p*-value**
Capric	C10:0	21	0.5	0.0-0.6	0.4	0.2	27	0.5	0.3-0.6	0.5	0.1	0.05*	0.21
Lauric	C12:0	21	1.0	0.8-1.1	1.0	0.1	27	1.0	0.3-1.1	1.0	0.2	0.81	0.66
Myristic	C14:0	21	1.0	0.9-1.2	1.0	0.1	27	1.0	0.9-1.2	1.0	0.1	0.76	0.11
Myristoleic	C14:1c9	21	2.0	1.5-2.3	2.0	0.2	27	2.1	1.4-2.4	2.0	0.2	0.38	0.28
Pentadecylic	C15:0	21	0.0	0.0-0.6	0.2	0.2	27	0.5	0.0-0.7	0.4	0.3	0.04*	0.05
Palmitic	C16:0	21	56.4	50.5-58.3	55.6	1.9	27	56.6	51.8-59.1	56.1	1.7	0.43	0.49
Stearic acid	C18:0	21	34.1	30.8-36.1	33.8	1.2	27	34.3	30.9-38.4	34.2	1.4	0.30	0.97
Elaidic	C18:1t9	21	1.1	0.0-1.5	0.8	0.6	27	0.0	0.0-1.5	0.5	0.6	0.10	0.03*
Oleic	C18:1c9	21	1.3	0.5-4.6	1.9	1.2	27	1.5	0.3-3.8	1.6	0.9	0.42	0.68
Vaccenic	C18:1c7	21	0.2	0.0-0.8	0.2	0.2	27	0.2	0.0-0.7	0.2	0.2	0.50	0.75
Linoleic (LA)	C18:2c9,12 (n-6)	21	0.2	0.0-1.3	0.3	0.3	27	0.2	0.0-1.3	0.2	0.3	0.57	0.76
Arachidic (AA)	C20:0	21	1.2	0.0-1.5	0.9	0.6	27	1.1	0.0-1.7	0.7	0.6	0.27	0.36
γ-Linolenic	C18:3c6,9,12 (n-6)	21	0.2	0.0-0.3	0.2	0.1	27	0.2	0.1-0.2	0.2	0.0	0.20	0.37
α-Linolenic (ALA)	C18:3c9,12,15 (n-3)	21	0.0	0.0-0.4	0.0	0.1	27	0.0	0.0-0.2	0.0	0.1	0.65	0.97
Eicosadienoic	C20:2c11,14 (n-6)	21	0.0	0.0	0.0	0.0	27	0.0	0.0-0.1	0.0	0.0	0.38	0.07
Behenic	C22:0	21	0.0	0.0	0.0	0.0	27	0.0	0.0-0.3	0.0	0.1	0.38	0.92
Eicosatrienoic (DGLA)	C20:3c8,11,14 (n-6)	21	0.3	0.1-0.5	0.3	0.1	27	0.3	0.1-0.5	0.3	0.1	0.58	0.82
Erucic	C22:1c13	21	0.0	0.0-0.8	0.2	0.3	27	0.0	0.0-0.7	0.1	0.2	0.13	0.02*
Eicosatrienoic (ETE)	C20:3c11,14,17 (n-3)	21	0.3	0.1-0.4	0.3	0.1	27	0.3	0.2-0.4	0.3	0.1	0.53	0.22
Arachidonic (ARA)	C20:4c5,8,11,14 (n-6)	21	0.3	0.0-0.8	0.4	0.3	27	0.3	0.0-1.4	0.3	0.3	0.57	0.94
Eicosopentaenoic (EPA)	C20:5c5,8,11,14,17 (n-3)	21	0.0	0.0	0.0	0.0	27	0.0	0.0	0.0	0.0	-	-
Lignoceric	C24:0	21	0.0	0.0-0.2	0.0	0.1	27	0.0	0.0-0.4	0.0	0.1	0.58	0.46
Nervonic	C24:1c15	21	0.0	0.0	0.0	0.0	27	0.0	0.0-0.2	0.0	0.0	0.22	0.69

Fatty acid	Structure	n	Median	Range	Mean	±SD	n	Median	Range	Mean	±SD	Difference in means between placebo and supplement groups (B)	Comparison of difference in means C=(B-A)
Docosapentaenoic (DPA)	C22:5c7,10,13,16, 19 (n-3)	21	0.0	0.0	0.0	0.0	27	0.0	0.0	0.0	0.0	-	-
Docosahexanoic (DHA)	C22:6c4,7,10,13,1 6,19 (n-3)	21	0.3	0.0-2.9	0.6	0.9	27	0.0	0.0-3.8	0.4	0.8	0.52	0.41
Total Fatty Acid content													
Total SFA	SFA	21	94.0	85.2-96.3	93.0	2.6	27	94.5	86.7-96.4	93.9	2.2	0.22	0.42
Total MUFA	MUFA	21	4.6	2.8-8.5	5.0	1.3	27	4.2	2.8-6.3.7	4.4	1.2	0.1	0.26
Total PUFA	PUFA	21	1.5	0.8-6.3	2.0	1.4	27	1.4	0.7-7.0	1.7	1.3	0.53	0.88
Total Omega-6 Fatty Acids	Total n-6	21	0.9	0.4-2.8	1.1	0.6	27	0.8	0.3-2.9	1.0	0.6	0.59	0.59
Total Omega-3 Fatty Acids	Total n-3	21	0.5	0.2-3.4	0.9	0.9	27	0.4	0.2-4.1	0.7	0.8	0.54	0.43
Fatty Acid Ratios		21											
SFA : PUFA	SFA : PUFA	21	64.2	13.6-125.1	64.0	32.4	27	68.9	12.4-145.0	74.0	36.1	0.37	0.38
Omega-6 : Omega-3 fatty acids	n-6 : n-3	21	1.9	0.6-5.0	2.0	1.2	27	1.8	0.6-6.4	2.0	1.2	0.94	0.21
AA : DHA Ratio		11	1.5	0.0-4.5	2.0	1.6	11	2.4	0.0-4.1	2.2	1.4	0.76	0.44

*p≤0.05

As data were normally distributed, means will be used for comparisons. At baseline, no statistically significant difference was found between the fatty acid composition of the semen of the placebo and supplement groups (Table 7.1).

After supplementation (Table 7.2) the difference in means (capric acid) between placebo group and supplement group was statistically significant (p=0.05), post-intervention the mean pentadecyclic acid (pentadecanoic acid) for the placebo group was 0.2±0.2% while the mean for the supplement group was 0.4±0.3%, that was statistically significant (p=0.04). When the difference in means between placebo and supplement groups were compared after the intervention period, the differences for pentadecyclic acid (p=0.05), elaidic acid (p=0.03) and erucic acid (p=0.02) was statistically significant.

After supplementation mean seminal SFAs of the placebo group was 93.0±2.6% and supplement group was 93.9±2.2 % (Table 7.2). The mean PUFA's at baseline of the placebo group was 2.0±1.5% and post-intervention 1.7±1.3%. The mean difference for both, as well as the differences from baseline to post-intervention was however not statistically significant. No statistically significant differences for between baseline and post-intervention for DHA was found.

After 90 days of supplementation, the mean omega-6 : omega-3 ratio stayed the same 2.0:1 (Table 7.1, Table 7.2), but there was a reduction between baseline and post-intervention in the supplement group (2.3:1 to 1.2:1). This change was not statistically significant. No other statistically significant differences in the other fatty acids or ratios were observed between baseline and after supplementation.

Table 7.3 reflects the results of the mean semen parameters at baseline and after intervention.

Table 7.3 Semen parameters at baseline and post-intervention

	Baseline							Post-intervention							
Variable	**Placebo group)**			**Supplement group**			**Difference in means between placebo and supplement groups (A)**	**Placebo group**			**Supplement group**			**Difference in means between placebo and supplement groups (B)**	**Comparison of difference in means (C = B-A)**
	N	**%**	**Mean (±SD)**	**n**	**%**	**Mean (±SD)**	***p*-value**	**n**	**%**	**Mean (±SD)**	**n**	**%**	**Mean (±SD)**	***p*-value**	***p*-value**
Semen volume (ml)	23	46	2.7 (1.0))	27	54	3.0 (1.5)	0.42	23	46	2.9 (0.8)	27	54	3.0 (1.3)	0.71	0.43
Sperm concentration (10^6 per ml)	23	46	41.1 (30.5)	27	54	42.8 (28.6)	0.84	23	46	38.5 (29.7)	27	54	42.5 (29.2)	0.63	0.74
Sperm total motility (%)	23	46	58.4 (15.7)	27	54	61.5 (12.1)	0.43	23	46	55.1 (14.6)	27	54	55.7 (13.5)	0.87	0.56
Progressive motility (PR) (%)	23	46	43.4 (14.7)	27	54	45.1 (11.4)	0.63	22	44	41.2 (11.9)	27	54	42.1 (11.2)	0.78	0.82
Non-progressive motility (NP) (%)	23	46	29.2 (7.4)	27	54	31.9 (11.3)	0.33	22	44	32.9 (8.5)	27	54	31.5 (8.0)	0.55	0.14
Immotility (IM) (%)	23	46	27.3 (10.0)	27	54	22.2 (7.7)	0.05*	22	44	25.7 (8.5)	27	54	25.7 (5.3)	0.96	0.11
Sperm morphology (normal forms %)	22	44	11.4 (4.0)	26	52	11.0 (3.2)	0.68	22	44	10.9 (4.3)	24	48	12.5 (2.6)	0.13	0.05*
pH	23	46	8.2 (0.3)	27	54	8.2 (0.4)	0.47	22	44	8.2 (0.3)	27	54	8.1 (0.3)	0.18	0.14

*p=≤0.05

Evaluated according to the WHO criteria in WHO laboratory manual for the Examination and processing of human semen. 2010. 5th Edition, Switzerland, 2010:224.

At baseline (Table 7.3) there was a statistically significant difference between the placebo and supplement groups when comparing the mean percentage for immotility of sperm. After supplementation, the only statistically significant change was an improvement in the percentage of sperm with normal forms (sperm morphology) (p=0.05) in the supplement group.

Correlation of individual fatty acids and sperm concentration at baseline, showed a positive correlation between vaccenic acid (r=0.54, p=<0001), linoleic acid (r=0.57, p=<0001), (r=0.47, p=0.0007), arachidonic acid (r=0.52, p=0.0002), docosahexanoic acid (r=0.59, p=<0001), total omega-6 fatty acids (r=0.52, p=0.0002) and total omega-3 fatty acids (r=0.57, p=<0001). A negative correlation was found between sperm concentration and the total SFAs (r=-0.5, p=0.0003) as well as the saturated fatty acid, stearic acid (r=-0.43, p=0.0024).

In Table 7.4 the correlation between fatty acids and change in sperm concentration, sperm motility and sperm morphology in the placebo and supplement groups are indicated.

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Table 7.4 Correlation of seminal fatty acid content with semen parameters

	Variable	n	%	Total SFA %		Total PUFA %		Omega-6		Omega-3		SFA : PUFA		Omega-6 : Omega-3	
Baseline	**Placebo group**			r	*p*-value	R	*p*-value	r	*p*-value	R	*p*-value	r	*p*-value	r	*p*-value
	Sperm concentration (10^6 per ml)	21	42	-0.55	0.0093*	0.67	0.001*	0.55	0.01*	0.69	0.0006*	-0.64	0.0019*	-0.51	0.02*
	Sperm motility (%)	20	40	-0.07	0.77	0.20	0.39	0.07	0.75	0.30	0.18	-0.26	0.26	-0.31	0.18
	Sperm morphology (%)	21	42	-0.8	0.74	0.08	0.73	0.07	0.78	0.08	0.72	-0.01	0.96	0.15	0.52
	Supplement group														
	Sperm concentration (10^6 per ml)	26	52	-0.47	0.02*	0.55	0.0038*	0.54	0.004*	0.49	0.01*	-0.52	0.0075*	0.02	0.92
	Sperm motility (%)	26	52	-0.50	0.35	0.28	0.17	0.17	0.40	0.32	0.11	-0.32	0.12	-0.29	0.15
	Sperm morphology (%)	25	50	-0.42	0.04*	0.36	0.08	0.35	0.08	0.32	0.12	-0.52	0.0075*	-0.17	0.43
Post-intervention	**Placebo group**														
	Sperm concentration (10^6 per ml)	21	42	-0.36	0.10	0.40	0.7	0.25	0.28	0.46	0.04*	0.46	0.04*	-0.34	0.13
	Sperm motility (%)	21	42	-0.27	0.24	0.26	0.26	0.17	0.46	0.29	0.2	-0.21	0.36	-0.26	0.26
	Sperm morphology (%)	20	40	-0.18	0.45	0.12	03.61	0.11	0.63	0.11	0.63	-0.06	0.79	-0.01	0.98
	Supplement group														
	Sperm concentration (10^6 per ml)	26	52	-0.24	0.24	0.44	0.03*	0.21	0.30	0.56	0.0028*	-0.27	0.19	-0.50	0.0099*
	Sperm motility (%)	26	52	0.11	0.58	-0.14	0.48	-0.20	0.31	0.08	0.68	0.08	0.68	-0.13	0.52
	Sperm morphology (%)	23	46	-0.40	0.06	0.27	0.22	0.35	0.10	0.18	0.42	-0.16	0.46	0.07	0.75

*p<0.05

In correlation analysis, significant correlations for seminal fatty acid content was only found for sperm concentration, with negative correlations with SFA's (-0.55, p=0093), SFA's : PUFA's (r=-0.64, *p*=0.02) and omega 6 : omega (r=-0.51, *p*=0.02) for the placebo group at baseline. Positive correlations for the same group were found between semen concentration with PUFA's (r=0.55, p=0.01), omega-6 (r=0.55, *p*=0.01), omega-3 (r=0.69, *p*=0.0006). The same trend regarding negative and positive correlation was observed in the supplement group at baseline, the only exception being the omega-6 : omega-3 that was not statistically significant. Negative correlations were also found between sperm morphology in the supplement group at baseline with SFA's (r=-0.42, *p*=0.04) and SFA : PUFA (r=-0.52, *p*=0.0075).

After the intervention period positive correlations were found in the placebo group between sperm concentration with omega-3 and SFA : PUFA (r=0.46, *p*=0.04). Positive correlations in the supplement group were observed between sperm concentration with PUFA's (r=0.44, *p*=0.03) and omega-3 (r=0.56, *p*=0.0028), with a negative correlation between sperm concentration and omega-6 : 0mega-3 (r=-0.50, *p*=0.0099).Discussion

7.5 Discussion

This study included men of a relative young age (median 24 years) and therefore age-related deterioration in semen parameters was not expected (Eskenazi et al 2003:447, Levitas et al 2007:45, Ng et al 2004:1811, Pasqualotto et al 2005:1087). There was no statistically significant difference in body weight or BMI between baseline and post-intervention (reported elsewhere), leading to the assumption that participants maintained their lifestyle during the study period. Although approximately 60% of participants presented with an overweight/ obese BMI classification, expected higher lean muscle mass in this younger and physically active population (reported elsewhere) could also have contributed to their higher BMI (Kay and Barratt 2009:239).

After intervention an unexpected statistically significant difference in means for pentadecyclic, elaidic and erucic acid between the placebo and supplement groups were found. The reasons for these changes are difficult to explain. Pentadecyclic or pentadecanoic acid is a saturated fatty acid, while elaidic acid is a long chain trans fatty acid and erucic acid a very long chain fatty acid with a *cis*-isomer form (Torun 2006:1976). Elaidic acid is the most abundant trans fatty acid in the human diet (Nielsen et al 2013:Online) and dietary intake of

trans fatty acids in young men, has been inversely related to total sperm count (Chavarro et al 2014:429,434,437), while trans fatty acids in sperm has been inversely related to sperm concentration (Chavarro et al 2011:1796). Erucic acid is a typical component of *Brassicaceae* oils like mustard or rape seed oil (Wendlinger et al 2014:393). Animal research had indicated that erucic oil may contribute to myocardial lipidosis and heart lesions in rats and some countries regulate its presence in fats and oils (Wendlinger et al 2014:393). Mean capric and pentadecyclic acid levels differed significantly between the two groups from baseline to after-intervention, as a result of lower levels in the placebo group after intervention. Capric acid is a medium chain saturated fatty acid (Torun 2006:1976) and the lower levels over time cannot be explained.

Although a lower mean for SFA's and an increased mean for PUFA's was observed in the supplement group when compared to the placebo group after intervention, these differences were not statistically significant. Another study investigating omega-3 supplementation did not report on changes in SFA's and PUFA's (Safarinejad et al 2010:44). No statistically significant differences were found for omega-6 : omega-3 ratio or any of the other fatty acids or fatty acid ratios after supplementation.

The only sperm parameter that showed a significant improvement after supplementation, was the percentage of sperm with normal forms (morphology). Not all causes of sperm morphological abnormalities are clear, and the specific effect of these abnormalities on fertility potential of sperm also need more research (Auger et al 2015:10).

7.6 Conclusion and recommendations

Supplementing a healthy group of young men with an omega-3 and multi vitamin-mineral supplement over a period of 90 days did not influence the fatty acid composition of their semen, but showed an improvement in the percentage of sperm with normal forms. The lack of effect, when compared to other studies, could be ascribed to the choice of healthy volunteers recruited for inclusion in the study. The slight trends observed in this relatively small study sample, encourages more research in this area, especially in a sample with abnormal semen parameters. Follow-up studies should focus on sub-fertile males, attempt to

increase sample size, extend the period of intervention and possibly increase the dosage of omega-3 supplementation.

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7.8 References

Aksoy Y, Aksoy H, Altinkaynak K, Aydin HR and Ozkan A (2006) Sperm fatty acid composition in subfertile men. *Prostaglandins, leukotrienes, and essential fatty acids* 75(2): 75–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16893631.

Auger J, Jouannet P and Eustache F (2015) Another look at human sperm morphology. *Human Reproduction* 31(1): dev251. Available at: http://humrep.oxfordjournals.org/content/early/2015/10/14/humrep.dev251.abstract.

Chavarro JE, Mínguez-Alarcón L, Mendiola J, Cutillas-Tolín A, López-Espín JJ and Torres-Cantero AM (2014) Trans fatty acid intake is inversely related to total sperm count in young healthy men. *Human reproduction (Oxford, England)* 29(3): 429–40. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24419496.

Conquer J, Martin J, Tummon I, Watson L and Tekpetey F (1999) Fatty acid analysis of blood serum, seminal plasma, and spermatozoa of normozoospermic vs. Asthernozoospermic males. *Lipids* 34(8): 793–799.

DeFilippis AP and Sperling LS (2006) Understanding omega-3's. *American Heart Journal* 151(3): 564–570.

Eskenazi B, Wyrobek a. J, Sloter E, Kidd S a., Moore L, Young S and Moore D (2003) The association of age and semen quality in healthy men. *Human Reproduction* 18(2): 447–454.

Farooqui A a., Horrocks L a. and Farooqui T (2000) Glycerophospholipids in brain: Their

metabolism, incorporation into membranes, functions, and involvement in neurological disorders. *Chemistry and Physics of Lipids* 106: 1–29.

Folch J, Lees M and Stanley GHS (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem*, 497–509. Available at: http://www.jbc.org/content/226/1/497.short.

Gallagher ML (2012) Intake: The Nutrients and Their Metabolism, In: Mahan KL, Escott-Stump S and Raymond J.L., *Krause's Food and the Nutrition Care Process*,13th edition, Philadelphia: W.B Saunders Company: 462-484.

Gibson RS. 2005. Anthropometric Assessment. *Principles of nutritional assessment*. 2nd edition. New York: Oxford University Press:6-283.

Hammadeh ME, Filippos A A and Hamad MF (2009) Reactive oxygen species and antioxidant in seminal plasma and their impact on male fertility. *International Journal of Fertility and Sterility* 3(3): 87–110.

Iammarrone E, Balet R, Lower a M, Gillott C and Grudzinskas JG (2003) Male infertility. *Best practice & research. Clinical obstetrics & gynaecology* 17(2): 211–229.

Kay VJ and Barratt CLR (2009) Male obesity: impact on fertility. *The British Journal of Diabetes & Vascular Disease* 9(5): 237–241. Available at: http://dvd.sagepub.com/cgi/doi/10.1177/1474651409343132.

Lee RD and Nieman DC. (2013) *Nutritional Assessment*, 6th edition, New York: McGraw-Hill:168,170,186.

Lenzi a, Gandini L, Maresca V, Rago R, Sgrò P, Dondero F and Picardo M (2000) Fatty acid composition of spermatozoa and immature germ cells. *Molecular human reproduction* 6(3): 226–231.

Levitas E, Lunenfeld E, Weisz N, Friger M and Potashnik G (2007) Relationship between age and semen parameters in men with normal sperm concentration: Analysis of 6022 semen samples. *Andrologia* 39(2): 45–50.

Mandal R, Badyakar D and Chakrabarty J (2014) Role of Membrane Lipid Fatty Acids in Sperm Cryopreservation. *Advances in Andrology* 1–9.

Ménézo YJ, Hazout A, Panteix G, Robert F, Rollet J, Cohen-Bacrie P, Chapuis F, Clément P and Benkhalifa M (2007) Antioxidants to reduce sperm DNA fragmentation: an unexpected adverse effect. *Reproductive BioMedicine Online*. Reproductive Healthcare Ltd, Duck End Farm, Dry Drayton, Cambridge CB23 8DB, UK 14(4): 418–421. Available at: http://www.sciencedirect.com/science/article/pii/S1472648310608875.

Ng KK, Donat R, Chan L, Lalak a, Di Pierro I and Handelsman DJ (2004) Sperm output of older men. *Human reproduction (Oxford, England)* 19(8): 1811–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15218002.

NHANES (2007) National Health and Nutrition Examinatory Survey. Anthropometry Procedures Manual. 1–1–5–16. Available at: http://www.cdc.gov/nchs/data/nhanes/nhanes_07_08/manual_an.pdf.

Opperman M (2013) What health professionals should know about omega-3 fatty acid supplements. *South African Journal of Clinical Nutrition* 26(2): 6–11. Available at: http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L369868166\nhttp://reference.sabinet.co.za/webx/access/electronic_journals/m_sajcn/m_sajcn_v26_n2_a3.pdf\nhttp://sfx.ub.rug.nl:9003/sfx_local?sid=EMBASE&issn=16070658&id=doi:&atitle=.

Park P and Goins R (1994) In Situ Preparation of Fatty Acid Methyl Esters for Analysis of Fatty Acid Composition in Foods. *Journal of Food Science* 59(6): 1262–1266.

Pasqualotto FF, Sobreiro BP, Hallak J, Pasqualotto EB and Lucon AM (2005) Sperm concentration and normal sperm morphology decrease and follicle-stimulating hormone level increases with age. *BJU international* 96(7): 1087–91. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16225533.

Safarinejad MR, Hosseini SY, Dadkhah F and Asgari MA (2010) Relationship of omega-3 and omega-6 fatty acids with semen characteristics, and anti-oxidant status of seminal plasma: a comparison between fertile and infertile men. *Clinical nutrition (Edinburgh, Scotland)*.

Elsevier Ltd 29(1): 100–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19666200.

Showell M, Brown J, Yazdani A, Stankiewicz M and Hart R (2012) Antioxidants for male subfertility (Review). (3).

Tavilani H, Doosti M, Nourmohammadi I, Mahjub H, Vaisiraygani A, Salimi S and Hosseinipanah SM (2007) Lipid composition of spermatozoa in normozoospermic and asthenozoospermic males. *Prostaglandins Leukotrienes and Essential Fatty Acids* 77(1): 45–50.

Tydeman-Edwards R (2012) Obesity, undernutrition and the double burden of disease in the Free State. (July). Available at: http://etd.uovs.ac.za/ETD-db/theses/available/etd-11212012-093519/unrestricted/Tydeman-EdwardsR.pdf.

Vendel Nielsen L, Krogager TP, Young C, Ferreri C, Chatgilialoglu C, Nørregaard Jensen O and Enghild JJ (2013) Effects of Elaidic Acid on Lipid Metabolism in HepG2 Cells, Investigated by an Integrated Approach of Lipidomics, Transcriptomics and Proteomics. *PLoS ONE* 8(9).

Wathes DC, Abayasekara DRE and Aitken RJ (2007) Polyunsaturated fatty acids in male and female reproduction. *Biology of reproduction* 77(2): 190–201. Available at: http://www.biolreprod.org/content/77/2/190.long.

Wendlinger C, Hammann S and Vetter W (2014) Various concentrations of erucic acid in mustard oil and mustard. *Food Chemistry*. Elsevier Ltd 153: 393–397. Available at: http://dx.doi.org/10.1016/j.foodchem.2013.12.073.

WHO (2005) The WHO STEPwise approach to chronic disease risk factor surveillance. *WHO STEPS Surveillance Manual*.

WHO (2010) Examination and processing of human semen. *Online* Fifth edit(10): 286. Available at: http://whqlibdoc.who.int/publications/2010/9789241547789_eng.pdf.

World Health Organization (WHO). 2006. BMI classification: Global Database on Body Mass Index. Available at: http://apps.who.int/bmi/index.jsp?introPage=intro_3.html. (Accessed: 24 November 2014).

Wong WY, Merkus HMW., Thomas CM., Menkveld R, Zielhuis GA and Steegers-Theunissen

RP. (2002) Effects of folic acid and zinc sulfate on male factor subfertility: a double-blind, randomized, placebo-controlled trial. *Fertility and Sterility* 77(3): 491–498. Available at: http://www.sciencedirect.com/science/article/pii/S0015028201032290.

Wong WY, Thomas CM., Merkus JMW., Zielhuis GA and Steegers-Theunissen RP. (2000) Male factor subfertility: possible causes and the impact of nutritional factors. *Fertility and Sterility* 73(3): 435–442. Available at: http://www.sciencedirect.com/science/article/pii/S0015028299005518.

Chapter 8 CONCLUSIONS AND RECOMMENDATIONS

8.1 Introduction

The main aim of this intervention study was to determine the effect of micro-nutrient and omega-3 supplementation on semen parameters by evaluating semen parameters and fatty acid composition of intact semen at baseline and after three months of nutrient supplementation. The study also investigated the effect of age, environmental-, lifestyle-, anthropometric and dietary factors on semen parameters. This chapter provide conclusions and recommendations based on the findings of the study that are presented in chapter four to seven in the form of four articles.

8.2 Effect of age, environmental-, lifestyle-, anthropometric- and dietary factors on semen parameters.

A relation between aging and sperm concentration and motility are described in overweight men (Eskandar et al 2012:1–4) and between aging and semen parameters (Tsao et al 2015:Online). In this younger study sample however, no correlation was found between age and semen parameters, probably because age related changes are only expected at an older age (Dunson et al 2004:51; Stewart and Kim 2011:498,499).

According to BMI classification the majority of participants were classified as overweight/ obese. Expected higher lean muscle mass in this younger and physically active population could however contributed to their higher BMI. Measurements of neck circumference showed that a large percentage of young males can be classified as overweight/ obese. When categorizing anthropometric measures and semen parameters according to acknowledged cut-off values, none of the anthropometric measures showed an association with semen concentration, -motility or -morphology levels. In other studies, the number of sitting hours per day is positively linked to daytime scrotal temperatures, which affects semen quality (Jung and Schuppe 2007:203,205,212). In this study, a weak negative correlation was found between the number of hours sitting per day and sperm morphology. Most men in this study did not use laptops computers, but more than half of participants spent more than four hours per day using electronic devices connected to Wi-Fi on a daily basis. A significant association between using electronic devices connected to Wi-Fi for four hours or more per day and a

lower sperm motility was found. With regard to cellular phones, no statistically significant association between where the cellular phone is carried and sperm parameters were found. Although more than half of participants in this study took hot baths, no significant association was found between the use of hot baths and below reference limits for sperm parameters. More than a third of participants wore tight fitting underwear or trousers, which may also contribute to an elevation in scrotal temperature and consequently poor semen quality (Dohle et al 2005:709). However no association was found between wearing tight fitting clothing and poor sperm parameters. Activity levels also did not show an association with sperm parameters.

Gaskins et al (2012:1) stated that a prudent dietary pattern, rich in fruits, vegetables, chicken, fish and whole grains may be an economical and safe way to improve percentage progressively motile sperm. Although the intake of vegetables and fruit are poor when compared to the South African Dietary Guidelines, no association with poor semen quality was found. Alcohol intake of more than five units per week however was significantly associated with a reduction in sperm concentration.

Although vegetable and fruit intake did not impact significantly on sperm parameters in this sample, the low intake remains a reason for concern in terms of other health considerations, which should be addressed. With regard to alcohol intake, not only women should be warned about the potential dangers of alcohol, but men planning to have children should be encouraged to limit or avoid alcohol intake at least three months before conception.

This study provided novel information on semen in a South African setting. From these results it seems that anthropometric measures, activity and most dietary factors do not influence semen parameters in healthy, young males, except for an association between time spent using electronic devices connected to Wi-Fi and sperm motility and the effect of alcohol consumption on sperm concentration. The high incidence of overweight and obesity when interpreting BMI and neck circumference as well as the low intake of vegetable and fruit amongst participants is however concerning in terms of long term metabolic health.

8.3 Anthropometric measurements, physical activity and semen parameters of healthy male volunteers

More than half of participants were classified as being overweight /obese according to BMI. However when applying calculated BAI, only less than a quarter were categorised as overweight/ obese. This discrepancy in estimating body adiposity by using the two different calculations could partly be explained by the influence of muscle mass on BMI (Kay and Barratt 2009:239). The contribution of muscle mass could have influenced the interpretation of BMI in this study sample of young active volunteers, reporting mostly moderate or high activity levels. Neck circumference is also used as an indicator of upper body fat distribution to identify overweight and obesity (Adamu et al 2013:82, Ben-Noun et al 2001:470, Preis et al 2010:3701, Saka et al 2014:570). In this sample, almost half of participants had a neck circumference of 38 cm or more, representing overweight (Yang et al 2010:2465,2467). At baseline and after three months, median WHR and WHtR were however classified as normal which would not indicate an increased risk for lifestyle diseases (Lee and Nieman 2013:185, Hammond and Litchford 2012:470).

The majority of participants indicated that they are moderately to highly active and spend less than eight hours per day sitting during the week. The level of activity did not have a statistically significant effect on any of the semen parameters, but the number of hours spent sitting showed a significant association with sperm morphology. If a male is seated, air does not circulate effectively around the scrotum resulting in less effective cooling (Sharpe 2010:1703). The duration of sitting during a work day is positively linked to daytime scrotal temperatures which may have a negative effect on semen quality (Jung and Schuppe 2007:203,205,212, Magnusdottir et al 2005:205). Although activity levels did not significantly influence semen parameters in this study, it has been documented that different training modalities might have an effect on semen parameters, especially as intensity and volume of exercise increase (Vaamonde et al 2009:1941,1943).

This study provided novel information on semen parameters in relation to anthropometric measures and activity in a South African setting. From these results it seems that anthropometric measures and activity do not influence semen parameters in healthy, young males, except for a significant association between time spent sitting and sperm morphology.

The high incidence of overweight and obesity when interpreting BMI and larger neck circumference amongst participants remains an area of concern in terms of long term metabolic health.

The possible effect of sitting hours on sperm morphology should be further investigated and potential fathers will need to evaluate their time spent sitting in this age where technology demands many hours sitting.

8.4 Effect of nutrient supplementation on anthropometry and semen parameters in healthy males

In this study, supplementation with a multi-vitamin mineral and an omega-3 supplement did not have a significant influence on anthropometric measures of a young group of apparently healthy males. From baseline to post intervention, no statistically significant changes were found for body weight, BMI, neck circumference, waist circumference, hip circumference, WHR or WHtR. As participants were requested to maintain their habitual lifestyle and eating habits; and because a multi-vitamin mineral supplement and an omega-3 supplement, with a very small energy contribution were used, no changes in mean anthropometric measurements due to the intervention were expected.

Although supplementation with various micronutrients (individual or combinations of a few nutrients) have shown a positive effect on semen parameters in other studies, multi-vitamin mineral supplementation within the limits of the RDA and UL in this study failed to show an effect on most semen parameters. A significant mean improvement from baseline to post-intervention however was shown for the percentage of sperm with normal morphology in the group that received the nutrient supplementation, implicating that nutrient supplementation might have the potential to improve sperm morphology.

8.5 Effect of omega-3 supplementation on semen parameters of healthy males

Supplementing a healthy group of young men with a multi vitamin-mineral and omega-3 supplement over a period of 90 days did not influence the fatty acid composition of their semen, but showed an improvement in the percentage of sperm with normal forms. The lack

of effect, when compared to other studies, could be ascribed to the choice of healthy volunteers recruited for inclusion in the study.

8.6 Limitations of the study

The main challenge of this study was recruiting volunteers, due to the sensitivity of obtaining semen samples for analysis. This severely limited the number of volunteers willing to participate in this study. If more resources are available, it is recommended that remuneration be paid to ensure a larger sample. Other geographic areas in South Africa could also be included, as habitual food intake can differ considerably.

This study do not report a substantial effect on sperm parameters after nutrient supplementation. The lack of effect in this study could have been influenced by the sample size, choice of younger, healthy volunteers and the prudent supplementation regime. The researcher recommends that follow-up studies could focus on sub-fertile males, attempt to increase sample size, extend the period of intervention and increase the dosage of supplementation.

8.7 Research application

Although no significant effect was found on the influence of age, most environmental-, lifestyle-, anthropometric- and dietary factors on semen parameters, this study cautions about the effect of alcohol consumption, time spent sitting per day and the use of electronic devices connected to Wi-Fi on sperm parameters in a South African setting. Results from this study add value when tailoring health message to address sperm health and serve as baseline research in this field in order to optimise sperm parameters, which could influence the health of future generations.

References

Academy of Nutrition and Dietetics (2014) Position of the academy of nutrition and dietetics: Dietary fatty acids for healthy adults. *Journal of the Academy of Nutrition and Dietetics* 114(1): 136–153.

Adams J a., Galloway TS, Mondal D, Esteves SC and Mathews F (2014) Effect of mobile telephones on sperm quality: A systematic review and meta-analysis. *Environment International*. Elsevier B.V. 70: 106–112. Available at: http://dx.doi.org/10.1016/j.envint.2014.04.015.

Adamu L, Asuku A, Taura M, Tela I, Datti S and Imam A (2013) Neck circumference: An upcoming tool of adiposity indices. *Nigerian Journal of Basic Clinical Sciences* 10(2): 82–85.

Afeiche M, Williams PL, Mendiola J, Gaskins AJ, Jørgensen N, Swan SH and Chavarro JE (2013) Dairy food intake in relation to semen quality and reproductive hormone levels among physically active young men. *Human reproduction (Oxford, England)* 28(8): 2265–75. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3712661&tool=pmcentrez&rendertype=abstract.

Agarwal A, Bragais FM and Sabanegh E (2008a) Assessing sperm function. *The Urologic clinics of North America* 35(2): 157–171, vii. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18423237.

Agarwal A, Desai NR, Makker K, Varghese A, Mouradi R, Sabanegh E and Sharma R (2009) Effects of radiofrequency electromagnetic waves (RF-EMW) from cellular phones on human ejaculated semen: an in vitro pilot study. *Fertility and Sterility*. Elsevier Ltd 92(4): 1318–1325. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0015028208033566.

Agarwal A, Desai NR, Ruffoli R and Carpi A (2008b) Lifestyle and testicular dysfunction: a brief update. *Biomedicine & pharmacotherapy = Biomédecine & pharmacothérapie* 62(8): 550–3. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18771892.

Agarwal A, Makker K and Sharma R (2008c) Clinical relevance of oxidative stress in male factor infertility: An update. *American Journal of Reproductive Immunology* 59(1): 2–11.

Agarwal A and Said TM (2011) Interpretation of Basic Semen Analysisn and Advanced Semen Testing, In Sabanegh ES, *Male Fetility: Problems and Solutions*, New York:Springer:15-22.

Agarwal A and Sekhon LH (2010) The role of antioxidant therapy in the treatment of male infertility. *Human fertility (Cambridge, England)* 13(4): 217–225.

Agarwal A, Sharma RK, Nallella KP, Thomas AJ, Alvarez JG and Sikka SC (2006) Reactive oxygen species as an independent marker of male factor infertility. *Fertility and sterility* 86(4): 878–85.

Agarwal A, Singh A, Hamada A and Kesari K (2011) Cell phones and male infertility: A review of recent innovations in technology and consequences. *International Braz J Urol* 37(4): 432–454.

Aggerholm AS, Thulstrup AM, Toft G, Ramlau-Hansen CH and Bonde JP (2008) Is overweight a risk factor for reduced semen quality and altered serum sex hormone profile? *Fertility and sterility* 90(3): 619–26. Available at:

http://www.sciencedirect.com/science/article/pii/S0015028207015282.

Aitken RJ and Baker M a. (2006) Oxidative stress, sperm survival and fertility control. *Molecular and Cellular Endocrinology* 250(1-2): 66–69.

Akin Y, Gulmez H, Bozkurt A, Nuhoglu B and Usta MF (2014) Usage of neck circumference as novel indicator of erectile dysfunction: a pilot study in Turkish population. *Andrologia* 1–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24147908.

Akmal M, Qadri JQ, Al-Waili NS, Thangal S, Haq A and Saloom KY (2006) Improvement in human semen quality after oral supplementation of vitamin C. *Journal of medicinal food* 9(3): 440–442. Available at: http://online.liebertpub.com/doi/abs/10.1089/jmf.2006.9.440\npapers://a66046c0-dbb4-40a6-bbd3-742e9e241be6/Paper/p23061.

Aksoy Y, Aksoy H, Altinkaynak K, Aydin HR and Ozkan A (2006) Sperm fatty acid composition in subfertile men. *Prostaglandins, leukotrienes, and essential fatty acids* 75(2): 75–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16893631.

Al-Azemi M, Omu A, Fatinikun T, Mannazhath N and Abraham S (2009) Factors contributing to gender differences in serum retinol and α-tocopherol in infertile couples. *Reproductive BioMedicine Online*. Reproductive Healthcare Ltd, Duck End Farm, Dry Drayton, Cambridge CB23 8DB, UK 19(4): 583–590. Available at: http://linkinghub.elsevier.com/retrieve/pii/S147264830900008X.

Alberti KGMM, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato K a., Fruchart JC, James WPT, Loria CM and Smith SC (2009) Harmonizing the metabolic syndrome: A joint interim statement of the international diabetes federation task force on epidemiology and prevention; National heart, lung, and blood institute; American heart association; World heart federation; International . *Circulation* 120(16): 1640–1645.

Andersen AG, Jensen TK, Carlsen E, Jørgensen N, Andersson AM, Krarup T, Keiding N and Skakkebaek NE (2000) High frequency of sub-optimal semen quality in an unselected population of young men. *Human reproduction (Oxford, England)* 15(2): 366–72. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10655308.

Anderson K, Nisenblat V and Norman R (2010) Lifestyle factors in people seeking infertility treatment - A review. *The Australian & New Zealand journal of obstetrics & gynaecology* 50(1): 8–20.

Arterburn L, Hall E and Oken H (2006) Distribution, interconversion, and dose response of n− 3 fatty acids in humans. *The American journal of clinical …* 83. Available at: http://ajcn.nutrition.org/content/83/6/S1467.short.

Ashwell M, Gunn P and Gibson S (2012) Waist-to-height ratio is a better screening tool than waist circumference and BMI for adult cardiometabolic risk factors: systematic review and meta-analysis. *Obesity reviews : an official journal of the International Association for the Study of Obesity* 13(3): 275–86. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22106927.

Ashwell M and Hsieh SD (2005) Six reasons why the waist-to-height ratio is a rapid and effective global indicator for health risks of obesity and how its use could simplify the international public health message on obesity. *International journal of food sciences and*

nutrition 56(5): 303–307.

Aswathappa J, Garg S, Kutty K and Shankar V (2013) Neck circumference as an anthropometric measure of obesity in diabetics. *North American Journal of Medical Sciences* 5(1): 28–31.

Attaman JA, Toth TL, Furtado J, Campos H, Hauser R and Chavarro JE (2012) Dietary fat and semen quality among men attending a fertility clinic. *Human reproduction (Oxford, England)* 27(5): 1466–74. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3329193&tool=pmcentrez&rendertype=abstract.

Auger J, Jouannet P and Eustache F (2015) Another look at human sperm morphology. *Human Reproduction* 31(1): dev251. Available at: http://humrep.oxfordjournals.org/content/early/2015/10/14/humrep.dev251.abstract.

Avendaño C, Mata A, Sanchez Sarmiento CA and Doncel GF (2012) Use of laptop computers connected to internet through Wi-Fi decreases human sperm motility and increases sperm DNA fragmentation. *Fertility and sterility* 97(1): 39–45.e2. Available at: http://www.sciencedirect.com/science/article/pii/S0015028211026781.

Baker J, Germaine M, John E and Moysich K (2005) Fertility Patterns Prior to Testicular Cancer Diagnosis on JSTOR. *Cancer Causes and Control* 16: 295–299. Available at: http://www.jstor.org/stable/20069468?seq=1#page_scan_tab_contents (accessed 20/01/16).

Bakos HW, Mitchell M, Setchell BP and Lane M (2010) The effect of paternal diet-induced obesity on sperm function and fertilization in a mouse model. *International journal of andrology* 34(5 Pt 1): 402–10. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20649934.

Battista N, Pasquariello N, Di Tommaso M and Maccarrone M (2008) Interplay between endocannabinoids, steroids and cytokines in the control of human reproduction. *Journal of neuroendocrinology* 20 Suppl 1(12): 82–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18426505.

Beckett GJ and Arthur JR (2005) Selenium and endocrine systems. *Journal of Endocrinology* 184(3): 455–465.

Begum H, Moniruddin ABM and Nahar K (2009) REVIEW ARTICLES ENVIRONMENTAL AND NUTRITIONAL ASPECT IN. 16–19.

Ben-Noun LL and Laor A (2006) Relationship between changes in neck circumference and cardiovascular risk factors. *Experimental and clinical cardiology* 11(1): 14–20. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2274839&tool=pmcentrez&rendertype=abstract.

Ben-Noun LL, Sohar E and Laor A (2001) Neck Circumference as a Simple Screening Measure for Identifying Overweight and Obese Patients. *Obesity Research* 9(8): 470–477. Available at: http://doi.wiley.com/10.1038/oby.2001.61.

Bergman RN, Stefanovski D, Buchanan TA, Sumner AE, Reynolds JC, Sebring NG, Xiang AH and Watanabe RM (2011) A Better Index of Body Adiposity. *Obesity*. Nature Publishing Group 19(5): 1083–1089. Available at: http://dx.doi.org/10.1038/oby.2011.38/nature06264.

Biekniek J, Kashanian J, Deibert C, Grober E, Brannigan R, Sandlow J and Jarvi K (2015)

DDeleterious effects of male obesity on reproductive parameters among a loarge multi-institutional cohort. *Fertility and Sterility*. Elsevier Ltd 104(3): e67. Available at: http://dx.doi.org/10.1016/j.fertnstert.2015.07.203.

Binder NK, Hannan NJ and Gardner DK (2012) Paternal Diet-Induced Obesity Retards Early Mouse Embryo Development, Mitochondrial Activity and Pregnancy Health. *PLoS ONE* 7(12): e52304. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3531483&tool=pmcentrez&rendertype=abstract.

Black RE, Allen LH, Bhutta Z a., Caulfield LE, de Onis M, Ezzati M, Mathers C and Rivera J (2008) Maternal and child undernutrition: global and regional exposures and health consequences. *The Lancet* 371(9608): 243–260.

Black RE, Victora CG, Walker SP, Bhutta Z a., Christian P, De Onis M, Ezzati M, Grantham-Mcgregor S, Katz J, Martorell R and Uauy R (2013) Maternal and child undernutrition and overweight in low-income and middle-income countries. *The Lancet* 382(9890): 427–451.

Bonde JP (1992) Semen quality in welders exposed to radiant heat. *British journal of industrial medicine* 49(1): 5–10. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1039225&tool=pmcentrez&rendertype=abstract (accessed 19/01/16).

Botha C, Wright H, Moss S and Kolbe-Alexander T (2013) 'Be active!'Revisiting the South African food-based dietary guideline for activity. *Food-Based Dietary Guidelines for South Africa* 26(3): 18–27.

Bouayed J and Bohn T (2010) Exogenous Antioxidants—Double-Edged Swords in Cellular Redox State: Health Beneficial Effects at Physiologic Doses versus Deleterious Effects at High Doses. *Oxidative Medicine and Cellular Longevity* 3(4): 228–237.

Braga DP de AF, Halpern G, Figueira R de CS, Setti AS, Iaconelli A and Borges E (2012) Food intake and social habits in male patients and its relationship to intracytoplasmic sperm injection outcomes. *Fertility and sterility* 97(1): 53–9. Available at: http://www.sciencedirect.com/science/article/pii/S001502821102677X.

Cade J, Thompson R, Burley V and Warm D (2002) Development, validation and utilisation of food-frequency questionnaires – a review. *Public Health Nutrition* 5(4): 567–587. Available at: http://journals.cambridge.org/abstract_S1368980002000794.

Campagne DM (2013) Can Male Fertility Be Improved Prior to Assisted Reproduction through The Control of Uncommonly Considered Factors? *International journal of fertility & sterility* 6(4): 214–223. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3850314&tool=pmcentrez&rendertype=abstract.

Campbell JM, Lane M, Owens JA and Bakos HW (2015) Paternal obesity negatively affects male fertility and assisted reproduction outcomes: a systematic review and meta-analysis. *Reproductive BioMedicine Online*. Reproductive Healthcare Ltd. 31(5): 593–604. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1472648315003776.

Carlsen E, Petersen JH, Andersson AM and Skakkebaek NE (2004) Effects of ejaculatory frequency and season on variations in semen quality. *Fertility and Sterility* 82(2): 358–366.

Charlton KE and Jooste P (2001) East salt sparingly - sprinkle , don ’ t shake ! *South African Journal of Clinical Nutrition* 14(3): S55–S64.

Charlton KE, Steyn K, Levitt NS, Jonathan D, Zulu J V and Nel JH (2007) Development and validation of a short questionnaire to assess sodium intake. *Public health nutrition* 11(1): 83–94.

Chavarro JE, Furtado J, Toth TL, Ford J, Keller M, Campos H and Hauser R (2011) Trans-fatty acid levels in sperm are associated with sperm concentration among men from an infertility clinic. *Fertility and Sterility*. Elsevier Ltd 95(5): 1794–1797. Available at: http://dx.doi.org/10.1016/j.fertnstert.2010.10.039.

Chavarro JE, Mínguez-Alarcón L, Mendiola J, Cutillas-Tolín A, López-Espín JJ and Torres-Cantero AM (2014) Trans fatty acid intake is inversely related to total sperm count in young healthy men. *Human reproduction (Oxford, England)* 29(3): 429–40. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24419496.

Chavarro JE, Toth TL, Wright DL, Meeker JD and Hauser R (2010) Body mass index in relation to semen quality, sperm DNA integrity, and serum reproductive hormone levels among men attending an infertility clinic. *Fertility and sterility*. Elsevier Ltd 93(7): 2222–31. Available at: http://www.sciencedirect.com/science/article/pii/S0015028209001502.

Cheah Y and Yang W (2011) Functions of essential nutrition for high quality spermatogenesis. *Advances in Bioscience and Biotechnology* 02(04): 182–197.

Chen J-W, Chen Y-H, Lin F-Y, Chen Y-L and Lin S-J (2003) Ginkgo biloba extract inhibits tumor necrosis factor-alpha-induced reactive oxygen species generation, transcription factor activation, and cell adhesion molecule expression in human aortic endothelial cells. *Arteriosclerosis, thrombosis, and vascular biology* 23(9): 1559–66. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12893683.

Chow V and Cheung AP (2006) Male infertility. *The Journal of reproductive medicine* 51(3): 149–56. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16674008 (accessed 20/01/16).

Cocuzza M, Athayde KS, Agarwal A, Sharma R, Pagani R, Lucon AM, Srougi M and Hallak J (2008) Age-Related Increase of Reactive Oxygen Species in Neat Semen in Healthy Fertile Men. *Urology* 71(3): 490–494.

Cocuzza M and Esteves SC (2014) Shedding light on the controversy surrounding the temporal decline in human sperm counts: a systematic review. *TheScientificWorldJournal* 2014: 365691. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3929517&tool=pmcentrez&rendertype=abstract.

Cohen BJ and Wood DL (2000) *The Human Body in Health and Disease*. 9th edition. Philadelphia: Lippincott Williams & Wilkins: 417-422.

Conquer J, Martin J, Tummon I, Watson L and Tekpetey F (1999) Fatty acid analysis of blood serum, seminal plasma, and spermatozoa of normozoospermic vs. Asthernozoospermic males. *Lipids* 34(8): 793–799.

Cooke L and Nelson SM (2011) Reproductive ageing and fertility in an ageing population. *The Obstetrician & Gynaecologist* 13(3): 161–168. Available at:

http://doi.wiley.com/10.1576/toag.13.3.161.27668.

Craig C, Marshall A, Sjostrom M, Bauman A, Booth M, Ainsworth B, Pratt M, Ekelund U, Yngve A, Sallis J and Oja P (2003) International Physical Activity Questionnaire: 12 Country Reliability and Validity. *Medicine & Science in Sports & Exercise* 35(8): 3508–1381. Available at: http://addssa.com/intranets/idrd/2011_10_activos_y_saludables/03_recursos/study_material/gestores/Articulos Ingles/Vigilancia y Evaluacion/1Q. International PAQ.pdf.

DeFilippis AP and Sperling LS (2006) Understanding omega-3's. *American Heart Journal* 151(3): 564–570.

Dohle GR, Colpi GM, Hargreave TB, Papp GK, Jungwirth a. and Weidner W (2005) EAU guidelines on male infertility. *European Urology* 48: 703–711.

Dott H and Glover T (1999) Sperm Production and Delivery in Mammals, including Man, In: Glover TD and Barratt CLR, *Male Fertility and Infertility*. New York: Cambridge University Press: 34-55.

Duits FH, Van Wely M, Van Der Veen F and Gianotten J (2010) Healthy overweight male partners of subfertile couples should not worry about their semen quality. *Fertility and Sterility*. Elsevier Ltd 94(4): 1356–1359. Available at: http://dx.doi.org/10.1016/j.fertnstert.2009.05.075.

Dunson DB, Baird DD and Colombo B (2004) Increased infertility with age in men and women. *Obstetrics and gynecology* 103(1): 51–56.

Dupont C, Faure C, Sermondade N, Boubaya M, Eustache F, Clément P, Briot P, Berthaut I, Levy V, Cedrin-Durnerin I, Benzacken B, Chavatte-Palmer P and Levy R (2013) Obesity leads to higher risk of sperm DNA damage in infertile patients. *Asian journal of andrology* 15(5): 622–5. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3881654&tool=pmcentrez&rendertype=abstract.

Egwurugwu JN, Nwafor a, Chike CPR, Ufearo CS, Uchefuna RC, Iwuji SC, Okwara JE and Alagwu E a (2011) The relationship between body mass index, semen and sex hormones in adult male. *Nigerian journal of physiological sciences : official publication of the Physiological Society of Nigeria* 26(1): 29–34. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22314983.

Eisenberg ML, Betts P, Herder D, Lamb DJ and Lipshultz LI (2013) Increased risk of cancer among azoospermic men. *Fertility and Sterility*. Elsevier Inc. 100(3): 681–685. Available at: http://dx.doi.org/10.1016/j.fertnstert.2013.05.022.

Eisenberg ML, Kim S, Chen Z, Sundaram R, Schisterman EF and Buck Louis GM (2014) The relationship between male BMI and waist circumference on semen quality: data from the LIFE study. *Human reproduction (Oxford, England)* 29(2): 193–200. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3896223&tool=pmcentrez&rendertype=abstract.

Eisenberg ML, Kim S, Chen Z, Sundaram R, Schisterman EF and Buck Louis GM (2015) The relationship between male BMI and waist circumference on semen quality: data from the LIFE study. *Human reproduction (Oxford, England)* 29(2): 193–200. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3896223&tool=pmcentrez&rendertype=abstract.

Eisenberg ML, Lathi RB, Baker VL, Westphal LM, Milki AA and Nangia AK (2013) Frequency of the male infertility evaluation: data from the national survey of family growth. *The Journal of urology*. Elsevier Inc. 189(3): 1030–4. Available at: http://www.sciencedirect.com/science/article/pii/S0022534712049476.

Erdemir F, Atilgan D, Markoc F, Boztepe O, Suha-Parlaktas B and Sahin S (2012) [The effect of diet induced obesity on testicular tissue and serum oxidative stress parameters]. *Actas urologicas españolas*. AEU 36(3): 153–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21959063.

Eskandar M, Al-Asmari M, Babu Chaduvula S, Al-Shahrani M, Al-Sunaidi M, Almushait M, Donia O and Al-Fifi S (2012) Impact of Male Obesity on Semen Quality and Serum Sex Hormones. *Advances in Urology* 2012: 1–4.

Eskenazi B, Kidd S a., Marks a. R, Sloter E, Block G and Wyrobek a. J (2005) Antioxidant intake is associated with semen quality in healthy men. *Human Reproduction* 20(4): 1006–1012.

Eskenazi B, Wyrobek a. J, Sloter E, Kidd S a., Moore L, Young S and Moore D (2003) The association of age and semen quality in healthy men. *Human Reproduction* 18(2): 447–454.

Eslamian G, Amirjannati N, Rashidkhani B, Sadeghi M-R and Hekmatdoost A (2012) Intake of food groups and idiopathic asthenozoospermia: a case-control study. *Human reproduction (Oxford, England)* 27(11): 3328–36. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22940769.

Evers JLH (2002) Female subfertility. *Lancet (London, England)* 360(9327): 151–9. Available at: http://www.sciencedirect.com/science/article/pii/S0140673602094175.

Farooqui A a., Horrocks L a. and Farooqui T (2000) Glycerophospholipids in brain: Their metabolism, incorporation into membranes, functions, and involvement in neurological disorders. *Chemistry and Physics of Lipids* 106: 1–29.

Fejes I, Koloszar S, Szöllösi J, Zavaczki Z and Pal A (2005) Is semen quality affected by male body fat distribution ? *Andrologia* 37: 155–159.

Ferguson-Smith AC and Patti ME (2011) You are what your dad ate. *Cell Metabolism*. Elsevier Inc. 13(2): 115–117. Available at: http://dx.doi.org/10.1016/j.cmet.2011.01.011.

Fernandez CDB, Bellentani FF, Fernandes GS a, Perobelli JE, Favareto AP a, Nascimento AF, Cicogna AC and Kempinas WDG (2011) Diet-induced obesity in rats leads to a decrease in sperm motility. *Reproductive biology and endocrinology : RB&E* 9: 32.

Folch J, Lees M and Stanley GHS (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem*, 497–509. Available at: http://www.jbc.org/content/226/1/497.short.

Fox SI (2011) *Human Physiology*. 12th edition. United States of America: McGraw-Hill:703-720.

Fronczak CM, Kim ED and Barqawi AB (2012) The insults of illicit drug use on male fertility. *Journal of andrology* 33(4): 515–28. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21799144.

Gallagher ML (2012) Intake: The Nutrients and Their Metabolism, In: Mahan KL, Escott-Stump S and Raymond J.L., *Krause's Food and the Nutrition Care Process*,13th edition, Philadelphia:

W.B Saunders Company: 462-484.

Garolla A, Torino M, Sartini B, Cosci I, Patassini C, Carraro U and Foresta C (2013) Seminal and molecular evidence that sauna exposure affects human spermatogenesis. *Human Reproduction* 28(4): 877–85. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23411620.

Gaskins AJ, Colaci DS, Mendiola J, Swan SH and Chavarro JE (2012a) Dietary patterns and semen quality in young men. *Human reproduction (Oxford, England)* 27(10): 2899–907. Available at: http://humrep.oxfordjournals.org/content/27/10/2899.short.

Gaskins AJ, Colaci DS, Mendiola J, Swan SH and Chavarro JE (2012b) Dietary patterns and semen quality in young men. *Human reproduction* 27(10): 2899–907. Available at: http://humrep.oxfordjournals.org/content/27/10/2899.short.

Gaskins AJ, Mendiola J, Afeiche M, Jørgensen N, Swan SH and Chavarro JE (2013) Physical activity and television watching in relation to semen quality in young men. *British Journal of Sports Medicine* 1–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23380634.

Gaur DS, Talekar MS and Pathak VP (2010) Alcohol intake and cigarette smoking: impact of two major lifestyle factors on male fertility. *Indian journal of pathology & microbiology* 53(1): 35–40. Available at: http://www.ijpmonline.org/article.asp?issn=0377-4929;year=2010;volume=53;issue=1;spage=35;epage=40;aulast=Gaur.

Gazvani MR, Buckett W, MLuckas MJ, Aird I a., Hipkin LJ and Lewis-Jones DI (1997) Conservative management of azoospermia following steroid abuse. *Human Reproduction* 12(8): 1706–1708.

Ghanayem BI, Bai R, Kissling GE, Travlos G and Hoffler U (2010) Diet-induced obesity in male mice is associated with reduced fertility and potentiation of acrylamide-induced reproductive toxicity. *Biology of reproduction* 82(1): 96–104. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2802115&tool=pmcentrez&rendertype=abstract.

Gharagozloo P and Aitken RJ (2011) The role of sperm oxidative stress in male infertility and the significance of oral antioxidant therapy. *Human reproduction (Oxford, England)* 26(7): 1628–40. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21546386.

Gibson RS. (2005) Anthropometric Assessment. *Principles of nutritional assessment*. 2nd edition. New York: Oxford University Press:6-283.

Gnoth C, Godehardt E, Frank-Herrmann P, Friol K, Tigges J and Freundl G (2005) Definition and prevalence of subfertility and infertility. *Human Reproduction* 20(5): 1144–1147.

Graybill F (1961) *An introduction to linear statistical models*. New York:McGraw-Hill.

Greco E, Iacobelli M, Rienzi L, Ubaldi F, Ferrero S and Tesarik J (2005) Reduction of the incidence of sperm DNA fragmentation by oral antioxidant treatment. *Journal of andrology* 26(3): 349–53. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15867002.

Greco E, Romano S, Iacobelli M, Ferrero S, Baroni E, Minasi MG, Ubaldi F, Rienzi L and Tesarik J (2005) ICSI in cases of sperm DNA damage: Beneficial effect of oral antioxidant treatment. *Human Reproduction* 20(9): 2590–2594.

Guenther WC (1977) Desk calculation of probabilities for the distribution of the sample

correlation coefficient. *The American Statician* 31(1): 45-48.

Guzick DS, Overstreet JW, Factor-Litvak P, Brazil CK, Nakajima ST, Coutifaris C, Carson SA, Cisneros P, Steinkampf MP, Hill JA, Xu D and Vogel DL (2001) Sperm morphology, motility, and concentration in fertile and infertile men. *The New England journal of medicine* 345(19): 1388–1393.

Hall E and Burt VK (2012) Male fertility: psychiatric considerations. *Fertility and sterility*. Elsevier Inc. 97(2): 434–9. Available at: http://www.sciencedirect.com/science/article/pii/S0015028211028032.

Hammadeh ME, Filippos A A and Hamad MF (2009) Reactive oxygen species and antioxidant in seminal plasma and their impact on male fertility. *International Journal of Fertility and Sterility* 3(3): 87–110.

Hammond KA and Litchford MD (2012) 'Nutrition Assessment: Clinical: Inflammation, Physical, and Functional Assessments',in Mahan KL, Escott-Stump S and Raymond J.L., *Krause's Food and the Nutrition Care Process*,13th edition, Philadelphia: W.B Saunders Company: 169,470.

Hammoud AO (2006) Obesity and Male Reproductive Potential. *Journal of Andrology* 27(5): 619–626.

Hammoud AO, Gibson M, Peterson CM, Meikle W and Carrell DT (2008a) Impact of male obesity on infertility: a critical review of the current literature. *Fertility and Sterility* 90(4): 897–904.

Hammoud AO, Wilde N, Gibson M, Parks A, Carrell DT and Meikle AW (2008b) Male obesity and alteration in sperm parameters. *Fertility and sterility*. Elsevier Ltd 90(6): 2222–5. Available at: http://www.sciencedirect.com/science/article/pii/S0015028207037752.

Haskell WL, Lee IM, Pate RR, Powell KE, Blair SN, Franklin B a., MacEra C a., Heath GW, Thompson PD and Bauman A (2007) Physical activity and public health: Updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. *Medicine and Science in Sports and Exercise* 39(8): 1423–1434.

Hawkes WC and Turek PJ (2001) Effects of dietary selenium on sperm motility in healthy men. *Journal of andrology* 22(5): 764–72. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11545288.

Hingorjo MR, Qureshi MA and Mehdi A (2012) Neck circumference as a useful marker of obesity: a comparison with body mass index and waist circumference. *JPMA. The Journal of the Pakistan Medical Association* 62(1): 36–40. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22352099.

Hjollund NHI, Bonde JPE, Henriksen TB, Giwercman A and Olsen J (2004) Reproductive effects of male psychologic stress. *Epidemiology (Cambridge, Mass.)* 15(1): 21–27.

Hoffstein V and Mateika S (1992) Differences in abdominal and neck circumferences in patients with and without obstructive sleep apnoea. *European Respiratory Journal* 5(4): 377–381.

Hofny ERM, Ali ME, Abdel-Hafez HZ, Kamal EE-D, Mohamed EE, Abd El-Azeem HG and Mostafa T (2010) Semen parameters and hormonal profile in obese fertile and infertile males. *Fertility*

and sterility. Elsevier Ltd 94(2): 581–4. Available at: http://www.sciencedirect.com/science/article/pii/S0015028209007559.

Homan GF, Davies M and Norman R (2007) The impact of lifestyle factors on reproductive performance in the general population and those undergoing infertility treatment: A review. *Human Reproduction Update* 13(3): 209–223.

Honig SC, Lipshultz LI and Jarow J (1994) Significant medical pathology uncovered by a comprehensive male infertility evaluation. *Fertility and sterility* 62(5): 1028–34. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7926114 (accessed 20/01/16).

Iammarrone E, Balet R, Lower a M, Gillott C and Grudzinskas JG (2003) Male infertility. *Best practice & research. Clinical obstetrics & gynaecology* 17(2): 211–229.

Inhorn MC and Patrizio P (2015) Infertility around the globe: new thinking on gender, reproductive technologies and global movements in the 21st century. *Human Reproduction Update* 21(4): 411–426. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25801630.

Institute of Medicine, Food and Nutrition Board. 2002. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. Washington: National Academy Press. Available at: http://www.nal.usda.gov/fnic/DRI/DRI_Tables/macronutrients.pdf / (Accessed 10 June 2013).

Institute of Medicine, Food and Nutrition Board. 2001. Dietary Reference Intakes for vitamin A, vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc. Washington: National Academy Press. Available at: http://fnic.nal.usda.gov/dietary-guidance/dietary-reference-intakes/dri-tables / (Accessed 10 June 2013).

Institute of Medicine, Food and Nutrition Board. 2000. Dietary Reference Intakes for vitamin C, vitamin E, Selenium and Carotenoids. Washington: National Academy Press. Available at: http://fnic.nal.usda.gov/dietary-guidance/dietary-reference-intakes/dri-tables / (Accessed 10 June 2013).

Institute of Medicine, Food and Nutrition Board. 1998. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic acid, Biotin, and Choline. Washington: National Academies Press. Available at: http://fnic.nal.usda.gov/dietary-guidance/dietary-reference-intakes/dri-tables / (Accessed 10 June 2013).

Institute of Medicine, Food and Nutrition Board. 2005. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. Washington: National Academies Press. Available at: http://fnic.nal.usda.gov/dietary-guidance/dietary-reference-intakes/dri-tables / (Accessed 10 June 2013).

Isidori A, Latini M and Romanelli F (2005) Treatment of male infertility. *Contraception* 72(4): 314–8. Available at: http://www.sciencedirect.com/science/article/pii/S0010782405001617.

Ivell R (2007) Lifestyle impact and the biology of the human scrotum. *Reproductive biology and endocrinology : RB&E* 5: 15.

Jacobs L and Steyn N (2013) If you drink alcohol, drink sensibly: Is this guideline still appropriate? *South African Journal of Clinical Nutrition* 23(1): 110–115.

Jarow JP, Espeland MA and Lipshultz LI (1989) Evaluation of the azoospermic patient. *The Journal of urology* 142(1): 62–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2499695 (accessed 20/01/16).

Jensen T, Andersson A, Jørgensen N, Andersen A, Carlsen E, Petersen J and Skakkebaek N (2004) Body mass index in relation to semen quality and reproductive hormones among 1,558 Danish men. *Fertility and sterility* 82(4): 863–70. Available at: http://www.sciencedirect.com/science/article/pii/S0015028204022241.

Jensen T, Heitmann B, Jensen M, Halldorsson T, Andersson A, Skakkebrek N, Joensen U, Lauritsen M, Christiansen P, Dalgård C, Lassen T and JØrgensen N (2013) High dietary intake of saturated fat is associated with reduced semen quality among 701 young Danish men from the general population. *American Journal of Clinical Nutrition* 97.

Jensen T, Jacobsen R, Christensen K, Nielsen N and Bostofte E (2009) Good semen quality and life expectancy: A cohort study of 43,277 men. *American Journal of Epidemiology* 170(5): 559–565.

Jensen TK, Gottschau M, Madsen JOB, Andersson A-M, Lassen TH, Skakkebaek NE, Swan SH, Priskorn L, Juul A and Jorgensen N (2014) Habitual alcohol consumption associated with reduced semen quality and changes in reproductive hormones; a cross-sectional study among 1221 young Danish men. *BMJ Open* 4(9): e005462–e005462. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4185337&tool=pmcentrez&rendertype=abstract.

Jensen TK, Heitmann BL, Jensen MB, Halldorsson TI, Andersson A, Lassen TH, Skakkebæk NE, Joensen UN, Lauritsen MP, Christiansen P, Dalga C and Jørgensen N (2012) High dietary intake of saturated fat is associated with reduced semen quality among 701 young Danish men from the general population. *American Journal of Clinical Nutrition* 114–118.

Jensen TK, Jacobsen R, Christensen K, Nielsen NC and Bostofte E (2009) Good semen quality and life expectancy: A cohort study of 43,277 men. *American Journal of Epidemiology* 170(5): 559–565.

Jensen TK, Sobotka T, Hansen M a, Pedersen AT, Lutz W and Skakkebaek NE (2008) Declining trends in conception rates in recent birth cohorts of native Danish women: a possible role of deteriorating male reproductive health. *International journal of andrology* 31(2): 81–92. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2440321&tool=pmcentrez&rendertype=abstract.

Jensenl TK, Jørgensen N, Punab M, Haugen TB, Suominen J, Zilaitiene B, Horte A, Andersen AG, Carlsen E, Magnus Ø, Matulevicius V, Nermoen I, Vierula M, Keiding N, Toppari J and Skakkebaek NE (2004) Association of In Utero Exposure to Maternal Smoking with Reduced Semen Quality and Testis Size in Adulthood: A Cross-Sectional Study of 1,770 Young Men from the General Population in Five European Countries. *American Journal of Epidemiology* 159(1): 49–58.

Jetté M, Sidney K and Blümchen G (1990) Metabolic equivalents (METS) in exercise testing, exercise prescription, and evaluation of functional capacity. *Clinical Cardiology* 13(8): 555–565.

De Jong a. ME, Menkveld R, Lens JW, Nienhuis SE and Rhemrev JPT (2014) Effect of alcohol intake and cigarette smoking on sperm parameters and pregnancy. *Andrologia* 46: 112–117.

De Jonge C, Lafromboise M, Bosmans E, Ombelet W, Cox A and Nijs M (2004) Influence of the abstinence period on human sperm quality. *Fertility and sterility* 82(1): 57–65.

Jørgensen N, Vierula M, Jacobsen R, Pukkala E, Perheentupa A, Virtanen HE, Skakkebaek NE and Toppari J (2011) Recent adverse trends in semen quality and testis cancer incidence among Finnish men. *International journal of andrology* 34(4 Pt 2): e37–48. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3170483&tool=pmcentrez&rendertype=abstract.

Jung a. and Schuppe HC (2007) Influence of genital heat stress on semen quality in humans. *Andrologia* 39(6): 203–215.

Jung A, Leonhardt F, Schill WB and Schuppe HC (2005) Influence of the type of undertrousers and physical activity on scrotal temperature. *Human Reproduction* 20(4): 1022–1027.

Jung A and Schuppe H (2007) Influence of genital heat stress on semen quality in humans. *Andrologia* 39(6): 203–215.

Jurewicz J, Hanke W, Radwan M and Bonde JP (2009) Environmental factors and semen quality. *International journal of occupational medicine and environmental health* 22(4): 305–29. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20053623.

Karavolos S, Stewart J, Evbuomwan I, McEleny K and Aird I (2013) Assessment of the infertile male. *The Obstetrician & Gynaecologist* 15(1): 1–9. Available at: http://doi.wiley.com/10.1111/j.1744-4667.2012.00145.x.

Kasturi SS, Tannir J and Brannigan RE (2008) The metabolic syndrome and male infertility. *Journal of andrology* 29(3): 251–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18222914.

Katz DL and Meller S (2014) Can we say what diet is best for health? *Annual review of public health* 35(February): 83–103. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24641555.

Kay VJ and Barratt CLR (2009) Male obesity: impact on fertility. *The British Journal of Diabetes & Vascular Disease* 9(5): 237–241. Available at: http://dvd.sagepub.com/cgi/doi/10.1177/1474651409343132.

Kefer JC, Agarwal A and Sabanegh E (2009) Role of antioxidants in the treatment of male infertility. 449–457.

Keskes-Ammar L, Feki-Chakroun N, Rebai T, Sahnoun Z, Ghozzi H, Hammami S, Zghal K, Fki H, Damak J and Bahloul A (2003) Sperm oxidative stress and the effect of an oral vitamin E and selenium supplement on semen quality in infertile men. *Archives of andrology* 49(2): 83–94. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12623744.

Kidd SA, Eskenazi B and Wyrobek AJ (2001) Effects of male age on semen quality and fertility: a review of the literature. *Fertility and Sterility* 75(2): 237–248. Available at: http://www.sciencedirect.com/science/article/pii/S0015028200016794.

Kilgallon SJ and Simmons LW (2005) Image content influences men's semen quality. *Biology letters* 1(3): 253–255.

Kleinhaus K, Perrin M, Friedlander Y, Paltiel O, Malaspina D and Harlap S (2006) Paternal age and spontaneous abortion. *Obstetrics and gynecology* 108(2): 369–377.

Koch OR, Pani G, Borrello S, Colavitti R, Cravero A, Farrè S and Galeotti T (2004) Oxidative stress and antioxidant defenses in ethanol-induced cell injury. *Molecular Aspects of Medicine* 25(1-2): 191–198.

Kolettis PN and Sabanegh ES (2001) Significant medical pathology discovered during a male infertility evaluation. *The Journal of urology* 166(1): 178–180.

Kort HI, Massey JB, Elsner CW, Mitchell-Leef D, Shapiro DB, Witt M a and Roudebush WE (2006) Impact of body mass index values on sperm quantity and quality. *Journal of andrology* 27(3): 450–2. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16339454.

Krausz C (2011) Male infertility: pathogenesis and clinical diagnosis. *Best practice & research. Clinical endocrinology & metabolism*. Elsevier Ltd 25(2): 271–85. Available at: http://www.sciencedirect.com/science/article/pii/S1521690X10000916.

Lambert E, Bohlmann T and Kolbe-Alexander T (2001) 'Be active' - Physical activity for health in South Africa. *South African Journal of Clinical Nutrition* 14(3): S12–S16.

Lanzafame F, La Vignera S, Vicari E and Calogero AE (2009) Oxidative stress and medical antioxidant treatment in male infertility. *Reproductive BioMedicine Online*. Reproductive Healthcare Ltd, Duck End Farm, Dry Drayton, Cambridge CB23 8DB, UK 19(5): 638–659. Available at: http://dx.doi.org/10.1016/j.rbmo.2009.09.014.

Larsson B, Svärdsudd K, Welin L, Wilhelmsen L, Björntorp P and Tibblin G (1984) Abdominal adipose tissue distribution, obesity, and risk of cardiovascular disease and death: 13 year follow up of participants in the study of men born in 1913. *British medical journal (Clinical research ed.)* 288(6428): 1401–4. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1441047&tool=pmcentrez&rendertype=abstract.

Lean ME, Han TS and Morrison CE (1995) Waist circumference as a measure for indicating need for weight management. *BMJ (Clinical research ed.)* 311(6998): 158–161.

Lee RD and Nieman DC. (2013) *Nutritional Assessment*, 6th edition, New York: McGraw-Hill:168,170,181,185,186.

Leedy PD and Omrod JE (2010) *Practical Research: Planning and Design*. 4th edition. New Jersey: Pearson Education International:28-29.

Lenzi a, Gandini L, Maresca V, Rago R, Sgrò P, Dondero F and Picardo M (2000) Fatty acid composition of spermatozoa and immature germ cells. *Molecular human reproduction* 6(3): 226–231.

Levitas E, Lunenfeld E, Weisz N, Friger M and Potashnik G (2007) Relationship between age and semen parameters in men with normal sperm concentration: Analysis of 6022 semen samples. *Andrologia* 39(2): 45–50.

Levy A, Fraser D, Katz M, Mazor M and Sheiner E (2005) Maternal anemia during pregnancy is an independent risk factor for low birthweight and preterm delivery. *European Journal of Obstetrics Gynecology and Reproductive Biology* 122(2): 182–186.

Li Y, Lin H, Li Y and Cao J (2011) Association between socio-psycho-behavioral factors and male semen quality: systematic review and meta-analyses. *Fertility and sterility*. Elsevier Ltd 95(1):

116–23. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20674912.

Louis JF, Thoma ME, Sørensen DN, McLain a C, King RB, Sundaram R, Keiding N and Buck Louis GM (2013) The prevalence of couple infertility in the United States from a male perspective: evidence from a nationally representative sample. *Andrology* 1(5): 741–748. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3752331&tool=pmcentrez&rendertype=abstract.

Love P and Sayed N (2001) Eat plenty of vegetables and fruits everyday. 14(3): S24–S32.

Lunenfeld B and Van Steirteghem a. (2004) Infertility in the third millenium: Implications for the individual, family and society: Condensed meeting report from the Bertarelli Foundation's Second Global Conference. *Human Reproduction Update* 10(4): 317–326.

Luque EM, Tissera A, Gaggino MP, Molina RI, Mangeaud A, Vincenti LM, Beltramone F, Larcher JS, Estofán D, Fiol de Cuneo M, Martini AC (2015) Body mass index and human sperm quality: neither one extreme nor the other. *Reproduction, Fertility and Development.* Available at: http://dx.doi.org/10.1071/RD15351

Lysen LK and Israel (2012) Nutrition in Weight Management, In: Mahan KL, Escott-Stump S and Raymond J.L., *Krause's Food and the Nutrition Care Process*,13th edition, Philadelphia: W.B Saunders Company: 462-484.

MacDonald a a, Herbison GP, Showell M and Farquhar CM (2010) The impact of body mass index on semen parameters and reproductive hormones in human males: a systematic review with meta-analysis. *Human reproduction update* 16(3): 293–311. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19889752.

Maconochie N, Doyle P, Prior S and Simmons R (2007) Risk factors for first trimester miscarriage - Results from a UK-population-based case-control study. *BJOG: An International Journal of Obstetrics and Gynaecology* 114(2): 170–186.

Magnusdottir E V, Thorsteinsson T, Thorsteinsdottir S, Heimisdottir M and Olafsdottir K (2005) Persistent organochlorines, sedentary occupation, obesity and human male subfertility. *Human reproduction (Oxford, England)* 20(1): 208–15. Available at: http://humrep.oxfordjournals.org/content/20/1/208.short.

Mah PM and Wittert G a. (2010) Obesity and testicular function. *Molecular and Cellular Endocrinology* 316(2): 180–186.

Mandal R, Badyakar D and Chakrabarty J (2014) Role of Membrane Lipid Fatty Acids in Sperm Cryopreservation. *Advances in Andrology* 1–9.

Martini AC, Tissera A, Estofán D, Molina RI, Mangeaud A, de Cuneo MF and Ruiz RD (2010) Overweight and seminal quality: a study of 794 patients. *Fertility and sterility* 94(5): 1739–43. Available at: http://www.sciencedirect.com/science/article/pii/S0015028209040461.

Mascarenhas MN, Flaxman SR, Boerma T, Vanderpoel S and Stevens G a. (2012) National, Regional, and Global Trends in Infertility Prevalence Since 1990: A Systematic Analysis of 277 Health Surveys. *PLoS Medicine* 9(12): 1–12.

McLachlan R and de Kretser D (2001) Male infertility: the case for continued research. *Medical Journal of Australia* 174(3): 116–117.

McPherson NO, Fullston T, Bakos HW, Setchell BP and Lane M (2014) Obese father's metabolic state, adiposity, and reproductive capacity indicate son's reproductive health. *Fertility and sterility*. Elsevier Inc. 101(3): 865–73. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24424359.

Meacham RB, Joyce GF, Wise M, Kparker A and Niederberger C (2007) Male infertility. *The Journal of urology* 177(6): 2058–66. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17509286.

Mendiola J, Torres-Cantero AM and Agarwal A (2009) Lifestyle factors and male infertility: an evidence-based review. *Arch Med Sci* 5(1A): S3–12.

Mendiola J, Torres-Cantero AM, Moreno-Grau JM, Ten J, Roca M, Moreno-Grau S and Bernabeu R (2009) Food intake and its relationship with semen quality: a case-control study. *Fertility and sterility* 91(3): 812–818. Available at: http://www.sciencedirect.com/science/article/pii/S0015028208000149.

Mendiola J, Torres-Cantero AM, Vioque J, Moreno-Grau JM, Ten J, Roca M, Moreno-Grau S and Bernabeu R (2010) A low intake of antioxidant nutrients is associated with poor semen quality in patients attending fertility clinics. *Fertility and Sterility* 93(4): 1128–1133.

Ménézo YJ, Hazout A, Panteix G, Robert F, Rollet J, Cohen-Bacrie P, Chapuis F, Clément P and Benkhalifa M (2007) Antioxidants to reduce sperm DNA fragmentation: an unexpected adverse effect. *Reproductive BioMedicine Online*. Reproductive Healthcare Ltd, Duck End Farm, Dry Drayton, Cambridge CB23 8DB, UK 14(4): 418–421. Available at: http://www.sciencedirect.com/science/article/pii/S1472648310608875.

Merhi ZO (2012) Challenging cell phone impact on reproduction: A Review. *Journal of Assisted Reproduction and Genetics* 29: 293–297.

Michalakis K, Mintziori G, Kaprara A, Tarlatzis BC and Goulis DG (2013) The complex interaction between obesity, metabolic syndrome and reproductive axis: A narrative review. *Metabolism: Clinical and Experimental*. Elsevier Inc. 62(4): 457–478. Available at: http://dx.doi.org/10.1016/j.metabol.2012.08.012.

Mieusset R, Bengoudifa B and Bujan L (2007) Effect of posture and clothing on scrotal temperature in fertile men. *Journal of andrology* 28(1): 170–175.

Mieusset R and Bujan L (1995) Testicular heating and its possible contributions to male infertility: a review. *International Journal of Andrology* 18(4): 169–184. Available at: http://dx.doi.org/10.1111/j.1365-2605.1995.tb00408.x.

Miller ERI, Pastor-barriuso R, Dalal D and Riemersma R a (2005) Review Meta-Analysis : High-Dosage Vitamin E Supplementation May Increase all Cause Mortality. *Ann Intern Med* 142: 37–46.

Mínguez-Alarcón L, Mendiola J, López-Espín JJ, Sarabia-Cos L, Vivero-Salmerón G, Vioque J, Navarrete-Muñoz EM and Torres-Cantero AM (2012) Dietary intake of antioxidant nutrients is associated with semen quality in young university students. *Human reproduction (Oxford, England)* 27(9): 2807–14. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22752607.

Muthusami KR and Chinnaswamy P (2005) Effect of chronic alcoholism on male fertility hormones and semen quality. *Fertility and sterility* 84(4): 919–24. Available at:

http://www.sciencedirect.com/science/article/pii/S0015028205012513.

Nachtigall RD (2006) International disparities in access to infertility services. *Fertility and sterility* 85(4): 871–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16580367.

Ng KK, Donat R, Chan L, Lalak a, Di Pierro I and Handelsman DJ (2004) Sperm output of older men. *Human reproduction (Oxford, England)* 19(8): 1811–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15218002.

Nguyen RHN, Wilcox AJ, Skjærven R and Baird DD (2007) Men's body mass index and infertility. *Human Reproduction* 22(9): 2488–2493.

NHANES (2004) National Health and Nutrition Examination Survey (NHANES) Anthropometry Procedures Manual. 1–1–3–27.

NHANES (2007) National Health and Nutrition Examinatory Survey. Anthropometry Procedures Manual. 1–1–5–16. Available at: http://www.cdc.gov/nchs/data/nhanes/nhanes_07_08/manual_an.pdf.

Nwajiaku L, Mbachu I and Ikeako L (2012) Prevalence , Clinical Pattern and Major Causes of Male Infertility in Nnewi , South East Nigeria : A Five Year Review. *Afrimedic Journal* 3(2): 16–19.

Olsen J and Høst Ramlau-Hansen C (2012) Dietary fats may impact semen quantity and quality. *Asian Journal of Andrology* 14: 511–512.

Ombelet W, Cooke I, Dyer S, Serour G and Devroey P (2008) Infertility and the provision of infertility medical services in developing countries. *Human reproduction update* 14(6): 605–21. Available at: http://humupd.oxfordjournals.org/content/14/6/605.

Onat A, Hergenç G, Yüksel H, Can G, Ayhan E, Kaya Z and Dursunoğlu D (2009) Neck circumference as a measure of central obesity: Associations with metabolic syndrome and obstructive sleep apnea syndrome beyond waist circumference. *Clinical Nutrition* 28(1): 46–51.

Opperman M (2013) What health professionals should know about omega-3 fatty acid supplements. *South African Journal of Clinical Nutrition* 26(2): 6–11. Available at: http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L369868166\nhttp://reference.sabinet.co.za/webx/access/electronic_journals/m_sajcn/m_sajcn_v26_n2_a3.pdf\nhttp://sfx.ub.rug.nl:9003/sfx_local?sid=EMBASE&issn=16070658&id=doi:&atitle=.

Paasch U, Grunewald S, Kratzsch J and Glander HJ (2010) Obesity and age affect male fertility potential. *Fertility and Sterility*. Elsevier Ltd 94(7): 2898–2901. Available at: http://dx.doi.org/10.1016/j.fertnstert.2010.06.047.

Pacey A a. (2009) Male fertility and infertility. *Obstetrics, Gynaecology and Reproductive Medicine*. Elsevier Ltd. 19(2): 42–47. Available at: http://dx.doi.org/10.1016/j.ogrm.2008.11.006.

Pacey A a. (2012) Assessment of male factor. *Best Practice and Research: Clinical Obstetrics and Gynaecology*. Elsevier Ltd 26(6): 739–746. Available at: http://dx.doi.org/10.1016/j.bpobgyn.2012.05.006.

Palmer NO, Bakos HW, Fullston T and Lane M (2012) Impact of obesity on male fertility, sperm

function and molecular composition. *Spermatogenesis* 2(4): 253–263. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3521747&tool=pmcentrez&rendertype=abstract.

Park P and Goins R (1994) In Situ Preparation of Fatty Acid Methyl Esters for Analysis of Fatty Acid Composition in Foods. *Journal of Food Science* 59(6): 1262–1266.

Pasquali R (2006) Obesity, fat distribution and infertility. *Maturitas* 54(4): 363–371.

Pasqualotto FF, Sobreiro BP, Hallak J, Pasqualotto EB and Lucon AM (2005) Sperm concentration and normal sperm morphology decrease and follicle-stimulating hormone level increases with age. *BJU international* 96(7): 1087–91. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16225533.

Pasqualotto FF, Umezu FM, Salvador M, Borges E, Sobreiro BP and Pasqualotto EB (2008) Effect of cigarette smoking on antioxidant levels and presence of leukocytospermia in infertile men: a prospective study. *Fertility and sterility* 90(2): 278–83. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18462724.

Peng X, Zeng X, Peng S, Deng D and Zhang J (2009) The association risk of male subfertility and testicular cancer: a systematic review. *PloS one* 4(5): e5591. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2680046&tool=pmcentrez&rendertype=abstract.

Petraglia F, Serour GI and Chapron C (2013) The changing prevalence of infertility. *International Journal of Gynecology and Obstetrics*. International Federation of Gynecology and Obstetrics 123(SUPPL. 2): S4–S8. Available at: http://dx.doi.org/10.1016/j.ijgo.2013.09.005.

Du Plessis SS, Cabler S, McAlister D a, Sabanegh E and Agarwal A (2010) The effect of obesity on sperm disorders and male infertility. *Nature reviews. Urology* 7(3): 153–161. Available at: http://dx.doi.org/10.1038/nrurol.2010.6.

Povey a. C, Clyma J a., McNamee R, Moore HD, Baillie H, Pacey a. a. and Cherry NM (2012) Modifiable and non-modifiable risk factors for poor semen quality: A case-referent study. *Human Reproduction* 27(9): 2799–2806.

Practice Committee of the American Society for Reproductive Medicine (2012) Diagnostic evaluation of the infertile male: A committee opinion. *Fertility and Sterility*. American Society for Reproductive Medicine 98(2): 294–301. Available at: http://dx.doi.org/10.1016/j.fertnstert.2012.05.033.

Practice Committee of the American Society for Reproductive Medicine (2015) Diagnostic evaluation of the infertile male: a committee opinion. *Fertility and Sterility*. American Society for Reproductive Medicine 103(3): e18–e25. Available at: http://linkinghub.elsevier.com/retrieve/pii/S001502821402528X.

Preis SR, Massaro JM, Hoffmann U, D'Agostino RB, Levy D, Robins SJ, Meigs JB, Vasan RS, O'Donnell CJ and Fox CS (2010) Neck circumference as a novel measure of cardiometabolic risk: the Framingham Heart study. *The Journal of clinical endocrinology and metabolism* 95(8): 3701–10. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2913042&tool=pmcentrez&rendertype=abstract.

Qin D-D, Yuan W, Zhou W-J, Cui Y-Q, Wu J-Q and Gao E-S (2007) Do reproductive hormones explain the association between body mass index and semen quality? *Asian journal of andrology* 9(6): 827–34. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17968470.

Quallich S (2006) Examining male infertility. *Urologic nursing* 26(4): 277–88; quiz 289. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16939045.

Racette S, Evans E, Weiss E, Hagberg J and Holloszy J (2006) Abdominal Adiposity Is a Stronger Predictor of Insulin Resistance Than Fitness Among 50–95 Year Olds. *Diabetes Care* 29(3): 673–678.

Ramlau-Hansen CH, Nohr EA, Thulstrup AM, Bonde JP, Storgaard L and Olsen J (2007) Is maternal obesity related to semen quality in the male offspring? A pilot study. *Human reproduction (Oxford, England)* 22(10): 2758–2762. Available at: http://www.scopus.com/inward/record.url?eid=2-s2.0-34548745728&partnerID=tZOtx3y1.

Rayman MP (2012) Selenium and human health. *The Lancet*. Elsevier Ltd 379(9822): 1256–1268. Available at: http://dx.doi.org/10.1016/S0140-6736(11)61452-9.

Reddy SP, James S, Sewpaul R, Koopman F, Funani N, Sifunda S, Josie J, Masuka P, Kambaran N and Omardien R (2010) *Umthente Uhlaba Usamila-The South African Youth Risk Behaviour Survey 2008*. *Cape Town: South African Medical Research Council*.

Ricciardi R, Metter EJ, Cavanaugh EW, Ghambaryan A and Talbot L a (2009) Predicting cardiovascular risk using measures of regional and total body fat. *Applied nursing research : ANR*. Elsevier Inc. 22(1): 2–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19171289.

Rives N, Perdrix A, Hennebicq S, Saïas-Magnan J, Melin M-C, Berthaut I, Barthélémy C, Daudin M, Szerman E, Bresson J-L, Brugnon F and Bujan L (2012) The semen quality of 1158 men with testicular cancer at the time of cryopreservation: results of the French National CECOS Network. *Journal of andrology* 33(6): 1394–401. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22837112.

Rolf C, Cooper TG, Yeung CH and Nieschlag E (1999) Antioxidant treatment of patients with asthenozoospermia or moderate oligoasthenozoospermia with high-dose vitamin C and vitamin E: a randomized, placebo-controlled, double-blind study. *Human Reproduction* 14(4): 1028–1033. Available at: http://humrep.oxfordjournals.org/cgi/content/abstract/14/4/1028.

Ronquest- Ross L-C, Vink N and Sigge GO (2015) Food consumption changes in South Africa since 1994. *South African Journal of Science* 111(9): 1–12. Available at: http://www.scielo.org.za/scielo.php?pid=S0038-23532015000500013&script=sci_arttext&tlng=en.

Rossouw J (2015) The diet-heart hypothesis , obesity and diabetes. *South African Journal of Clinical Nutrition* 28(1): 38–44.

Rossouw L, Burger RP and Burger R (2012) The fertility transition in South Africa: A retrospective panel data analysis. *Development Southern Africa* 29(5): 738–755.

Safarinejad MR, Asgari SA, Farshi A, Ghaedi G, Kolahi AA, Iravani S and Khoshdel AR (2013) The effects of opiate consumption on serum reproductive hormone levels, sperm parameters, seminal plasma antioxidant capacity and sperm DNA integrity. *Reproductive toxicology (Elmsford, N.Y.)*. Elsevier Inc. 36: 18–23. Available at:

http://www.ncbi.nlm.nih.gov/pubmed/23207164.

Safarinejad MR, Hosseini SY, Dadkhah F and Asgari MA (2010) Relationship of omega-3 and omega-6 fatty acids with semen characteristics, and anti-oxidant status of seminal plasma: a comparison between fertile and infertile men. *Clinical nutrition (Edinburgh, Scotland)*. Elsevier Ltd 29(1): 100–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19666200.

Safarinejad MR and Safarinejad S (2009) Efficacy of Selenium and/or N-Acetyl-Cysteine for Improving Semen Parameters in Infertile Men: A Double-Blind, Placebo Controlled, Randomized Study. *Journal of Urology*. American Urological Association 181(2): 741–751. Available at: http://dx.doi.org/10.1016/j.juro.2008.10.015.

Saka M, Türker P, Ercan A, Kiziltan G and Baş M (2014) Is neck circumference measurement an indicator for abdominal obesity? A pilot study on Turkish Adults. *African health sciences* 14(3): 570–575. Available at: http://www.scopus.com/inward/record.url?eid=2-s2.0-84906924509&partnerID=tZOtx3y1.

Schmid TE, Eskenazi B, Baumgartner a., Marchetti F, Young S, Weldon R, Anderson D and Wyrobek a. J (2007) The effects of male age on sperm DNA damage in healthy non-smokers. *Human Reproduction* 22(1): 180–187.

Scholtz S, Vorster H, Matshego L and Vorster H (2001) Foods from animals can be eaten everyday - not a conundrum! *South African Journal of Clinical Nutrition* 14(3): S39–S47.

Schonfeldt HC, Pretorius B and Hall N (2013) Food-Based Dietary Guidelines for South Africa: 'Fish, chicken, lean meat and eggs can be eaten daily' 8. *S Afr J Clin Nutr* 26(3): 66–76.

Schonfeldt HC, Pretorius B and Hall N ((n.d.)) 8. 'Fish, chicken, lean meat and eggs can be eaten daily': a food-based dietary guideline for South Africa. *South African Journal of Clinical Nutrition*, S66–S76. Available at: http://sajcn.co.za/index.php/SAJCN/article/view/747 (accessed 19/01/16).

Selit I, Basha M, Maraee a, El-Naby SH, Nazeef N, El-Mehrath R and Mostafa T (2013) Sperm DNA and RNA abnormalities in fertile and oligoasthenoteratozoospermic smokers. *Andrologia* 45(1): 35–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22564026.

Sermondade N, Faure C, Fezeu L, Shayeb a. G, Bonde JP, Jensen TK, Van Wely M, Cao J, Martini a. C, Eskandar M, Chavarro JE, Koloszar S, Twigt JM, Ramlau-Hansen CH, Borges E, Lotti F, Steegers-Theunissen RPM, Zorn B, Polotsky a. J, La Vignera S, Eskenazi B, Tremellen K, Magnusdottir E V., Fejes I, Hercberg S, Lévy R and Czernichow S (2012) BMI in relation to sperm count: An updated systematic review and collaborative meta-analysis. *Human Reproduction Update* 19(3): 221–231.

Sharma R, Biedenharn KR, Fedor JM and Agarwal A (2013) Lifestyle factors and reproductive health: taking control of your fertility. *Reproductive biology and endocrinology : RB&E*. Reproductive Biology and Endocrinology 11(1): 66. Available at: http://www.rbej.com/content/11/1/66.

Sharpe RM (2010) Environmental/lifestyle effects on spermatogenesis. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 365(1546): 1697–1712.

Shayeb a. G, Harrild K, Mathers E and Bhattacharya S (2011) An exploration of the association

between male body mass index and semen quality. *Reproductive BioMedicine Online* 23(6): 717–723. Available at: http://dx.doi.org/10.1016/j.rbmo.2011.07.018.

Shefi S and Turek PJ (2006) Definition and current evaluation of subfertile men. *International Braz J Urol* 32(4): 385–397.

Sheiner EK, Sheiner E, Hammel RD, Potashnik G and Carel R (2003) Effect of occupational exposures on male fertility: literature review. *Industrial health* 41(2): 55–62.

Sherwood L (2013) *Human Physiology*. United States of America: McGraw-Hill:761-812.

Sheynkin Y, Jung M, Yoo P, Schulsinger D and Komaroff E (2005) Increase in scrotal temperature in laptop computer users. *Human Reproduction* 20(2): 452–455.

Showell M, Brown J, Yazdani A, Stankiewicz M and Hart R (2012) Antioxidants for male subfertility (Review). (3).

Showell MG, Brown J, Yazdani A, Stankiewicz MT and Hart RJ (2011) Antioxidants for male subfertility. *Cochrane database of systematic reviews (Online)*, CD007411.

Sikka SC (2004) Role of oxidative stress and antioxidants in andrology and assisted reproductive technology. *J Androl* 25(1): 5–18. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14662779.

Simmons LW, Firman EC, Rhodes G and Peters M (2004) Human sperm competition: testis size, sperm production and rates of extrapair copulations. (1995): 297–302.

Sjöström CD, Håkangård AC, Lissner L and Sjöström L (1995) Body compartment and subcutaneous adipose tissue distribution--risk factor patterns in obese subjects. *Obesity research* 3(1): 9–22. Available at: http://onlinelibrary.wiley.com/doi/10.1002/j.1550-8528.1995.tb00116.x/abstract\nhttp://www.ncbi.nlm.nih.gov/pubmed/7712364.

Smuts CM and Wolmarans PW (2013) 10. The importance of the quality or type of fat in the diet: a food-based dietary guideline for South Africa. *South African Journal of Clinical Nutrition*, S87–S99. Available at: http://sajcn.co.za/index.php/SAJCN/article/view/750 (accessed 19/01/16).

De Souza GL and Hallak J (2011) Anabolic steroids and male infertility: A comprehensive review. *BJU International* 108(11): 1860–1865.

Stewart AF and Kim ED (2011) Fertility concerns for the aging male. *Urology*. Elsevier Inc. 78(3): 496–9. Available at: http://www.sciencedirect.com/science/article/pii/S0090429511006200.

Stewart TM, Liu DY, Garrett C, Jørgensen N, Brown EH and Baker HWG (2009) Associations between andrological measures, hormones and semen quality in fertile Australian men: inverse relationship between obesity and sperm output. *Human reproduction (Oxford, England)* 24(7): 1561–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19351657.

Suleiman SA, Eamin Ali M, Zaki ZMS, El-Malik EM and Nasr MA (1996) Lipid peroxidation and human sperm motility: protective role of vitamin E. *Journal of Andrology* 17(5): 530–537. Available at: http://onlinelibrary.wiley.com/doi/10.1002/j.1939-4640.1996.tb01830.x/abstract.

Tavilani H, Doosti M, Nourmohammadi I, Mahjub H, Vaisiraygani A, Salimi S and Hosseinipanah SM (2007) Lipid composition of spermatozoa in normozoospermic and asthenozoospermic males. *Prostaglandins Leukotrienes and Essential Fatty Acids* 77(1): 45–50.

Teerds KJ, de Rooij DG and Keijer J (2011) Functional relationship between obesity and male reproduction: from humans to animal models. *Human reproduction update* 17(5): 667–83. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21546379.

Thomas B and Bishop J. (2007) *Manual of Dietetic Practice, In conjunction with British Dietetic Association.* 4th edition. London : Blackwell Publishing:324.

Thonneau P, Bujan L, Multigner L and Mieusset R (1998) Occupational heat exposure and male fertility: a review. *Human reproduction (Oxford, England)* 13(8): 2122–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9756281.

Tikkiwal M, Ajmera RL and Mathur NK. 1987. Effect of zinc administration on seminal zinc and fertility of oligospermic males. *Indian journal of physiology and pharmacology* 31(1): 30–4. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3666872 (accessed 20/01/16).

Toshimori K (1998) Maturation of mammalian spermatozoa: Modifications of the acrosome and plasma membrane leading to fertilization. *Cell and Tissue Research* 293: 177–187.

Travison TG, Araujo AB, Kupelian V, O'Donnell AB and McKinlay JB (2007) The relative contributions of aging, health, and lifestyle factors to serum testosterone decline in men. *The Journal of clinical endocrinology and metabolism* 92(2): 549–555.

Tremellen K (2008) Oxidative stress and male infertility — a clinical perspective. 14(3): 243–258.

Tsao C-W, Liu C-Y, Chou Y-C, Cha T-L, Chen S-C and Hsu C-Y (2015) Exploration of the Association between Obesity and Semen Quality in a 7630 Male Population. *PloS one* 10(3): e0119458. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4379020&tool=pmcentrez&rendertype=abstract.

Tydeman-Edwards R (2012) Obesity, undernutrition and the double burden of disease in the Free State. (July). Available at: http://etd.uovs.ac.za/ETD-db/theses/available/etd-11212012-093519/unrestricted/Tydeman-EdwardsR.pdf.

USDA (2010) *Dietary guidelines for Americans*. . Available at: http://www.health.gov/dietaryguidelines/dga2010/DietaryGuidelines2010.pdf.

Vaamonde D, Da Silva-Grigoletto ME, Garcia-Manso JM, Barrera N and Vaamonde-Lemos R (2012) Physically active men show better semen parameters and hormone values than sedentary men. *European Journal of Applied Physiology* 112(9): 3267–3273. Available at: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=medl&AN=22234399\nhttp://openurl.ac.uk/athens:_edu//lfp/LinkFinderPlus/Display?sid=OVID:Ovid+MEDLINE%28R%29&id=pmid:22234399&id=10.1007%2Fs00421-011-2304-6&issn=1439-6319&isbn=&volume=11.

Vaamonde D, Da Silva-Grigoletto ME, García-Manso JM, Vaamonde-Lemos R, Swanson RJ and Oehninger SC (2009) Response of semen parameters to three training modalities. *Fertility and sterility* 92(6): 1941–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19013565

(accessed 02/12/15).

Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M and Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. *The international journal of biochemistry & cell biology* 39(1): 44–84.

Van Heerden IV and Parry CDH (2001) If you drink alcohol, drink sensibly, *South African Journal of Clinical Nutrition* 14(3): S71.

Varshini J, Srinag BS, Kalthur G, Krishnamurthy H, Kumar P, Rao SBS and Adiga SK (2012) Poor sperm quality and advancing age are associated with increased sperm DNA damage in infertile men. *Andrologia* 44(SUPPL.1): 642–649.

Te Velde ER and Pearson PL (2002) The variability of female reproductive ageing. *Human Reproduction Update* 8(2): 141–154.

Vendel Nielsen L, Krogager TP, Young C, Ferreri C, Chatgilialoglu C, Nørregaard Jensen O and Enghild JJ (2013) Effects of Elaidic Acid on Lipid Metabolism in HepG2 Cells, Investigated by an Integrated Approach of Lipidomics, Transcriptomics and Proteomics. *PLoS ONE* 8(9).

Venter CS and van Eyssen E (2001) More legumes for better overall health, *South African Journal of Clinical Nutrition* 14(3): S32.

Vorster H, Badham J and Venter C (2013) An introduction to the revised food-based dietary guidelines for South Africa. *South African Journal of Clinical Nutrition* 26(3): 100–104.

Vorster HH, Love P and Browne C (2001) Development of food-based dietary guidelines for South Africa — the process, *South African Journal of Clinical Nutrition* 14(3): S3.

Vorster HH and Nell TA (2001) Eat plenty of vegetables and fruits every day, *South African Journal of Clinical Nutrition* 14(3): S24.

Vorster H, Wenhold F, Wright H, Wentzel-Viljoen E, Venter C and Vermaak M (2013) 'Have milk, maas or yoghurt every day': a food-based dietary guideline for South Africa. *South African Journal of Clinical Nutrition* 26(3): S57–S65.

Van Waeleghem K, De Clercq N, Vermeulen L, Schoonjans F and Comhaire F (1996) Deterioration of sperm quality in young healthy Belgian men. *Human reproduction (Oxford, England)* 11(2): 325–9.

Walsh TJ, Croughan MS, Schembri M, Chan JM and Turek PJ (2009) Increased risk of testicular germ cell cancer among infertile men. *Archives of internal medicine* 169(4): 351–6. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2881689&tool=pmcentrez&rendertype=abstract.

Walsh TJ, Schembri M, Turek PJ, Chan JM, Carroll PR, Smith JF, Eisenberg ML, Van Den Eeden SK and Croughan MS (2010) Increased risk of high-grade prostate cancer among infertile men. *Cancer* 116(9): 2140–7. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2893877&tool=pmcentrez&rendertype=abstract.

Wathes DC, Abayasekara DRE and Aitken RJ (2007) Polyunsaturated fatty acids in male and female reproduction. *Biology of reproduction* 77(2): 190–201. Available at:

http://www.biolreprod.org/content/77/2/190.long.

Wendlinger C, Hammann S and Vetter W (2014) Various concentrations of erucic acid in mustard oil and mustard. *Food Chemistry*. Elsevier Ltd 153: 393–397. Available at: http://dx.doi.org/10.1016/j.foodchem.2013.12.073.

Wentzel-Viljoen E, Steyn K, Ketterer E and Charlton CE (2001) 12. 'Use salt and foods high in salt sparingly': a food-based dietary guideline for South Africa. *South African Journal of Clinical Nutrition*, S105–S113. Available at: http://sajcn.co.za/index.php/SAJCN/article/view/753 (accessed 19/01/16).

WHO (1995) Physical Status: the Use and Interpretations of Anthropometry. , 1–463.

WHO (2000) Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organization Technical Report Series*, i–xii, 1–253. Available at: http://libdoc.who.int/trs/WHO_TRS_894.pdf\nhttp://www.ncbi.nlm.nih.gov/pubmed/11234459.

WHO (2003) Diet, Nutrition and the Prevention of Chronic Disease. *World Health Organization* 1–160.

WHO (2004) Public health Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet* 363: 157–163. Available at: http://www.thelancet.com/journals/a/article/PIIS0140-6736(03)15268-3/fulltext.

WHO (2005) The WHO STEPwise approach to chronic disease risk factor surveillance. *WHO STEPS Surveillance Manual*.

Wolmarans P and Oosthuizen W (2001) Eat fats sparingly — implications for health and disease, *South African Journal of Clinical Nutrition* 14(3): S48.

World Health Organization (WHO). 2006. BMI classification: Global Database on Body Mass Index. Available at: http://apps.who.int/bmi/index.jsp?introPage=intro_3.html. (Accessed: 24 November 2014).

World Health Organization (WHO). 2006. BMI classification: Global Database on Body Mass Index. Available at: http://apps.who.int/bmi/index.jsp?introPage=intro_3.html. (Accessed: 24 November 2014).

WHO (2010) Examination and processing of human semen. *Online* Fifth edit(10): 286. Available at: http://whqlibdoc.who.int/publications/2010/9789241547789_eng.pdf.

WHO (2011) Waist Circumference and Waist-Hip Ratio Report of a WHO Expert Consultation. , 8–11.

World Health Organization (WHO). 2015. Obesity and overweight. WHO Fact Sheet N°311. Available at: http://www.who.int/mediacentre/factsheets/fs311/en/. (Accessed: 22 June 2015).

Winters SJ, Wang C, Abdelrahaman E, Hadeed V, Dyky MA and Brufsky A (2006) Inhibin-B levels in healthy young adult men and prepubertal boys: is obesity the cause for the contemporary decline in sperm count because of fewer Sertoli cells? *Journal of andrology* 27(4): 560–564. Available at:

http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=16582411&retmode=ref&cmd=prlinks.

Wong WY, Merkus HMW., Thomas CM., Menkveld R, Zielhuis GA and Steegers-Theunissen RP. (2002) Effects of folic acid and zinc sulfate on male factor subfertility: a double-blind, randomized, placebo-controlled trial. *Fertility and Sterility* 77(3): 491–498. Available at: http://www.sciencedirect.com/science/article/pii/S0015028201032290.

Wong WY, Thomas CM., Merkus JMW., Zielhuis GA and Steegers-Theunissen RP. (2000) Male factor subfertility: possible causes and the impact of nutritional factors. *Fertility and Sterility* 73(3): 435–442. Available at: http://www.sciencedirect.com/science/article/pii/S0015028299005518.

Wong WY, Zielhuis GA, Thomas CMG, Merkus HMWM and Steegers-Theunissen RPM (2003) New evidence of the influence of exogenous and endogenous factors on sperm count in man. *European journal of obstetrics, gynecology, and reproductive biology* 110(1): 49–54. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12932871.

Wu Q and Suzuki M (2006) Parental obesity and overweight affect the body-fat accumulation in the offspring: The possible effect of a high-fat diet through epigenetic inheritance. *Obesity Reviews* 7(2): 201–208.

Yang GR, Yuan S, Fu H, Wan G, Zhu L, Bu X, Zhang J, Du X, Li Y, Ji Y, Gu X and Li Y (2010) Neck Circumference Positively Related With Central Obesity, Overweight, and Metabolic Syndrome in Chinese Subjects With Type 2 Diabetes: Beijing Community Diabetes Study 4. *Diabetes Care* 33(11): 2465–2467.

Zar JH (1984) *Biostatistical analysis*. 2nd edition. Englewood Cliffs, New Jersey: Prentice Hall.

Zhang E, Zhang H, Zang Z, Chen J and Zhang B (2014) Association of Body Mass Index with Semen Quality and Sexual Hormone Levels among Men in Intrauterine Insemination. (July): 1861–1865.

Zini A and Al-Hathal N (2011) Antioxidant therapy in male infertility: fact or fiction? *Asian journal of andrology* 13(3): 374–381.

Zitzmann M (2013) Effects of age on male fertility. *Best practice & research. Clinical endocrinology & metabolism*. Elsevier Ltd 27(4): 617–28. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24054934.

Addendums

Addendum A: Advertisement to recruit participants

Advertisement for research

Male research participants needed!

The Departments of Obstetrics and Gynaecology and Nutrition and Dietetics from the University of the Free State are recruiting 100 healthy male volunteers (18 to 45 years) to take part in a study on the "Impact of nutrient supplementation on semen parameters". The study will entail two semen analyses performed at the start and two analyses after three months of supplementation with a daily nutrient supplement. Results of the analysis will be provided to participants on completion of the study.

If you are interested to participate in this study, please contact the Department of Obstetrics and Gynaecology at 051-405 3272 or the Department of Nutrition and Dietetics at 051-401 2894 during office hours or send an e-mail to Nutrition @ufs.ac.za.

Advertensie vir navorsingsprojek

Manlike deelnemers benodig vir 'n navorsingsprojek!

Die Departemente Obstetrie en Ginekologie en Voeding en Dieetkunde van die Universiteit van die Vrystaat werf graag 100 gesonde, manlike vrywilligers (18 – 45 jaar) om aan 'n studie, "Impak van voedingstofsupplementasie op semenparameters" deel te neem. Die studie behels twee semenontledings voor en twee semenonledings na drie maande se gebruik van 'n daaglikse voedingstofaanvulling of alternatief. Resultate van die ontledings sal aan deelnemers na afloop van die studie verskaf word.

Indien u belangstel om aan die studie deel te neem, kontak die Departement Obstetrie en Ginekologie by 051-405 3272 of die Departement Voeding en Dieetkunde by 051-401 2894 tydens kantoorure of stuur 'n e-pos aan Nutrition@ufs.ac.za.

Addendum B: Permission to advertise on campus of the University of the Free State

UFS·UV

23 June 2014

School of Medicine
University of the Free State

Dear Miss du Toit

CSA Research Committee: Study approval and registration

With reference to your application for approval by registration with the College of Student Affairs (CSA) Research Committee of your study, *Impact of nutrient supplementation on semen parameters,* submitted on 5 June 2014, I am pleased to report committee approval granted for your study to engage the student population for the purposes of the research.

Your study is registered with the CSA Research Desk for its full duration, which desk is appointed to offer you support in further detailing access to and data collection among students. Also, please note that Dr WP Wahl is appointed to serve as your principal contact and you're requested to please contact him for further arrangements.

Kindly also note to schedule the submission of the required report of findings to the Research Desk upon completion of the study, as relfected in the research timeline you provided for the study.

Please do not hesitate to contact Mr. Vhugala Nthakheni, CSA Secretary, with further queries or requests for support.

Yours sincerely,

B Rudi Buys VDM, Dean of Student Affairs

CC: Dr. L Lategan
Dr. L Lange
Dr. WP Wahl
Mr. V Nthakheni

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Bloemfontein 9301, South Africa/Suid-Afrika
PO Box/Posbus 339
Bloemfontein 9300
South Africa/Suid-Afrika
www.ufs.ac.za

Addendum C: Information document and informed consent (English)

DEPARTMENT OF NUTRITION AND DIETETICS

UNIVERSITY OF THE FREE STATE

INFORMATION SHEET FOR RESEARCH PARTICIPANTS

Impact of Nutrient Supplementation on Semen Parameters

Dear participant

As a postgraduate student from the Department of Nutrition and Dietetics I would like to invite you to participate in this research project. Before you decide whether you would like to participate, I want to make sure that you understand what it is all about by explaining to you why I do the research and what the research study entails. Please read through the following information sheet and feel free to ask me or a medical practitioner any questions; or to discuss it with anybody you might feel more comfortable with.

Why do we do the research?

I, Elmine du Toit a PhD student, am conducting this research project as part of my post graduate studies at the University of the Free State. We realise that research is very important to improve both health and the wellbeing of society. This research aims to contribute to improving semen parameters and fertility in males.

Many nutrition and lifestyle factors may contribute to poor semen quality. With this research study I want to identify the nutrition and life-style factors in males that might affect semen quality. I will provide you with nutrient supplements or alternatives for 90 days to measure the effect on semen parameters.

Why is information on your nutrition and lifestyle important for this study?

It is important to identify day to day habits which include usual diet, exercise and smoking. Researching these factors will help improve our understanding of these factors on semen parameters. If the nutrient supplements affect semen parameters, nutrient supplementation can be used to obtain healthy sperm or as part of future treatment.

Do I really have to participate in the research?

I would really appreciate it if you decide to participate in the study. Participation is voluntary. You can decide whether or not to take part and are free to withdraw at any time without reason. You will not be discriminated against if you decide not to take part in the study. If you decide to participate in this research, you will be asked to complete and sign a consent form provided.

What will happen during the research?

I need at least 100 participants to participate in the research. The research study will take place at the Department of Obstetrics and Gynaecology. You will be provided with a unique number to protect your identity in this study. You will be weighed and your height, neck, waist, and hip circumference will be measured. You will need to provide two semen samples two to three days apart. For three days before you give the first sample until after you have given the last sample you need to abstain from any sexual activity. You will also be asked to complete a questionnaire on nutrition and lifestyle factors that will take 10-15 minutes to complete. You will be supplied with nutrient supplements or alternatives for 90 days that you will have to take every morning and evening. You will need to come back to the Department of Obstetrics and Gynaecology to provide another two semen samples two to three days apart and abstain from sexual activity for three days before you give the first sample until after you have given the last sample.

What do I have to do?

If you agree to participate, we will ask the following from you:

- To provide two semen samples two to three days apart;
- To complete a questionnaire honestly and to the best of your ability;
- To allow us to measure your weight, height, neck, waist, and hip circumference;
- Not to take any other supplement(s) than what you have been provided with during the three months of the study.
- To continue with your habitual food and fluid intake for 3 months;
- To drink the nutrient supplements or alternatives every morning and evening for 90 days;
- To make an appointment to provide two semen samples two to three days apart after 90 days of supplement use.

We plan the following schedule:

Day 1 and 4 of the study

Provide a semen samples on each of these two days. You need to abstain from any sexual activity for three days before the first semen sample until after you have given the second semen sample.

On day 1 weight, height, neck, waist, and hip circumference will be measured and a questionnaire completed. Nutrient supplements or alternatives will be provided for 90 days. Please start taking the products on the day you receive it.

Supplementation day 1-90

Please remember to drink the product provided every morning and every evening. If you forgot to take the supplements or alternatives, please take it as soon as you remember.

After 90 days (3 months)

Please make an appointment to provide two semen samples at the Department of Obstetrics and Gynaecology and please return your empty product containers. You will need to provide a semen samples on two days with two to three days between each sample. You need to abstain from any sexual activity for three days before the first semen sample until after you have provided the second semen sample.

Are there any risks, side effects or discomfort that I can expect?

As the amount of each nutrient in the supplement will not exceed the safe upper limit, and neither of the products provided will carry any risk or cause any side effect.

What are the benefits of participating in the study?

Research always provides benefit by developing the world. It will not cost you any money to participate in this study and you will receive the analysis of you semen sample and nutrition supplements free of charge.

What will we do with the results of the study?

All results from the study will be handled confidentially and your name will not be placed on any reports. It will not be possible to identify you as a participant of the study in any research reports. Our records might be inspected for quality control and statistical analysis and all efforts will be made to keep personal information confidential by providing you with a unique number. The results of the study may be published in medical journals and / or presented at scientific meetings, but confidentiality will be maintained. You can request a copy of the study results after the study has been completed.

Who can I contact for more information?

You are welcome to ask us any questions you may have concerning the study and your participation in it. If you have any other questions during the research, you are welcome to contact Ms Elmine du Toit at 062 179 3390 or your medical practitioner.

If you have any problems or complaints with the study, you can contact the secretary of the Ethics Committee of the Faculty of Health Sciences, University of the Free State at telephone 051-405 2812.

Thank you for your time and considering taking part in this research study!

Kind regards, Elmine du Toit RD (SA)

Consent form **Ethics committee reference number: ECUFS NR 08/2014**

DEPARTMENT OF NUTRITION AND DIETETICS

FACULTY OF HEALTH SCIENCES

Impact of Nutrient Supplementation on Semen Parameters

I have been asked to participate in this research study and the study has been explained to me by_____.

Please tick the following if you agree:

1	I have read and understand the Information document for the above mentioned study and had the opportunity to ask questions.	
2	I understand that it is my choice to participate in the research study and that I can withdraw from it at any time without any reason, without discrimination or any of my rights affected.	
3	I understand that I can be removed from the study without my consent.	
4	I understand that this study has received ethical approval from the Ethics Committee, Faculty of Health Sciences, University of the Free State.	
5	I understand that research records may be audited for quality control and that data analysis will be done anonymously and hereby provide permission to these authorities and scientists to access my data and study records.	
6	I understand that the results from this research study will be presented as oral or written reports and understand that I will not be identified by name in any reports or publications.	
7	I understand what will be expected from me in this research study and voluntarily agree to participate.	
8	I give consent that I will be reminded of my follow-ups after two and a half months of supplement use.	
9	I undertake to drink the supplements or alternatives provided daily.	

Research participant:	Name:	
	Signature:	
	Date:	
Researcher:	Name:	
	Signature:	
	Date:	
Witness:	Name:	
	Signature:	
	Date:	

Cell phone number: _____

Inligtingstuk en ingeligte toestemming (Afrikaans)

DEPARTEMENT VOEDING EN DIEETKUNDE

UNIVERSITEIT VAN DIE VRYSTAAT

INLIGTINGSTUK VIR DEELNEMERS

Impak van voedingstofsupplementasie op semen parameters

Geagte deelnemer

As ‘n nagraadse student in die Departement Voeding en Dieetkunde, nooi ek u graag om aan hierdie navorsingsprojek deel te neem. Voor u besluit om deel te neem, wil ek seker maak dat u die volgende aangaande hierdie projek verstaan. Ek verduidelik graag hoekom ek hierdie navorsing gaan doen en wat die navorsingstudie behels. Lees asseblief die volgende inligtingstuk en rig enige vrae aan my of aan die mediese praktisyn of bespreek die inligtinge met wie ookal u op u gemak voel.

Hoekom doen ons navorsing?

Ek, Elmine du Toit, ‘n PhD student, doen hierdie navorsingsprojek as deel van my nagraadse studies by die Universiteit van die Vrystaat onder leiding van my studieleiers. Ons besef dat navorsing baie belangrik is vir die gesondheid en welstand van die gemeenskap. Hierdie navorsing het ten doel om by te dra tot die verbetering semenparameters vrugbaarheid in mans.

Baie voeding- en leefstylfaktore mag bydra tot lae semenkwaliteit. Met hierdie navorsingsprojek wil ek graag vasstel watter voeding- en leefstylfaktore semenkwaliteit beïnvloed. Ek sal ook aan u ‘n voedingstofaanvullingt vir 90 dae verskaf om die effek daarvan op spermparameters te meet.

Waarom is inligting oor u voeding en leefstyl belangrik vir hierdie studie?

Dit is belangrik om daaglikse gewoontes soos dieet, oefening en rook te identifiseer. Navorsing oor hierdie faktore sal help om die invloed daarvan op die semenparameter te verstaan. Indien die voedingstofaanvulling semenkwaliteit verbeter, kan voedingstofaanvullingse ook in toekomstige behandeling gebruik word.

Moet ek regtig aan die studie deelneem?

Ek sal dit regtig waardeer indien u aan hierdie studie sal deelneem. Deelname is vrywillig. U kan self besluit om deel te neem of nie en u kan enige tyd aan die studie onttrek, sonder om

‘n rede te verskaf. Daar sal nie teen u gediskrimineer word indien u sou besluit om nie aan die navorsing deel te neem nie. Sou u instem om aan die navorsing deel te neem, sal gevra word dat u ‘n toestemmingsvorm voltooi en onderteken.

Wat gaan tydens die navorsing gebeur?

Ek benodig ten minste 100 persone om aan hierdie navorsing deel te neem. Die navorsingstudie sal plaasvind by die Departement Obstetrie en Ginekologie, Universiteit van die Vrystaat. U sal van ‘n unieke nommer voorsien word om u identiteit in hierdie studie te beskerm. U gewig, lengte, middel-, heup- en nekomtrek sal gemeet word. U moet twee semenmonsters twee tot drie dae uitmekaar verskaf. U moet u weerhou van enige seksuele aktiwiteit vir drie dae voordat u die eerste monster verskaf totdat u die laaste monster verskaf het. U sal ook gevra word om ‘n vraelys oor voeding- en leefstylfaktore te voltooi. Die vraelys sal 10 – 15 minute neem om te voltooi. U sal van ‘n vitamien-voedingstofaanvullingste vir 90 dae voorsien word wat u elke oggend moet drink. Na drie maande moet u asseblief weer na die Departement Obstetrie en Ginekologie, Universiteit van die Vrystaat kom om nog twee semenmonster twee tot drie dae uitmekaar te verskaf en weerhou van enige seksuele aktiwiteit vir drie dae voordat u die eerste monster verskaf totdat u die laaste monster verskaf het.

Wat moet ek doen?

Indien u besluit om deel te neem, sal dit die volgende van u vereis:

- Om twee semenmonster twee tot drie dae uitmekaar te verskaf; Om ‘n vraelys eerlik en na die beste van u vermoë te voltooi;
- Om ons toe te laat om u gewig, lengte, middel-, heup- en nekomtrek te neem;
- Om nie enige ander supplemente as die supplemente wat aan u verskaf is vir die drie maande van hierdie studie te gebruik nie;
- Om vir die 3 maande van hierdie studie te eet soos wat u gewoonlik geëet het;
- Om die voedingstofaanvullings wat aan u verskaf is elke oggend en aand vir 90 dae te drink;
- Om ‘n afspraak te maak om nog twee semenmonsters twee tot drie dae uitmekaar te verskaf 90 dae nadat u die supplement begin gebruik het.

Ons beplan die volgende skedule:

Dag 1 en 4

Verskaf ‘n semenmonster op elk van die twee dae. U moet u weerhou van enige seksuele aktiwiteite vir drie voordat u die eerste monster verskaf totdat u die laaste monster verskaf het. Op dag 1 sal u gewig, lengte, middel-, heup- en nekomtrek gemeet en ‘n vraelys voltooi word. Voedingstofaanvullings sal vir 90 dae verskaf word. Begin asseblief die supplement drink op die dag wat u dit ontvang.

Supplementasie dag 1-90

Onthou asseblief om elke oggend en aand u supplement te drink. Die supplement sal slegs effektief wees indien u dit daagliks drink. Indien u vergeet het om u supplement te drink, drink die supplement asseblief so gou as wat u onthou.

Na 90 dae (3 maande)

Maak asseblief ‘n afspraak om weer twee semenmonsters twee tot drie dae uitmekaar te verskaf. U moet u weerhou van enige seksuele aktwiteit vir drie dae voordat u die eerste semenmonster verskaf totdat u die laaste semenmonster verskaf het. Bring asseblief ook u leë voedingstofaanvullinghouers saam.

Kan ek enige risiko’s, newe-effekte of ongemak verwag?

Aangesien die voedingstowwe in die supplement nie die veilige bogrens vir inname oorskrei nie hou die supplement nie enige risiko of newe effekte in nie.

Wat is die voordele van deelname aan die studie?

Navorsing hou altyd voordele vir ontwikkeling in. Indien fondse beskikbaar is sal u vergoeding vir reisonkoste ontvang nadat die studie voltooi is. Deelname aan die studie is gratis en u sal die ontleding van u semenmonster en voedingstofaanvulling ontvang om vir 90 dae te drink.

Wat gaan ons met die resultate van die studie doen?

Alle resultate van die studie sal vertroulik hanteer word en u naam gaan in geen verslae verskyn nie. Dit sal nie moontlik wees om u as deelnemer in enige navorsingsverslae te identifiseer nie. Ons rekords mag vir kwaliteitskontrole en statistiese ontleding nagegaan word en moeite sal gedoen word om u persoonlike inligting vertroulik te hou deur aan u ‘n unieke nommer te gee. Die resultate van die studie mag in mediese tydskrifte gepubliseer word en/of tydens wetenskaplike vergaderings voorgelê word, maar vertroulikheid sal behou word. U kan ‘n kopie van die studie se resultate van ons aanvra nadat die studie voltooi is.

Wie kan ek kontak vir meer inligting?

U is welkom om vir ons enige vrae oor die studie en u deelname daaraan te vra. As u enige ander vrae gedurende die ondersoek het, is u welkom om Me. Elmine du Toit by 062 179 3390 of u mediese praktisyn te kontak.

Indien u enige probleme of klagtes in verband met die studie het, kan u die sekretaresse van die Etiekkomitee van die Fakulteit Gesondheidswetenskappe, Universiteit van die Vrystaat, kontak by 051-405 2812.

Dankie vir u tyd en dat u oorweeg om aan hierdie navorsingstudie deel te neem!

Vriendelike groete

Elmine du Toit,
RD (SA)

Toestemmingsvorm **Etiekkomitee verwysingsnommer:ECUFS NR 08/2014**

DEPARTEMENT VOEDING EN DIEETKUNDE

FAKULTEIT GESONDHEIDSWETENSKAPPE

Impak van voedingstofsupplementasie op semenparameters

Ek is gevra om aan hierdie navorsingstudie deel te neem en die studie is deur ____ _____aan my verduidelik.

Merk asseblief met 'n regmerk indien jy met die volgende saam stem:

1	Ek het die inligtingsdokument vir bogenoemde studie gelees, ek verstaan dit en ek het die geleentheid gehad om vrae te vra.	
2	Ek verstaan dat dit my keuse is om aan die navorsingstudie deel te neem, en dat ek enige tyd sonder rede kan ontrek sonder dat teen my gediskrimineer sal word of dat my regte geraak word.	
3	Ek verstaan dat ek sonder my toestemming van die studie verwyder kan word.	
4	Ek verstaan dat hierdie studie deur die Etiekkomitee and die Fakulteit Gesondheidwetenskappe, Universiteit van die Vrystaat goedgekeur is.	
5	Ek verstaan dat die navorsingsrekords as deel van kwaliteitskontrole geïnspekteer kan word en dat data-ontleding anoniem gedoen sal word. Hiermee verleen ek toestemming aan persone in beheer en wetenskaplikes om toegang tot my data en mediese rekords te verkry.	
6	Ek verstaan dat die resultate van hierdie navorsingstudie as mondelinge of geskrewe verslag aangebied sal word en dat ek nie by name in enige verslag of publikasie identifiseer sal word nie.	
7	Ek verstaan wat van my in die navorsingstudie verwag word en stem vrywillig in om deel te neem.	
8	Ek verleen toestemming dat ek herinner word aan my opvolgafsprake na twee en'n half maande van supplement gebruik.	
9	Ek onderneem om die produkte wat aan my verskaf word soggens en saans te drink.	

Deelnemer:	Naam:	
	Handtekening:	
	Datum:	
Navorser:	Naam:	
	Handtekening:	
	Datum:	
Getuie:	Naam:	
	Handtekening:	
	Datum:	

Selfoonnommer: _____

Addendum D: Questionnaires

Impact of Nutrient Supplementation on Semen Parameters

Participant study code ☐☐☐

Date of birth? d d m m y y

For Office Use ☐☐☐

☐☐☐☐☐☐

d d m m y y

Please indicate your choice with an X in the block ☒.
Please complete all questions and do not leave any questions unanswered.
Please mark only one answer at each question.
Use the serving size as a guide to determine how much you usually eat, if you eat greater sizes than the medium serving: indicate it as L, if less than a medium serving indicate as S.

THIS SECTION WILL INCLUDE QUESTIONS ON YOUR DIETARY INTAKE.

1. During the past month, how often did you eat fruit?

Include fresh, frozen or canned fruit, do not include fruit juice.

None or less than 1 a week	1 time per week	2-3 times per week	4-6 times per week	1 x day	2 x day	3 x day	> 3 day
1	2	3	4	5	6	7	8

1.2. What portion size do you usually eat at a time?

S	M	L
¼ cup or size of 1 ping pong ball 	1 cup or 1 fist size 	2 cups or 2 fist sizes 
1	2	3

2. During the past month, how often did you eat vegetables?

Include fresh, frozen or canned, also vegetables used in stews and dishes.

None or less than 1 a week	1 time per week	2-3 times per week	4-6 times per week	1 x day	2 x day	3 x day	> 3 day
1	2	3	4	5	6	7	8

2.1. What portion size do you usually eat?

S	M	L
¼ cup or size of 1 ping pong ball 	1 cup or 1 fist size 	2 cups or 2 fist size 
1	2	3

For Office Use

3. List the 5 vegetables and/or fruit you eat most

1._____

2._____

3._____

4._____

5._____

4. How often do you eat the following?

	None or less than 1 a week	1 time per week	2-3 times per week	4-6 times per week	1 x day	2 x day	3 x day	> 3 day
Spaghetti, whole-wheat	1	2	3	4	5	6	7	8
Barley, pearled	1	2	3	4	5	6	7	8
Bran flakes	1	2	3	4	5	6	7	8
Bran muffin	1	2	3	4	5	6	7	8
Oatmeal, instant or cooked	1	2	3	4	5	6	7	8
Popcorn, air-popped	1	2	3	4	5	6	7	8
Brown rice, cooked	1	2	3	4	5	6	7	8
Bread, rye	1	2	3	4	5	6	7	8
Bread/rolls, whole-wheat or multigrain	1	2	3	4	5	6	7	8
White bread/rolls	1	2	3	4	5	6	7	8
Brown bread/rolls	1	2	3	4	5	6	7	8
Legumes/Beans/Soy	1	2	3	4	5	6	7	8

5. For the next section, select the option that you use most

Do you use more:		**Or more:**			
White bread/rolls	1	Brown/whole-wheat bread or rolls	2	**Or none**	3
Porridge (maize, maltabella)	1	Porridge (Oats)	2	**Or none**	3
Refined Cereals (Cornflakes, Rice Crispies, Frosties)	1	Hi Fiber Cereals (All Bran, Hi-Fiber Bran, Muesli)	2	**Or none**	3

6.1. How often would you use milk / milk products (Include yogurt, milk in tea and coffee, milk drinks, hot chocolate, milo, milkshakes)?

None or less than 1 a week	1 time per week	2-3 times per week	4-6 times per week	1 x day	2 x day	3 x day	> 3 day
1	2	3	4	5	6	7	8

For Office Use

6.2. On average how much milk/ milk products would you use (include yogurt, milk in tea and coffee, milk drinks, hot chocolate milo, milkshakes) in a day? | | | | | | ml

6.3. Which milk product do you use most? (select one)

Milk skim 0 %	1
Milk low fat 1 %	2
Milk low fat 2 %	3
Milk full cream	4
Maas/Amazi	5
Soy milk	6
Do not use milk at all	7

7.1. How often do you eat the following?

	None or less than 1 a week	1 time per week	2-3 times per week	4-6 times per week	1 x day	2 x day	3 x day	> 3 day
Beef	1	2	3	4	5	6	7	8
Chicken	1	2	3	4	5	6	7	8
Pork	1	2	3	4	5	6	7	8
Liver	1	2	3	4	5	6	7	8
Seafood (including fish)	1	2	3	4	5	6	7	8

7.2. Do you remove the visible fat on your meat before eating?

Yes 1 No 2

7.3. What portion size do you usually eat?

S	M	L
Smaller than a medium portion	Size of palm of hand = 120g 	Bigger than a medium portion
1	2	3

7.4. How many eggs do you eat a week (include eggs used in dishes)? | | | |

8.1. How often do you eat the following?

	None or less than 1 a week	1 time per week	2-3 times per week	4-6 times per week	1 x day	2 x day	3 x day	> 3 day
Bacon	1	2	3	4	5	6	7	8
Russians /salami / Vienna's	1	2	3	4	5	6	7	8
Biltong with fat	1	2	3	4	5	6	7	8
Cheese or cheese spread, regular	1	2	3	4	5	6	7	8

For Office Use

8.2. What portion size do you usually eat?

S	M	L
Smaller than a medium portion	30g or the size of thumb 	Bigger than a medium portion
1	2	3

8.3. How often do you eat the following?

	None or less than 1 a week	1 time per week	2-3 times per week	4-6 times per week	1 x day	2 x day	3 x day	> 3 day
Nuts (all nuts including peanuts)	1	2	3	4	5	6	7	8

8.4. What portion size do you usually eat?

S	M	L
Smaller than a medium portion	¼ cup or size of 1 ping pong ball 	Bigger than a medium portion
1	2	3

8.5. For the next section, select the option that you use most

	Do you use more:		Or more:			
8.5.1	Butter or margarine (wrapped in foil or paper)	1	Oils and soft margarines in tub	2	Or none	3
8.5.2	Mayonnaise, regular	1	Mayonnaise reduced or low fat	2	Or none	3
8.5.3	Salad dressings, regular	1	Salad dressing reduced or low fat	2	Or none	3
8.5.4	Fried potato chips/ French fries	1	Oven baked chips	2	Or none	3
8.5.5	Fats / oils when cooking	1	Extra butters / margariine / oils at the table	2	Or none	3
8.5.6	Non dairy creamer (e.g. cremora)	1	Low-fat or skimmed milk	2	Or none	3

8.6. How often do you eat meals prepared at home?

(exclude pre-prepared meals/ready to eat meals)

None or less than 1 a week	1 time per week	2-3 times per week	4-6 times per week	1 x day	2 x day	3 x day	> 3 day
1	2	3	4	5	6	7	8

For Office Use

8.7. How often do you have take-aways? (e.g. burgers, pizza, pies, KFC)

None or less than 1 a week	1 time per week	2-3 times per week	4-6 times per week	1 x day	2 x day	3 x day	> 3 day
1	2	3	4	5	6	7	8

8.8. Which cooking method is used most at home when preparing foods?
(mark only one)

Fried, stir-fried or sautéed in fat/ oil/ margarine or butter	1
Grilled / braaied	2
Steamed or Boiled	3

9.1. How often do you eat the following?

	None or less than 1 a week	1 time per week	2-3 times per week	4-6 times per week	1 x day	2 x day	3 x day	> 3 day
Vegetable or meat spreads: Marmite / Oxo	1	2	3	4	5	6	7	8
Popcorn / Chips	1	2	3	4	5	6	7	8
Gravy (made with stock)	1	2	3	4	5	6	7	8
Chutney, Atchar, Worcester sauce Tomato sauce	1	2	3	4	5	6	7	8
Soup (any)	1	2	3	4	5	6	7	8

9.2. Do you add salt to your food before tasting it?

Yes 1 No 2

10.1. How often do you drink the following?

	None or less than 1 a week	1 time per week	2-3 times per week	4-6 times per week	1 x day	2 x day	3 x day	> 3 day
Water	1	2	3	4	5	6	7	8
100 % fruit juice or vegetable juice	1	2	3	4	5	6	7	8
Sweetened juice beverage/ drink	1	2	3	4	5	6	7	8
Soft drink / cold drink e.g. Coke	1	2	3	4	5	6	7	8
Artificially sweetened soft drink / cold drink e.g. Coke Zero	1	2	3	4	5	6	7	8
Sports drinks (e.g. Powerade)	1	2	3	4	5	6	7	8
Energy drinks (e.g. Play, Red Bull)	1	2	3	4	5	6	7	8
Tea / coffee with sugar	1	2	3	4	5	6	7	8
Tea / coffee without sugar	1	2	3	4	5	6	7	8

For Office Use

10.2. How much do you drink at a time?

	None	< ¾ cup	1 cup	1 ½ cup	2 cups	>2 ½ cup
		175 ml	250 ml	375 ml	500ml	625ml
Water	1	2	3	4	5	6
100 % fruit juice or vegetable juice	1	2	3	4	5	6
Sweetened juice beverage/ drink	1	2	3	4	5	6
Soft drink / cold drink e.g. Coke	1	2	3	4	5	6
Artificially sweetened soft drink / cold drink e.g. Coke Zero	1	2	3	4	5	6
Sports drinks (e.g. Powerade)	1	2	3	4	5	6
Energy drinks (e.g. Play, Red Bull)	1	2	3	4	5	6
Tea / coffee with sugar	1	2	3	4	5	6
Tea / coffee without sugar	1	2	3	4	5	6

10.3. How often do you drink the following ?

	None or less than 1 a week	1 time per week	2-3 times per week	4-6 times per week	1 x day	2 x day	3 x day	> 3 day
Hard liquor (shots) 25ml	1	2	3	4	5	6	7	8
Beer, ales, wine cooler 340ml	1	2	3	4	5	6	7	8
Wine (red, rosé or white) 120ml	1	2	3	4	5	6	7	8

10.4. How many units do you drink at a time?

	None	1 units	2 units	3 units	4 units	≥5 units
Hard liquor (shots) 25ml	1	2	3	4	5	6
Beer, ales, wine cooler 340ml	1	2	3	4	5	6
Wine (red, rosé or white) 120ml	1	2	3	4	5	6

10.5.How many days in the week do you **not** drink alcohol? ☐

THIS SECTION WILL INCLUDE QUESTIONS ON YOUR LIFESTYLE.
Mark with a ☒ and please write in print

For Office Use

11. Do you currently smoke or have you smoked cigarettes in the last three months? Yes [1] No [2]

11.1. If yes, how many cigarettes in a day ? [ ][ ][ ]

11.2. How many packs do you smoke per week ? [ ][ ][ ]

11.3. How many years have you been smoking ? [ ][ ]

11.4. Are you regularly exposed to secondary /passive smoke at home or work ? Yes [1] No [2]

12.1. Do you use nutritional supplements?
(include multivitamins/mineral, commercial protein and energy shakes etc)
Yes [1] No [2]

12.2. If yes, what is the name(s) of the supplement(s) you use ?

_____

_____

(Please include photocopy of labels/label)

13. Have you used any recreational drugs in the past 3 months?
Yes [1] No [2]

14. Do you work with a laptop on your lap?
Yes [1] No [2]

15. How many hours a day do you use electronic devices connected to Wi-Fi (tablets, smartphones, laptops)?

None	1-3 hours	4-8 hours	> 8 hours
1	2	3	4

16. Where do you carry your cellphone?

Belt	Hip pocket	Shirt	Other
1	2	3	4

17. Do you wear tight fitting underwear or trousers (example cycling shorts or tight fitting jeans)?
Yes [1] No [2]

18. Do you use a sauna? Yes [1] No [2]

19. Do you take a hot bath? Yes [1] No [2]

20. How stressful would you say your life or work is at the moment?
(rate from 1 – 10; 1 being the lowest and 10 the highest) Mark with a X

1	2	3	4	5	6	7	8	9	10

THIS SECTION WILL INCLUDE QUESTIONS ON YOUR ACTIVITY LEVEL

Mark with a ☒ and please write in print

For Office Use

21. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, aerobics, or fast bicycling?
Think about only those physical activities that you did for at least 10 minutes at a time.

☐ days a week or ☐ none

a) How much time in total did you usually spend on one of those days doing vigorous physical activities?

☐☐ hours
☐☐☐ minutes

22. Again, think only about those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

☐ days a week or ☐ none

a) How much time in total did you usually spend on one of those days doing moderate physical activities?

☐☐ hours
☐☐☐ minutes

23. During the last 7 days, on how many days did you walk for at least 10 minutes at a time? This includes walking at work and at home, walking to travel from place to place, and any other walking that you did solely for recreation, sport, exercise or leisure.

☐ days a week or ☐ none

a) How much time in total did you usually spend walking on one of those days?

☐☐ hours
☐☐☐ minutes

24. The next question is about the time you spent sitting on weekdays while at work, at home, while doing course work and during leisure time. This includes time spent sitting at a desk, visiting friends, reading traveling on a bus or sitting or lying down to watch television.

During the last 7 days, how much time in total did you usually spend sitting on a week day?

☐☐ hours
☐☐☐ minutes

25. Would you say the questions on activity that you have answered represent what you would have usually done in the last 3 months?

Yes ☐1 No ☐2

Impak van Voedingstofsupplementasie op Semenparameters

Vir kantoorgebruik

Deelnemer nommer | | | | | |

Geboortedatum? | d | d | m | m | j | j |

d d m m j j

Dui asseblief u keuse met 'n X in die blokkie aan ☒.
Antwoord asseblief alle vrae en moet nie enige vrae onbeantwoord laat nie.
Merk asseblief net een blokkie by elke vraag.
Gebruik die porsiegrootte ("serving size") as 'n riglyn om aan te dui hoeveel u gewoonlik eet. Indien u meer as die medium grootte eet, dui dit as L (large) aan, indien u minder as die medium grootte een, dui dit as S (small) aan.

1. Hoeveel vrugte het u die afgelope maand geëet?

Sluit vars, bevrore en ingemaakte vrugte in, moet nie vrugtesap insluit nie.

Geen of minder as 1 keer per week	1 keer per week	2-3 keer per week	4-6 keer per week	1 x dag	2 x dag	3 x dag	> 3 dag
1	2	3	4	5	6	7	8

1.2. Watter porsiegrootte eet u gewoonlik per keer?

S	M	L
¼ koppie of grootte van 1 tafeltennisbal 	1 koppie of 1 vuisgrootte 	2 koppies of 2 vuisgroottes 
1	2	3

2. Hoeveel groente het u die afgelope maand geëet?

Sluit vars, bevrore, geblikte en groente wat in bredies en geregte gebruik is in.

Geen of minder as 1 keer per week	1 keer per week	2-3 keer per week	4-6 keer per week	1 x dag	2 x dag	3 x dag	> 3 dag
1	2	3	4	5	6	7	8

2.1. Watter porsiegrootte eet u gewoonlik per keer?

S	M	L
¼ koppie of grootte van 1 tafeltennisbal	1 koppie of 1 vuisgrootte 	2 koppies of 2 vuisgroottes 
1	2	3

Vir kantoorgebruik

3. Lys die 5 groente en/of vrugte wat u die meeste eet

1._____

2._____

3._____

4._____

5._____

4. Hoe dikwels eet u die volgende?

	Geen of minder as 1 keer per week	1 keer per week	2-3 keer per week	4-6 keer per week	1 x dag	2 x dag	3 x dag	> 3 dag
Spaghetti, volgraan	1	2	3	4	5	6	7	8
Gort	1	2	3	4	5	6	7	8
Bran flakes	1	2	3	4	5	6	7	8
Bran muffin	1	2	3	4	5	6	7	8
Hawermout/*oats*, kits of gaar	1	2	3	4	5	6	7	8
Popcorn, air-popped	1	2	3	4	5	6	7	8
Bruinrys	1	2	3	4	5	6	7	8
Rogbrood	1	2	3	4	5	6	7	8
Brood /rolletjies, volgraan of *multigrain*	1	2	3	4	5	6	7	8
Witbrood/rolletjies	1	2	3	4	5	6	7	8
Bruinbrood/rolletjies	1	2	3	4	5	6	7	8
Peulgroente/Droë bone/Soja	1	2	3	4	5	6	7	8

5. Vir die volgende afdeling, kies die opsie wat u die meeste gebruik

Gebruik u meer:		**Of meer:**			
Witbrood/rolletjies	1	Bruin/volgraan brood of rolletjies	2	**Of geen**	3
Pap (mieliepap, maltabella)	1	Pap (hawermout/*oats*)	2	**Of geen**	3
Verfynde grane (*Cornflakes, Rice Crispies, Frosties*)	1	Hoë vesel ontbytgrane (*All Bran, Hi-Fiber Bran, Muesli*)	2	**Of geen**	3

6.1. Hoe dikwels gebruik u melk / melkprodukte (Sluit jogurt, melk in tee en koffie, melkdranke, *hot chocolate*, *milo*, melkskommel in)?

Geen of minder as 1 keer per week	1 keer per week	2-3 keer per week	4-6 keer per week	1 x dag	2 x dag	3 x dag	> 3 dag
1	2	3	4	5	6	7	8

Vir kantoorgebruik

6.2. Hoeveel melk/melkprodukte gebruik u gemiddeld per dag
(sluit jogurt, melk in tee en koffie, melkdranke, *hot chocolate* *milo*, melkskommels in)? | | | | | | ml

6.3. Watter melkprodukte gebruik u die meeste? (kies een)

Melk afgeroom (*skim*) 0 %	1
Melk lae vet 1 %	2
Melk lae vet 2 %	3
Melk volroom	4
Maas/Amazi	5
Sojamelk	6
Gebruik glad nie melk nie	7

7.1. Hoe dikwels eet u die volgende?

	Geen of minder as 1 keer per week	1 keer per week	2-3 keer per week	4-6 keer per week	1 x dag	2 x dag	3 x dag	> 3 dag
Beesvleis	1	2	3	4	5	6	7	8
Hoender	1	2	3	4	5	6	7	8
Varkvleis	1	2	3	4	5	6	7	8
Lewer	1	2	3	4	5	6	7	8
Vis en seekos	1	2	3	4	5	6	7	8

7.2. Verwyder u sigbare vet aan die vleis voordat u eet?
Ja 1 Nee 2

7.3. Watter porsiegrootte eet u gewoonlik?

S	M	L
Kleiner as 'n medium porsie	Grootte van 'n handpalm = 120g 	Groter as 'n medium porsie
1	2	3

7.4. Hoeveel eiers eet u per week (sluit eiers wat in geregte gebruik word in)? | | | |

8.1. Hoe dikwels eet u die volgende?

	Geen of minder as 1 keer per week	1 keer per week	2-3 keer per week	4-6 keer per week	1 x dag	2 x dag	3 x dag	> 3 dag
Spek/*Bacon*	1	2	3	4	5	6	7	8
Russians / salami / Vienna's	1	2	3	4	5	6	7	8
Biltong met vet	1	2	3	4	5	6	7	8
Kaas of kaassmeer.gewone	1	2	3	4	5	6	7	8

Vir kantoorgebruik

8.2. Watter porsiegrootte eet u gewoonlik?

S	M	L
Kleiner as 'n medium porsie	30g of 'n duimgrootte 	Groter as 'n medium porsie
1	2	3

8.3. Hoe dikwels eet u die volgende?

	Geen of minder as 1 keer per week	1 keer per week	2-3 keer per week	4-6 keer per week	1 x dag	2 x dag	3 x dag	> 3 dag
Neute (sluit alle neute, asook grondbone in)	1	2	3	4	5	6	7	8

8.4. Watter porsiegrootte eet u gewoonlik?

S	M	L
Kleiner as 'n medium porsie	¼ koppie of grootte van 1 tafeltennisbal 	Groter as 'n medium porsie
1	2	3

8.5. Vir die volgende afdeling, kies die opsie wat u die meeste gebruik

	Gebruik u meer:		**Of meer:**			
8.5.1	Botter of harde vet (in foelie of papier)	1	Olie en sagte margarien (in bakkie)	2	**Of geen**	3
8.5.2	Mayonnaise, gewone	1	Mayonnaise verlaagde of lae vet	2	**Of geen**	3
8.5.3	Slaaisous, gewone	1	Slaaisous verlaagde of lae vet	2	**Of geen**	3
8.5.4	Slap chips	1	*Oven chips*	2	**Of geen**	3
8.5.5	Vet / olie vir gaarmaak	1	Ekstra botter/margarien/ olie aan tafel	2	**Of geen**	3
8.5.6	Nie-suiwelverromer (bv. cremora)	1	Lae vet of afgeroomde melk	2	**Of geen**	3

8.6. Hoe gereeld eet u maaltye wat tuis berei word?
(sluit voorafbereide/gereed om te eet/*ready to eat* maaltye uit)

Geen of minder as 1 keer per week	1 keer per week	2-3 keer per week	4-6 keer per week	1 x dag	2 x dag	3 x dag	> 3 da
1	2	3	4	5	6	7	8

Vir kantoorgebruik

8.7. Hoe gereeld eet u wegneem-etes bv. burgers, pizza, pasteie, KFC?

Geen of minder as 1 keer 'n week	1 keer per week	2-3 keer per week	4-6 keer per week	1 x dag	2 x dag	3 x dag	> 3 dag
1	2	3	4	5	6	7	8

8.8. Watter gaarmaakmetode word tuis die meeste vir voedselbereiding gebruik?
(merk slegs een)

Braai, roerbraai of soteer in vet/ olie/ margarien of botter	1
Rooster / braai oor kole	2
Stoom of Kook	3

9.1. Hoe gereeld eet u die volgende ?

	Geen of minder as 1 per week	1 keer per week	2-3 keer per week	4-6 keer per week	1 x dag	2 x dag	3 x dag	> 3 dag
Groente- of vleissmeer: Marmite / Oxo	1	2	3	4	5	6	7	8
Popcorn / Harde chips	1	2	3	4	5	6	7	8
Bruin- / vleissous (gemaak van aftreksel)	1	2	3	4	5	6	7	8
Blatjang, Atjar, Worcestersous, Tamatiesous	1	2	3	4	5	6	7	8
Sop (enige soort)	1	2	3	4	5	6	7	8

9.2. Voeg u sout by u kos voordat u daaraan geproe het?

Ja 1 Nee 2

10.1. Hoe gereeld drink u die volgende?

	Geen of minder as 1 per week	1 keer per week	2-3 keer per week	4-6 keer per week	1 x dag	2 x dag	3 x dag	> 3 dag
Water	1	2	3	4	5	6	7	8
100 % vrugtesap of groentesap	1	2	3	4	5	6	7	8
Versoete vrugtesap/ vrugtedrank	1	2	3	4	5	6	7	8
Versoete koeldrank bv. Coke	1	2	3	4	5	6	7	8
Kunsmatig versoete koeldrank bv. Coke Zero	1	2	3	4	5	6	7	8
Sportdrankie (bv. Powerade)	1	2	3	4	5	6	7	8
Energiedrankie (bv. Play, Red Bull)	1	2	3	4	5	6	7	8
Tee / koffie met suiker	1	2	3	4	5	6	7	8
Tee / koffie sonder suiker	1	2	3	4	5	6	7	8

Vir kantoorgebruik

10.2. Hoeveel drink u per keer?

	Geen	< ¾ K	1 K	1½ K	2 K	>2½ K
		175 ml	250 ml	375 ml	500ml	625ml
Water	1	2	3	4	5	6
100 % vrugtesap of groentesap	1	2	3	4	5	6
Versoete vrugtesap/ vrugtedrank	1	2	3	4	5	6
Koeldrank bv. Coke	1	2	3	4	5	6
Kunsmatig versoete koeldrank bv Coke Zero	1	2	3	4	5	6
Sportdrankie (bv. Powerade)	1	2	3	4	5	6
Energiedrankie (bv. Play, Red Bull)	1	2	3	4	5	6
Tee / koffie met suiker	1	2	3	4	5	6
Tee / koffie sonder suiker	1	2	3	4	5	6

10.3. Hoe gereeld drink u die volgende?

	Geen of minder as 1 keer per week	1 keer per week	2-3 keer per week	4-6 keer per week	1 x dag	2 x dag	3 x dag	> 3 dag
Sterk drank / h*ard liquor* sopies /*shots* 25ml	1	2	3	4	5	6	7	8
Bier, *ales, wine cooler* 340ml	1	2	3	4	5	6	7	8
Wyn (rooi, rosé of wit) 120ml	1	2	3	4	5	6	7	8

10.4. Hoeveel eenhede drink u per keer?

	Geen	1 een-heid	2 een-hede	3 een-hede	4 een-hede	≥5 ee+I27 3n-hede
Sterk drank / *Hard liquor* sopies / *shots* 25ml	1	2	3	4	5	6
Bier, *ales, wine cooler* 340ml	1	2	3	4	5	6
Wyn (rooi, rosé of wit) 120ml	1	2	3	4	5	6

10.5.Hoeveel dae per week drink u **nie** alkohol nie? ☐

HIERDIE AFDELING SLUIT VRAE OOR U LEEFSTYL IN.
Merk met 'n ☒ en skryf asseblief in drukskrif

Vir kantoorgebruik

11. Rook u tans of het u in die afgelope drie maande sigarette gerook? Ja [1] Nee [2]

11.1. Indien ja, hoeveel sigarette per dag? [ ][ ][ ]

11.2. Hoeveel pakkies rook u per week ? [ ][ ][ ]

11.3. Hoeveel jaar rook u al? [ ][ ]

11.4. Word u gereeld tuis of by die werk aan sekondêre/ /passiewe rook blootgestel? Ja [1] Nee [2]

12.1. Gebruik u enige voedingsupplemente?
(insluitend multivitamien/mineraal, kommersiële proteïen- en energie *shakes* ens.)
Ja [1] Nee [2]

12.2. Indien ja, wat is die naam/name van die supplement(e) wat u gebruik?

_____

_____

(Sluit asseblief 'n fotostaat van die etiket of etiket in)

13. Het u enige dwelms in die afgelope 3 maande gebruik?
Ja [ ] Nee [2]

14. Werk u met 'n skootrekenaar op u skoot?
Ja [1] Nee [2]

15. Hoeveel uur per dag gebruik u elektroniese toestelle wat aan WiFi gekoppel is (tablette, slimfone, skootrekenaars)?

Geen	1-3 ure	4-8 ure	> 8 ure
1	2	3	4

16. Waar dra u u selfoon?

Belt	Broek-sak	Hemp	Ander
1	2	3	4

17. Dra u styfpassende onderklere of broeke (bv. fietsrybroeke of styfpassende jeans)?
Ja [1] Nee [2]

18. Sauna u? Ja [1] Nee [2]

19. Bad u in warm water? Ja [1] Nee [2]

20. Hoe stresvol is u lewe of werk tans?
Dui aan van 1 – 10; 1 is die laagste en 10 die hoogste) Merk met 'n X

1	2	3	4	5	6	7	8	9	10

HIERDIE AFDELING SLUIT VRAE OOR U AKTIWITEITSVLAK IN
Merk met 'n ☒ en skryf asseblief in drukskrif

Vir kantoorgebruik

21. Gedurende die afgelope 7 dae, op hoeveel dae het u intense fisieke oefening gedoen soos optel van swaar voorwerpe, spit, aerobiese oefening, of vinnig fiets gery?
Dink slegs aan daardie fisieke aktiwiteite wat u vir ten minste 10 minute per keer gedoen het.

☐ dae per week of ☐ geen

a) Hoeveel tyd in totaal het u gewoonlik per dag aan intense fisieke aktiwiteite spandeer?

☐☐ ure
☐☐☐ minute

22. Dink weer slegs aan daardie fisieke aktiwiteite wat u vir ten minste 10 minute per keer gedoen het.
Gedurende die afgelope 7 dae, op hoeveel dae het u matige fisieke aktiwiteite gedoen soos dra van ligte items, fietsry teen 'n matige spoed, of dubbelspel tennis? Moet nie stap insluit nie.

☐ dae per week of ☐ geen

a) Hoeveel tyd in totaal het u gewoonlik per dag aan matige fisieke aktiwiteit spandeer?

☐☐ ure
☐☐☐ minute

23. Gedurende die afgelope 7 dae, op hoeveel dae het u vir ten minste 10 minute per keer gestap? Dit sluit in stap by die werk en by die huis, stap van plek tot plek, en enige stap wat u net vir ontspanning, sport, oefening en genot gedoen het.

☐ dae per week of ☐ geen

a) Hoeveel tyd in totaal het u gewoonlik per dag gestap?

☐☐ ure
☐☐☐ minute

24. Die volgende vraag handel oor die hoeveelheid tyd wat u weeksdae sit by die werk, by die huis, terwyl u kursuswerk doen en sit vir ontspanning. Dit sluit die hoeveelheid tyd in wat u sit by 'n lessenaar, vriende besoek, lees terwyl u bus ry of sit of lê terwyl u televisie kyk.

Gedurende die afgelope 7 dae, hoeveel tyd in totaal het u gewoonlik gesit op 'n weeksdag?

☐☐ ure
☐☐☐ minute

25. Sou u sê dat die vrae wat u oor u aktiwiteit geantwoord het verteenwoordigend is van wat u die afgelope 3 maande gedoen het?

Ja ☐ 1 Nee ☐ 2

Addendum E: Nutrient analysis of supplements

Centrum® Adult	
Amount Per Serving (1 tablet)	**% Daily Value**
Vitamin A 3,500 IU (29% as Beta-Carotene)	70%
Vitamin C 60 mg	100%
Vitamin D 1,000 IU	250%
Vitamin E 30 IU	100%
Vitamin K 25 μg	31%
Thiamin 1.5 mg	100%
Riboflavin 1.7 mg	100%
Niacin 20 mg	100%
Vitamin B_6 2 mg	100%
Folic Acid 400 μg	100%
Vitamin B_{12} 6 μg	100%
Biotin 30 μg	10%
Pantothenic Acid 10 mg	100%
Calcium 200 mg	20%
Irson 18 mg	100%
Phosphorus 20 mg	2%
Iodine 150 μg	100%
Magnesium 50 mg	13%
Zinc 11 mg	73%
Selenium 55 μg	79%
Copper 0.5 mg	25%
Manganese 2.3 mg	115%
Chromium 35 μg	29%
Molybdenum 45 mcg	60%
Chloride 72 mg	2%

Centrum® Adult	
Potassium 80 mg	2%
Nickel 5 μg	*
Silicon 2 mg	*
Tin 10 μg	*
Vanadium 10 μg	*

* Daily Value not established.

Ingredients: Calcium Carbonate, Potassium Chloride, Dibasic Calcium Phosphate, Magnesium Oxide, Microcrystalline Cellulose, Ascorbic Acid (Vit. C), Ferrous Fumarate, dl-Alpha Tocopheryl Acetate (Vit. E), Maltodextrin.**Contains < 2% of:** Beta-Carotene, BHT (to preserve freshness), Biotin, Calcium Pantothenate, Cholecalciferol (Vit. D_3), Chromium Picolinate, Corn Starch, Crospovidone, Copper Sulfate, Cyanocobalamin (Vit. B_{12}), Folic Acid, Gelatin, Hydrogenated Palm Oil, Magnesium Stearate, Manganese Sulfate, Modified Corn Starch, Niacinamide, Nickelous Sulfate, Phytonadione (Vit. K), Polyethylene Glycol, Polyvinyl Alcohol, Potassium Iodide, Pregelatinized Corn Starch, Pyridoxine Hydrochloride (Vit. B_6), Riboflavin (Vit. B_2), Silicon Dioxide, Sodium Ascorbate (to preserve freshness), Sodium Metavanadate, Sodium Molybdate, Sodium Selenate, Stannous Chloride, Talc, Thiamine Mononitrate (Vit. B_1), Titanium Dioxide, Tocopherols (to preserve freshness), Vitamin A Acetate, Yellow 6 Lake, Zinc Oxide.

Centrum® MYNUTRIENTS™ Omega-3 MiniGel

Ingredients	**Amount per MiniGel**
Total Omega-3	366 mg
EPA	220m mg
DHA	110 mg
Other Omega-3's	36 mg

Other ingredients:

Glycerine, gelatin (bovine), purified water, tocopherols, sunflower oil, citric acid, canola oil & natural flavour

Gluten free; suitable for diabetics

Potential allergens:

Fish, soybean oil, sulphur dioxide and sulphites

A varied, balanced diet and a healthy lifestyle are important. This product should not be used as a substitute for a balanced diet.

Addendum F: South African Journal of Clinical Nutrition Author Guidelines

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All named authors must give consent to publication. Authorship should be based only on substantial contribution to: (i) conception, design, analysis and interpretation of data; (ii) drafting the article or revising it critically for important intellectual content; (iii) final approval of the version to be published. All three of these conditions must be met (Uniform requirements for manuscripts submitted to biomedical journals; www.icmje.org/index.html).

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Authors must declare all sources of support for the research and any association with the product or subject that may constitute conflict of interest.

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Identifying information should not be published in written descriptions, photographs, and pedigrees unless the information is essential for scientific purposes and the patient (or parent or guardian) gives informed written consent for publication. Informed consent for this purpose requires that the patient be shown the manuscript to be published. (www.icmje.org)

Ethnic classification
Work that is based on or contains reference to ethnic classification must indicate the rationale for this.

Manuscripts
Short items are more likely to appeal to our readers and therefore to be accepted for publication. Manuscript should not exceed 4000 words in total all contents inclusive.

Original articles of 4 000 words or less, with up to 6 tables or illustrations, should normally report observations or research of relevance to the field of nutrition. References should preferably be limited to no more than 25.

Short reports or scientific letters, which include case reports, side effects of nutrient supplements/drugs and brief or negative research findings should be 1000 words or less, with 1 table or illustration and no more than 6 references.

Editorials, Opinions, Issues in the field of nutrition. should be about 1000 words and are welcome, but unless invited, will be subjected to the SAJCN peer review process.

Review articles are rarely accepted unless invited.

Letters to the editor, if intended for the correspondence column, should be marked 'for publication', signed by all authors and presented in triple spacing. Letters should be no longer than 400 words with only one illustration or table.

Obituaries should not exceed 400 words and may be accompanied by a photograph.

Manuscript preparation

- Please submit your manuscript electronically at www.sajcn.co.za
- Research articles should have a structured abstract not exceeding 250 words (50 for short reports) comprising: Objectives, Design, Setting, Subjects, Outcome measures, Results and Conclusions.
- A second abstract should be written in simple and clear spoken language highlighting the reason(s) that the research work was undertaken, the key findings and the key recommendations **WITHOUT**, overtly or covertly implying or containing any claims of whatsoever nature, but rather explaining how the work will help scientists (and/or lay persons) better understand and address the topic of investigation. The abstract should not exceed an absolute maximum of 75 words. In addition, please also include a < 140 character, "strong" message that can be used for social media.
- Refer to articles in recent issues for guidance on the presentation of headings and subheadings.
- Abbreviations should be spelt out when first used in the text and thereafter used consistently.

- Scientific measurements should be expressed in SI units except: blood pressure should be given in mmHg and haemoglobin values in g/dl.

If in doubt, refer to www.icmje.org/index.html

Illustrations

1. Figures consist of all material that cannot be set in type, such as photographs and line drawings.
2. Tables and legends for illustrations should appear on separate sheets and should be clearly identified.
3. Line drawings should be arranged to conserve vertical space. Note that reduction to 80 mm for a single column or 170 mm for double columns should not render lettering illegible. Explanations should be included in the legend and not on the figure itself.
4. Figure numbers should be clearly marked on the back of prints and the top of illustrations should be indicated.
5. If any tables or illustrations submitted have been published elsewhere, written consent to republication should be obtained by the author from the copyright holder and the author(s).
6. A limited number of illustrations are free at the discretion of the editor. Colour illustrations are encouraged but are charged to the author.

A quote will be provided on request. Consider sponsorship.

References
References should be inserted in the text as superior numbers and should be listed at the end of the article in numerical and not in alphabetical order.
Authors are responsible for verification of references from the original sources.

References should be set out in the Vancouver style and approved abbreviations of journal titles used; consult the List of Journals in Index Medicus for these details.

Names and initials of all authors should be given unless there are more than six, in which case the first three names should be given followed by et al. First and last page numbers should be given.

Journal references should appear thus:
1. Price NC . Importance of asking about glaucoma. BMJ 1983; 286: 349-350.

Book references should be set out as follows:
1. Jeffcoate N. Principles of Gynaecology. 4th ed. London: Butterworth, 1975: 96-101.
2. Weinstein L, Swartz MN. Pathogenic properties of invading microorganisms. In: Sodeman WA jun, Sodeman WA, eds. Pathologic Physiology: Mechanisms of Disease. Philadelphia: WB Saunders, 1974: 457-472.

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In the event of a manuscript needing revision following the peer review process, all revision changes to the original manuscript should be made using the "track changes" function in Microsoft Word, or in any other such similar format so as to facilitate the speedy completion of the review process. In the event of an "author-reviewer" difference of opinion, the author(s) should state their opinion in writing in the text, which should be bracketed. Revised manuscripts which do not conform to this revision format will be returned to the authors for editing.

Revised manuscript should be resubmitted electronically within 3 weeks of receipt thereof.

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As part of the submission process, authors are required to check off their submission's compliance with all of the following items, and submissions may be returned to authors that do not adhere to these guidelines.

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2. The submission file is in Microsoft Word, or RTF file format
3. When available, the URLs to access references online are provided, including those for open access versions of the reference. The URLs are ready to click (e.g., http://pkp.sfu.ca).
4. The text is single-spaced; uses a 12-point font; employs italics, rather than underlining (except with URL addresses); and all illustrations, figures, and tables are placed within the text at the appropriate points, rather than at the end.
5. The text adheres to the stylistic and bibliographic requirements outlined in the Author Guidelines, which is found in About the Journal.
6. If submitting to a peer-reviewed section of the journal, the instructions in Ensuring a Blind Review have been followed.
7. The manuscript has an abstract.
8. The second abstract should be written in simple and clear spoken language highlighting the reason(s) that the research work was undertaken, the key findings and the key recommendations WITHOUT, overtly or covertly implying or containing any claims of whatsoever nature, but rather explaining how the work will help scientists (and/or lay persons) better understand and address the topic of investigation. The abstract should not exceed an absolute maximum of 75 words. In addition, please also include a < 140 character, "strong" message that can be used for social media.

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Addendum G: South African Family practice Author Guidelines

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The author must always retain a copy. All the named authors must have approved the final manuscript. Pages should be numbered consecutively in the lower right corner. Please note that the Original Research section will follow a ";print-short, web-long"; policy, which means that only the abstracts will be published in print, with the full article published on the web. Some review articles may also be published under these provisions.

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1. *Original research* (Between 1000 and 3500 words):
2. *Letters to the Editor* (Up to 400 words):
3. *Scientific Letters* (Less than 600 words): A short abstract is required (125-150 words) and should be structured under the following headings: background, methods, results and conclusion. One table or graph and not more than 5 references.
4. *Review/CPD articles* (Up to 1800 words): Most review articles are published as part of the continuous professional development (CPD) programme of SAFP. A scientific editor is appointed to approve topics, invite authors and to review the articles before they are independently peer-reviewed. All articles are reviewed by a family physician as well a topic specialist. Review articles outside the CPD programme are welcomed. Once accepted they may be published in full in the printed journal OR a 250 word abstract will be published in print with the full article available online.
5. *Opinions (Open Forum)* (Between 1000 and 3500 words).
6. *Editorials* (Between 600 - 800 words): Scientific editorials can be used to highlight progress in any scientific field related to family medicine.

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